A TEXT-BOOK OF HISTOLOGY

BREMER
WEATHERFORD

A TEXT-BOOK of HISTOLOGY

ARRANGED UPON AN EMBRYOLOGICAL BASIS

BY

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SIXTH EDITION OF "LEWIS AND STOHR"

FIVE HUNDRED AND NINETY-EIGHT ILLUSTRATIONS

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PREFACE TO THE SIXTH EDITION

This is a presentation of the sixth edition of A Text-Book of Histology arranged upon an Embryological Basis by Frederic T. Lewis, which was originally a free translation of Phillip Stöhr's widely used *Lehrbuch der Histologie*. The second edition was changed materially and numerous citations and references to original papers added. The third, fourth and fifth editions were extensively rewritten by J. Lewis Bremer. Recent advances in histology have necessitated a further rewriting but the spirit and form of the previous editions have been adhered to, with emphasis upon development as essential for the understanding of structure and function. The book is a digest of a vast and growing literature, requiring consultation of both the classical and more recently published investigations with selected references to more than seven hundred of them. Of the five hundred and ninety-eight illustrations, three hundred and two are new to this edition; each chosen as a visual explanation of the text.

I am greatly indebted to my colleagues in the Department of Anatomy; Dr. F. T. Lewis and Dr. J. L. Bremer, the former editors of this book and to Dr. G. B. Wislocki, each of whom has been extremely helpful. I am particularly appreciative of the permission given by my former teacher, Professor Wilhelm von Möllendorff, Zürich, to use illustrations from the several works under his editorship. Dr. S. B. Wolbach, Dr. A. B. Dawson and Dr. Shields Warren, all members in other departments of Harvard University have been most generous in giving me either preparations from their collections or lending original drawings used in illustrating their works. Acknowledgment is made to the several authors and publishers who have allowed me the use of illustrations from their publications. Many of the new figures have been meticulously drawn by Miss Etta Piotti from original preparations and others redrawn from illustrations in the literature by either Miss Piotti or Miss Evelyn Glidden. Mr. Leo Talbert and Mr. Irving F. Rider are responsible for the excellent photography.

HAROLD L. WEATHERFORD.

HARVARD MEDICAL SCHOOL.

PREFACE TO THE FIFTH EDITION

The increasing vitality of the embryological and histological sciences within the last few years seems to require a rather radical revision of this textbook with the incorporation of much new material. More emphasis has been given to the normal functional changes in the various cells and to their activities in the living state, as correlated with the usual histological picture. The newer conceptions of the various hormones have been briefly included, and because of their importance the section on the endocrine glands has been advanced to a position ahead of the sections dealing with those organs in which their actions are best recognized. In general, however, the book follows the same plan as in former editions, and emphasis is still laid more on development and the resulting form than on function, except as the latter helps to explain the morphology.

No modern Textbook of Histology can hope to maintain reasonable dimensions if it is to enter exhaustively into the known details of the present subdivisions of the subject; larger handbooks are available for this purpose. To supply the deficiencies, I have greatly enlarged the bibliography of pertinent references, so that the interested student, with access to a good library, may search for further details in the original publications. This is to his advantage, for there he will find that the literature not only records essentially new discoveries, but also, especially in recent years, just as often tends to question formerly accepted ideas. New technical methods of approach to old problems alter the picture. Even slight familiarity with the literature will disclose the active vitality of modern anatomical science.

Many of my colleagues have helped me in this edition. Professor H. L. Weatherford has written the short section of 'the use of stains,' which replaces the former one on 'microscopic technique.' Professor George R. Minot has provided the new drawing of 'blood,' and Dr. Henry Jackson, Jr., has, himself, drawn the 'bone marrow' and aided in writing that part of the text. To Professor G. B. Wislocki and to Drs. A. L. Grafflin, T. L. Terry, and M. J. Eisenberg I am indebted for advice and assistance in writing of the hypophysis, kidney, eye, and teeth, and for many original illustrations supplied by them. I am indeed fortunate in having such a group at hand to call on, and I hereby thank them all gratefully. Other new illustrations for the book are borrowed from various publications, or are original; devised by me and drawn by Miss Piotti, Miss Cabot or Mr. Schumann. I thank them also for their patience and accuracy.

J. L. BREMER.

HARVARD MEDICAL SCHOOL

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J. L. Bremer.

Harvard Medical School

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MICROSCOPIC ANATOMY

Microscopy

Microscopic anatomy is the study of the minute structure of animals and plants, at present for general purposes limited by the resolving powers of the compound microscope. Of its two larger subdivisions, cytology[1] pertains to individual cells—their origin, structure, and functions; while histology[2] is concerned with the organization of aggregates of cells and the intercellular materials into tissues. The study of the several fundamental tissues is called general histology and that of the organs—special histology or organology.

DEVELOPMENT OF THE MICROSCOPE

The origin of the compound microscope has been a subject of no little speculation and controversy since a number of claimants have sought the credit for its invention. It is difficult to evaluate all these claims because the early history of the microscope and of the telescope parallel one another. Presumably the earliest compound microscope was made about 1590 by Zacharias Janssen, with the help of his father Hans, a spectacle-maker in Middleburg, the ancient capital of Zeeland in Holland.

Pierre Borel in his '*De vero telescopii inventore cum brevi omnium conspiciliorum historia. Accessit etiam Centuria observationum microscopicarum.*' Hag. Comitum (1655) included among other documents, a letter from Willem Boreel, the Dutch envoy to France, who wrote that as a child in Middelburg he knew Zacharias very well and had often heard it said that he and his father invented the microscope. He also stated in the letter that one of the early instruments was presented to Prince Maurice, the commander of the Dutch forces and a later one to Albert, Archduke of Austria, the governor of Holland. While in England in 1619, Boreel was shown this microscope which the Archduke had given to the Dutchman, Cornelius Drebbel, mathematician to King James I.

Professor P. Harting[3] stated that according to the Middelburg parish-register Zacharias was born in 1577 and would therefore have been only thirteen years old in

[1] The name *cytology* (cytologie) was probably introduced by J. B. Carnoy, 'La Biologie Cellulaire,' Lierre, 1884.

[2] The term *histology* was introduced by C. Mayer, 'Ueber Histologie und eine neue Eintheilung der Gewebe des menschlichen Körpers,' Bonn, 1819.

"Histology is the study of the texture of the so-called fundamental systems or tissues of the animal body, and of the origins and laws of their normal and abnormal development" (trans., p. 20). Carl Friedrich Heusinger, 'System der Histologie. Erster Theil. Histographie,' Eisenach, 1822.

[3] HARTING, 1859.

1590. It seems highly probable then that he must have *accidently placed two convex* lenses in the proper relative position in making his microscope. In 1866 two old optical instruments were given to the Museum of the Zeeland Scientific Society, both of them having been in the donor's family for several *generations and traditionally* believed to be early forms of inventions by Zacharias Janssen. Professor Harting examined these instruments and identified one as a compound microscope and the other as a telescope. The microscope was crudely made of tinned-iron plates soldered *together* into three tubes—an outer one into which slide two smaller tubes fitted with lenses. When closed the magnification was about three times and when withdrawn about nine times. This instrument is much more primitive than the one presented to the Archduke Albert, which according to Borel, was an upright affair of gilt brass supported upon *three dolphins* As the *Archduke* came to Zeeland in *1605* it is assumed that the instrument given him was of about that date. Professor Harting referring to the 'Middelburg microscope' wrote (translation)—"In spite of the fact that all direct proofs fail, I consider it by *no means improbable that the tradition is true* and that this instrument is one of the oldest microscopes, which by Hans and Zacharias Janssen or by the first alone were manufactured long before they brought to a finish instruments more accurate and *optically more* complete which were destined *for Prince Maurice and Archduke Albert.*" John Mayall Jr. inspected these same instruments left to the Museum and wrote (Journal Royal Microscopic Society, 1889, page 164) that he would "unhesitatingly affirm the microscope to be older than the so-called Galileo microscopes," still extant in Florence.

While the microscope was evidently invented in Holland it was developed and named in Italy. Galileo writing in 1610, said that he had learned of an ocular instrument made by a certain Dutchman, by means of which an object could be made to appear distant or near and that this information stimulated him to apply himself to the problem and arrive at its solution by reasoning These first crude instruments devised by Galileo were apparently not microscopes but telescopes It seems evident that he did not become acquainted with the compound microscope until instruments copied by Drebbel from Zacharias reached *Rome in 1622*

Within the next two years Galileo presented microscopes to a number of his friends. Portions of letters accompanying the gifts are reproduced by Govi[1]. One of these, dated September 23, 1624 was addressed to Federigo Cesi, the founder and head of the society of scholars—the Accademia dei Lincei, of which Galileo himself was a member. During the years following, this society did much to encourage microscopic studies.

Francesco Selluti, also a member of the Accademia, published in 1625 probably the first illustrations made from microscopic observations on bees which he curiously interpolated (p. 52) in his Italian translation of *the poems of Persius* (1630). The papal physician, Giovanni Faber in a letter to Cesi dated April 13, 1625, suggested the name '*microscopio*' since the Accademia had already coined the name '*telescopio.*' The versatile Jesuit, Athanasius Kircher ('Ars Magna Lucia et Umbrae'—Rome, 1646) aside from his many speculations told how microscopes should be made and urged those using them to procure the best of instruments.

Tremendous progress was made in the development of the microscope during the second half of the seventeenth century the instrument apparently being received with mixed enthusiasm. Robert Hooke[2] wrote in his 'Micrographia'—"my faithful *Mercury*, my Microscope" while the diarist Samuel Pepys, (August 13, 1664) after paying five pounds ten shillings for a microscope considered it "a great price, but a most curious bauble it is." Although there is no record that Pepys ever saw anything with his micro-

[1] Govi, 1888. [2] Hooke, 1665

scope, his contemporaries did. Marcello Malpighi in Italy, Antonie van Leeuwenhoek and Jan Swammerdam in Holland, and Robert Hooke and Nehemiah Grew in England made such fundamental discoveries with their instruments, that this period has been referred to as the "golden age of microscopy." Leeuwenhoek especially skillful in lens grinding made his own microscopes and a catalogue drawn up for the auction of his effects on May 29, 1747 listed 247 completed instruments and 172 mounted lenses. There were three lenses made of quartz but there is no evidence according to Dobell that he ground lenses from diamonds as has sometimes been reported. Professor Harting examined the Leeuwenhoek microscope in Utrecht and found it to have a magnifying power of no less than 270 diameters.

One of the greatest defects of these early microscopes was chromatic aberration—an object placed under the lens would shimmer with all the colors of the spectrum. Leading physicists and mathematicians worked on the problem and in 1757 John Dolland, an English mechanician, using the calculations of Chester More Hall (1722) made the first achromatic glass. It was, however, some time before this glass was used for making lenses for microscopes. Amici of Modena equipped his microscopes with achromatic lenses in 1816 and Charles Chevalier,[1] the Paris maker in 1824.

Perhaps the first microscope made in America was produced by Edward Bromfield,[2] a graduate of Harvard in the class of 1742. A portrait of Bromfield painted in 1745 and now hanging in the anatomical laboratory at Harvard shows him with his microscope which was an upright instrument supported by three metal scrolls. The Reverend Thomas Prince, writing in the American Magazine, Boston for November 30, 1746 tells of Bromfield and his skill in microscopy and lens making.

In 1847, the year following the establishment in Germany of the firm of Carl Zeiss, Charles A. Spencer began making microscopes commercially at Canastota, New York. His lenses were awarded highest honors at the Paris exhibition of 1878 when shown in competition with those of the best makers of Europe.

During the second half of the nineteenth century a number of manufacturers began making and improving upon microscopes in England, on the continent and in America. Many of their improvements were reported in the Journal of the Royal Microscopical Society, founded in 1839, which has been responsible for fostering standards such as the 'Society screw-thread' permitting an interchange of optical equipment from one instrument to another. The Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopische Technik established in 1884 has also been a medium for publication of improvements in microscopes and methods for microscopy.

For further reading on the history of the microscope see: Singer, 1914. Notes on the early history of microscopy, Proc. Roy. Soc. Med., vol. 7, pt. 2. Sect. Hist. Med., pp. 247–279. Disney, Hill and Baker, 1928. *Origin and Development of the Microscope.* The Royal Microscopical Society, London. Clay and Court, 1932. The history of the microscope, compiled from original instruments and documents, up to the introduction of the achromatic microscope. Charles Griffin and Company, Limited, London. Dobell, 1932. Antony van Leeuwenhoek and his "little animals." Harcourt, Brace and Company, New York. Woodruff, L. L., 1940, Microscopy before the Nineteenth Century, Biological Symposia, Vol. I, pp. 5–36.

USE OF THE MICROSCOPE

Within the last years most of the makers of microscopes have evolved a type suitable for students' use, provided with oculars of two

[1] Chevalier, 1839. [2] Hagen, 1870.

degrees of magnification and with three objectives, 'low power' (16 mm.), 'high dry' (4 mm.), and 'oil immersion' (2 mm.). The figures indicate the distance in millimeters of the objective from the section when the specimen is in focus. Such microscopes also have coarse and fine adjustment for regulating the position of the optical apparatus, and beneath the stage a mirror, one side plane, one concave, for directing the light, and an iris diaphragm and Abbé condenser for regulating the amount of

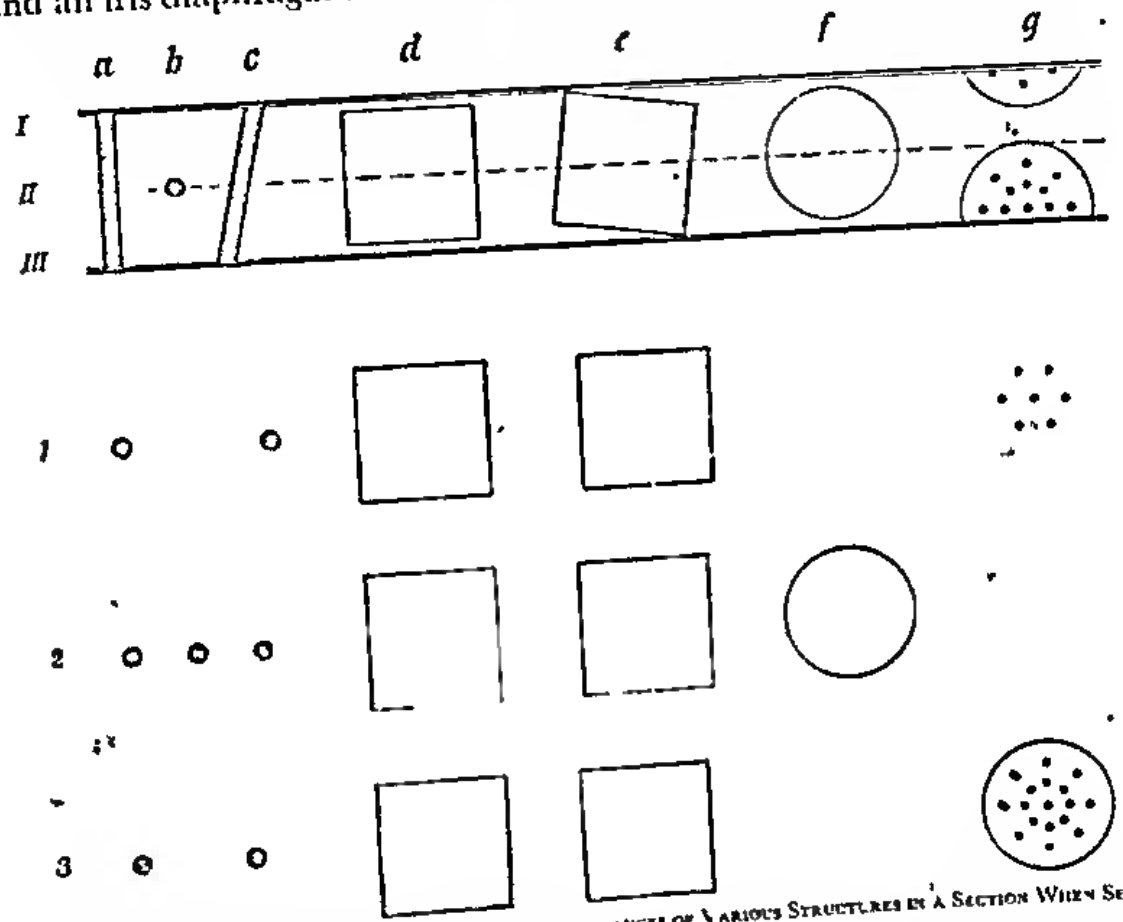


Fig. 1.—Diagram to Demonstrate the Different Appearance of Various Structures in a Section When Seen at Different Focal Planes.

The upper row represents a side view of the section between the coverglass at *I* and the glass slide at *III*. Below are three views such as would be seen through the microscope 1, (across both pages) when the focal plane is at or just below the coverglass (*I*), 2, at the focal plane *II*, 3, just above the slide (*III*)

light and focussing it on the specimen through the hole in the stage. Since the condenser is designed to transmit parallel light rays, the plane side of the mirror should be used unless disconcerting images of window bar or electric light filaments appear, when the concave side may be substituted. The iris diaphragm acts as in a photographic camera, the smaller the aperture the sharper the picture until a limit is reached on account of lack of light. This limit changes with different specimens, and the best result must be sought for each. For distinguishing color in the section, a wider aperture is often useful.

Always examine a specimen first with a low power objective and then with a high power. In focusing the microscope, have the objective drawn

away from the slide and focus down. This should be done *cautiously*, with a portion of the specimen actually beneath the lens; if there are only cover glass and balsam there, the objective will probably be driven down upon the slide. Unless one is sure that stained tissue is in the field, the slide should be moved back and forth as the objective is being lowered.

In the use of the ordinary microscope and of the usual histological or embryological slide one is in the condition of a one-eyed individual

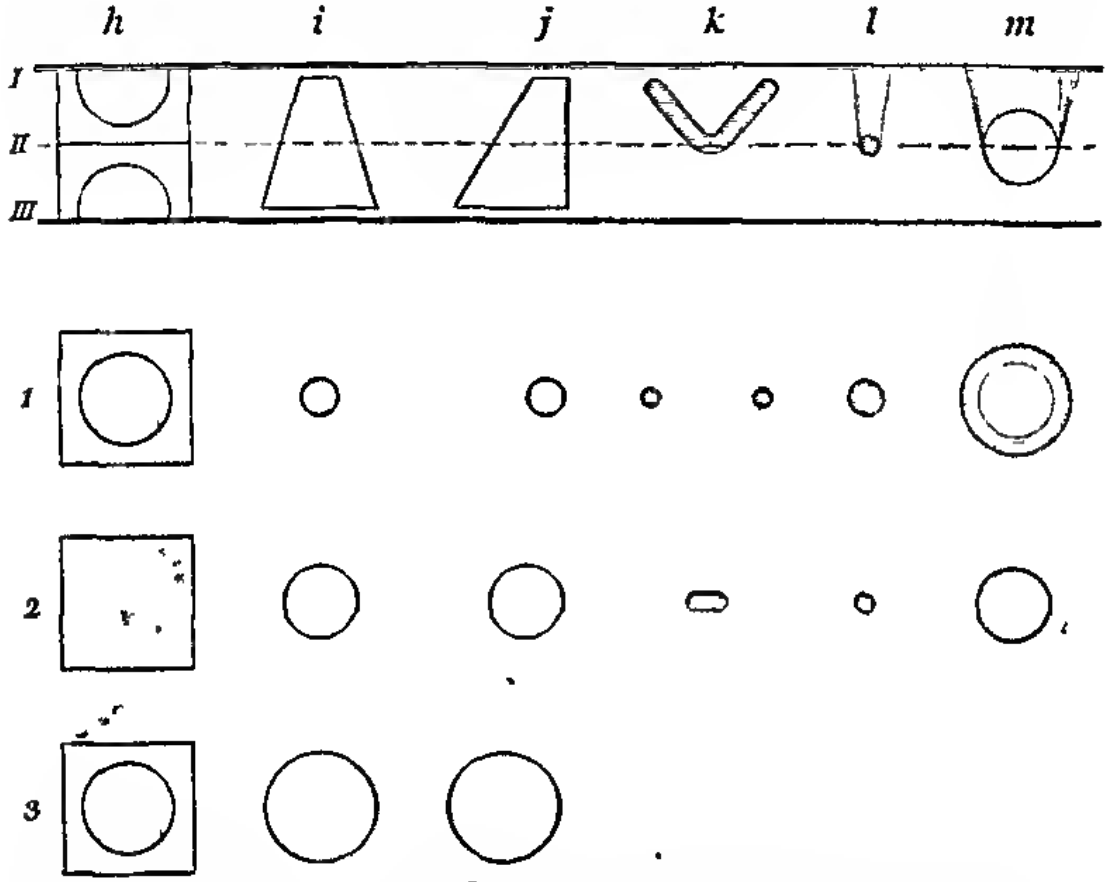


FIG. 1 (*Continued*).

a, vertical rod, b, granule; c, slanting rod, d, hollow cube; e, tilted hollow cube, f, hollow sphere, g, two spheres containing granules, only partly contained in the section, h, two spheres within hollow cubes, i, truncated hollow cone; j, tilted cone; k, bent rod, l, refractive granule; m, refractive sphere (e g an air bubble).

looking at a transparent stained-glass window. The perspective shown by a two-eyed vision and by the shadows on a moulded surface is lacking, and must be replaced, especially when one is using the higher objectives, by the careful manipulation of the fine adjustment. The focal plane, *i.e.* the level at which the clearest image is given, is very narrow with the highest powers, so that even in the thinnest sections all parts, from top to bottom, are not in focus at the same time. The constant use of the fine adjustment, changing the focal plane from one level to another in the specimen, and thus allowing a perfect view of all parts of it in the field, distinguishes an experienced microscopist from a beginner. This is very important, because the eye, which always attempts to bring objects in

focus, will strive in vain to do what the hand can do readily. Eye strain can be avoided by the continual use of the fine adjustment.

Monocular vision is fortunately greatly aided by a narrow focal plane. If one of two objects in a section is in clear focus and the other near it looks hazy, but becomes clear as the focus is lowered, the relative position of the two is readily understood. A rod-shaped structure running vertically through a specimen, from top to bottom, will be in focus at all levels of the focal plane, though appearing always in cross section as a round dot (Fig. 1, a). A spherical granule, on the other hand, though resembling the rod at a certain level, will disappear at higher or lower levels (b). A slanting rod will be seen at different positions in relation to other objects in the slide and will seem to move as the focal plane is changed (c appears as a round dot which moves toward a as the objective is lowered). A horizontally placed rod will of course appear as such, but only at one position of the fine adjustment. Thus to the high powers of the microscope the thinnest sections have the third dimension, depth, which can be investigated.

The use of transmitted light as in a transparency also entails certain limitations which must be understood in order to make full use of the usual microscopic specimens. Only those structures which partially block the light rays are distinctly visible, and to block the rays objects must have a certain density or thickness or both. To the naked eye a glass slide held to the light may be invisible, in that one can see through it; but the same slide turned edgewise is readily seen, because the depth of material is now greater. On the other hand an ink spot on the glass slide is visible because of its density of color.

Transferring these ideas to microscopic sections, we can understand that many structures actually present in a specimen may be invisible because we can see through them. Thus the thin top and bottom walls of a hollow cube might be invisible, while the perpendicular sides would be plainly seen (d). At a certain point in the tilting of a thin plane from the horizontal to the vertical position one passes from invisibility to visibility. Tilted planes which are vertical enough to be visible will appear to move, as the plane of focus is altered, just as a tilted rod seems to move (e). If a plane, seen as a line, does not change its position on focussing, it must be perpendicular to the surface of the specimen.

The same principle holds for a hollow sphere. The more horizontal top and bottom are transparent, while the more perpendicular parts of the sides are opaque. The sphere thus appears as a circle (f). If only the top or bottom of a sphere is present in a section (the remainder having been sliced away), its presence may be revealed only by its contents (g).

Sections are made as transparent as possible by being infiltrated with oil or balsam, which has nearly the same index of refraction as the glass

of the slide or cover-glass. Air has a different index, so that any light ray not absolutely in the line of vision will be bent in coming from the specimen to the objective of the microscope, and deliver a distorted image to the eye. Since the rays from the Abbé condenser are nearly parallel, this distortion is slight and negligible for the low power and even for the high-dry objective; but with the highest power even this distortion is rectified by a drop of oil between cover-glass and objective, immersing the lens in oil of the proper index of refraction. This oil should always be removed from the lens, after use, by gentle wiping with lens paper (a fresh piece each time, as the oil is apt to collect dust which might scratch the lens surface).

Opaque objects in transparent specimens will stop the light and therefore appear dark. Refractile objects, which have the property of bending the light rays, will show a dark contour, increasing in size at higher focus (l). If also opaque, such a refractive object will simply appear to grow larger, but if itself transparent, though refractive (like a bubble of air in oil), it may change from dark to light at varying focal planes (m). Partly refractive structures, when stained, may throw their color above them, though themselves out of focus and invisible at a given level. Too deep a stain often spoils a specimen for the observation of delicate details.

In Figs. 1 and 1 (cont.) the preceding paragraphs have been summarized in a diagram. The student should continually remember the third dimension, depth, in studying any microscopic section.

In 'dark-field illumination' a powerful light is thrown on the specimen obliquely, while light from below is shut off. A bright slanting beam of sunlight reveals dust particles in the air, which are otherwise invisible. By the same principle the dark-field microscope shows granules and other structures of ultra-microscopic size. The points of reflected light are seen rather than the actual granules. The method reveals their presence and any motion they may have, without indicating much as to their actual size or character. It is of use chiefly for the study of living tissues.

METHODS FOR STUDYING CELLS AND TISSUES

When cells and tissues are removed from the body they are either colorless or only faintly tinged. Living cells and bits of tissues may be studied under the microscope mounted in their own fluids, in blood plasma, in amniotic fluid or in physiologically balanced salt solutions. It is, however, not always possible to make out details in the way of structure in these preparations because the refractive indices of the different constituents are so nearly the same. The addition of dilute solutions of different dyes such as methylene blue, crystal violet, Bismarck brown and methyl green to the mounting medium stains certain of the

cellular constituents, at the same time allowing the cells and tissues to be studied in a comparatively fresh condition. Other dyes of relatively low

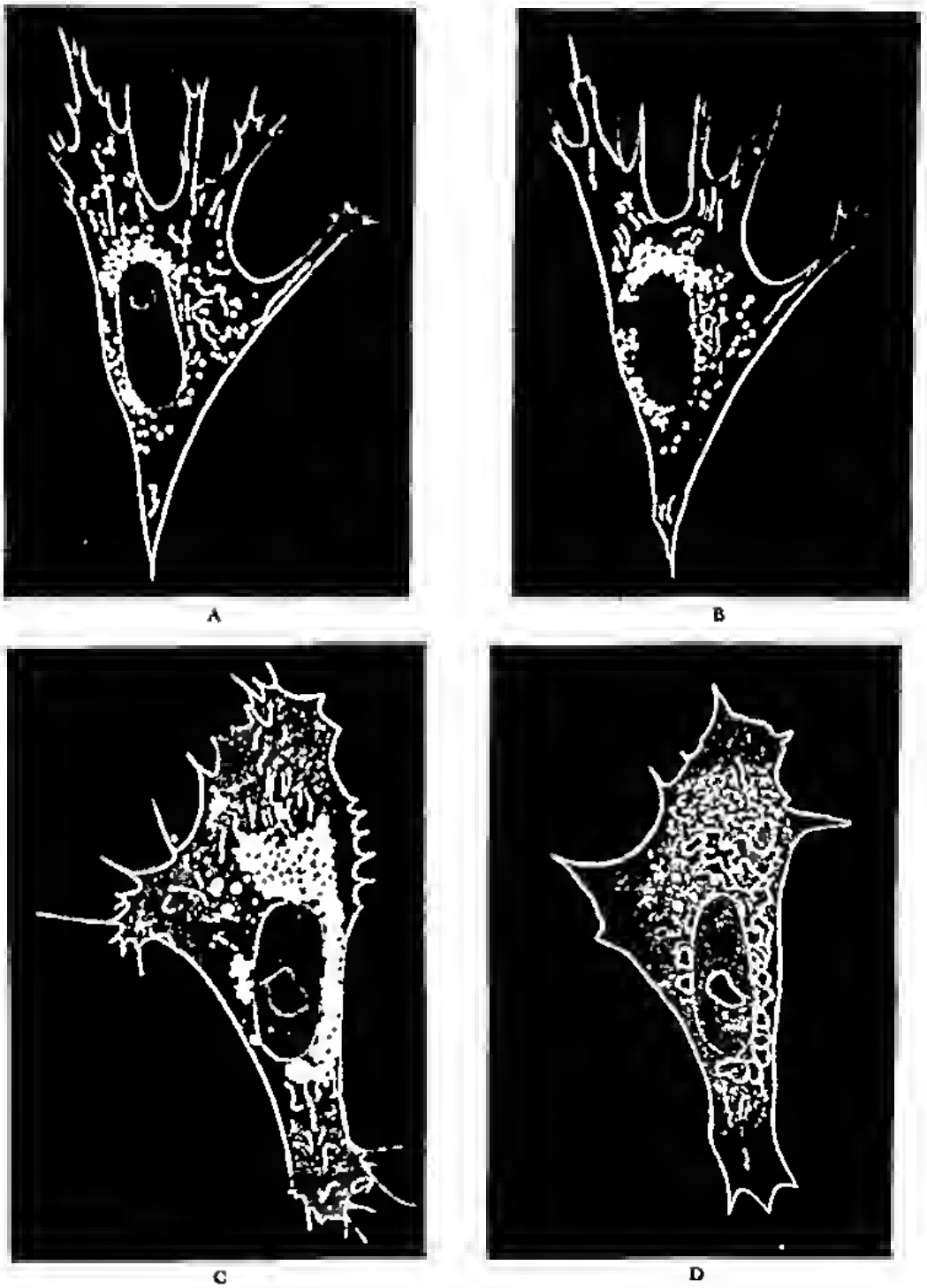


FIG. 2.—CONNECTIVE TISSUE CELLS BEFORE AND AFTER FIXATION.

A, normal living cell; B, after fixation by 2% osmic acid—shows little alteration of form and internal structure; C, normal living cell; D, after fixation by corrosive sublimate. Coalescence of fat globules and loss of the finer processes at the surface. (Strangeways and Canti.)

toxicity—as neutral red and Janus green B. in very dilute solutions applied directly or injected intravenously, have been widely used for

demonstrating more or less selectively certain granules and other formations in the cytoplasm. All these preparations are only temporary.

The development of special methods for studying living cells and tissues has opened up whole new fields of investigation in experimental cytology and histology. Knowledge of the physical structure and the chemistry of living matter has been advanced by micromanipulative methods which allow cells to be stimulated, injected and dissected under the microscope. By tissue culture or explantation living cells and tissues and even small embryos may be grown in suitable media outside the body and their growth and differentiation studied. Also living tissue may be transplanted elsewhere in the body and in successful 'takes' continue to grow: transplantations into the anterior chamber of the eye are particularly useful in studying the growth of blood vessels. Vascular growth and tissue reactions may be observed over long periods, even months, through transparent windows inserted into the rabbit's ear.

Permanent preparations are obtained after fixation, dehydration and embedding in a suitable medium as celloidin or paraffin and sectioning on a microtome. Fixation arrests post-mortem changes in the cell and renders insoluble many of its constituents. Generally the ideal in choosing a method of fixation is one to maintain as life-like appearance as possible, although this may be sacrificed if the observer wants to bring out prominently some particular structure. Dehydration performed after washing out of the fixation fluid, is usually accomplished by successive treatments with alcohols of increasing strengths. Embedding is the infiltration of a tissue with a suitable medium which when solidified holds it together and allows it to be sectioned. In preparation for embedding in paraffin, the alcohol is replaced by some liquid with which it is miscible and the paraffin soluble—chloroform, benzol, oil of cedarwood, etc.

Sections may be stained, or in some instances the entire block of tissue is stained before cutting. In the process of staining, solutions of dyes cause the constituents of the tissues to display different intensities of color since some have an affinity for one dye and not for another. These permanent preparations while valuable for the study of cellular relations and structural details do not always permit a full appreciation of cellular function and when studied exclusively, a student not infrequently carries away ideas of cells and tissues as pictures in terms of fixation and staining.

For those interested in microscopic technique and the methods employed in making the preparations described in this book, manuals are to be found in the laboratory or library. Standard manuals in English are those of Bensley, Guyer, Lee, Mallory, and McClung. The files of Stain Technology contain much information on the history and development of stains and staining, besides recent advances in these subjects.

Cytology

THE CELL

A fundamental concept in biology is that all plants and animals are composed of small structural elements called cells (Latin, *cellula;* Greek, κύτος, cytos). The lowest forms of animals and of plants are alike in being single cells throughout life. The more complex organisms are groups of cells, which have been derived by process of repeated division from a single cell, the fertilized ovum. Thus the human body, which begins as one cell, becomes in the adult an aggregation of cells variously modified and adapted to perform special functions. Since the various tissues making up the organs of the body are masses of essentially similar cells, the problems of their functional activity are the problems of the functions of a single one of their cells. Changes occurring within or in the environment of any cell or groups of cells may lead to malfunction and disease: it is evident, therefore, that cytology, the science of cells, is a basis for both physiology and pathology.

The idea of the cellular organization of plants and animals was developed gradually from the time of the discovery and naming of the cell by Robert Hooke[1] in 1665, but it remained for the botanist Schleiden[2] in 1838 and the anatomist Schwann[3] in the following year to enunciate a definite *cell theory.* Perhaps the clearest expression of the theory is in Schwann's own words. He wrote: "The elementary parts of all tissues are formed of cells in an analogous, though very diversified manner, so that it may be asserted, *that there is one universal principle of development for the elementary parts of organisms, however different, and that this principle is the formation of cells.*"[4] Notwithstanding its faults and inadequacies, the cell theory gave an astonishing stimulus to histological investigation. Leaders among plant and animal histologists quickly appreciated the significance of the theory and from the results of the long series of investigations that followed a foundation was laid for modern cytology and histology.

A cell is a unit mass of living matter. For descriptive purposes, it is the most useful unit, although as such it does not preclude the existence of smaller living units. Certainly, the bacteria are living, while at the

[1] Hooke, 1665 [2] Schleiden, 1838. [3] Schwann, 1839.
[4] Schwann, 1847.

same time they fail to satisfy the usual definitions for cells. A cell may be defined as a structural element of limited dimensions, which under certain conditions can react to external stimuli and perform the functions of assimilation, growth, and reproduction. It is described as a mass of protoplasm containing a nucleus. A third element, the centrosome, is found in the cells of animals, but it is doubtful whether it exists in the cells of the higher plants. It becomes prominent when a cell is about to divide. Some authorities regard the centrosome as a temporary structure, which forms shortly before division begins and disappears after it is completed. Others consider it as a permanent and essential part of a cell, which accordingly consists of cytoplasm, nucleus, and centrosome.

Protoplasm. A cell is an organized mass of living material, consisting of *karyoplasm* or nuclear substance and the *cytoplasm* surrounding it. The term *protoplasm* is used to denote all living material irrespective of its particular differentiation into karyoplasm and cytoplasm. "Protoplasm is an extraordinary complex heterogeneous system of numerous phases and components" (Bayliss).

Before attempting to describe protoplasm, one must distinguish between the living material and the dead and fixed structure. The usual material for histological and pathological study comprises sections of tissues or organs fixed, *i.e.*, killed and preserved, and stained. In a few instances in man and readily in animals cells can be removed while still living for study by spreading in thin sheets or smears, and may even be stained. Tissue culture, the growth of living cells outside the body under conditions in which they may be readily studied with the microscope, has now added a valuable means of comparing the living and the dead, and though for the student the method is still too unwieldy for ordinary use, the appearances seen in fixed and stained tissues may often be rendered more intelligible by descriptions of the living cells, and both the living and the dead cells should be studied whenever possible.

In living cells, 'the cytoplasm is a colorless, translucent substance in which there may or may not be embedded granules and vacuoles in varying numbers.'[1] The basis, or *hyaloplasm*, is a soft, viscid, colloidal mass, very slightly alkaline and extremely sensitive to chemical changes. It is ordinarily more than three-fourths water, and the remainder consists of salts and organic substances, some in solution and some in a colloidal state. The organic materials are classed as proteins, glycogen or some allied carbohydrates, and lipoid (fat-like) bodies.

The granules and other structures embedded in the mass may be seen to move about slowly or to vibrate rapidly, according to their kind. The basis is therefore more or less fluid. The fluidity may be increased

[1] Chambers, 1924.

by weak alkalies or ether, when the activity of the embedded particles is also increased; or decreased by acids, which cause a cessation of all motion and a coagulation of the hyaloplasm.

In most cells 'the presence of visible granules, fibrils, and vacuoles in the hyaline matrix is such a universal feature that most of our conceptions as to the visible structure of protoplasm have been based on the assumption that these inclusions form an integral part of the protoplasmic structure. However, . . . we must regard them rather as specialized differentiations.'

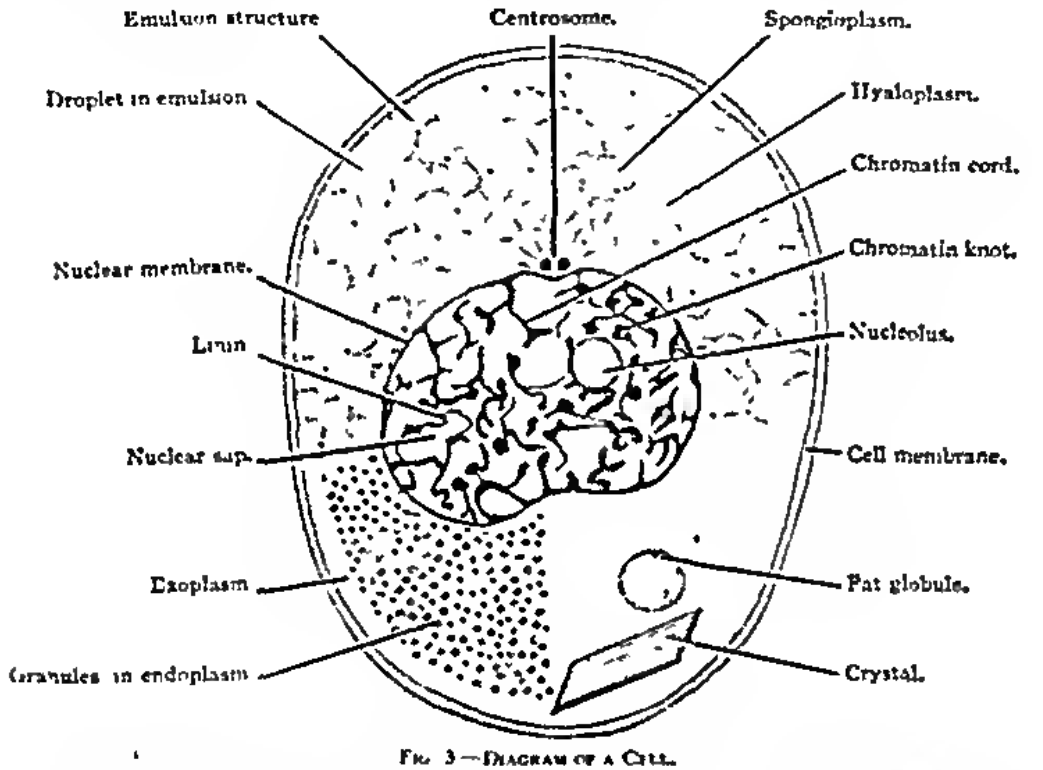


FIG. 3.—DIAGRAM OF A CELL.
In the four quadrants different types of protoplasmic structure are represented—namely, foam-like, fibrillar, granular, and homogeneous

Even the ground substance of protoplasm, in which the granules or fibrils are embedded, is not necessarily homogeneous. According to Bütschli's interpretation it has the structure of foam or of an emulsion—that is, it consists of minute droplets of one substance completely surrounded by walls of another substance. In these walls, granules and filaments may be lodged, as seen at the margins of the upper left quadrant of Fig. 3. The complex chemical activities of a cell are said to be manifestly impossible in any homogeneous mass; but in such a heterogeneous medium as an emulsion, they are conceivable. In other words, the vital qualities of protoplasm may not depend so much on hypothetical complex and unstable living molecules, as upon the interaction of various substances, made possible by their arrangement in droplets and investing films.

The protoplasm of the nucleus is different from that of the cytoplasm. it is more translucent, has a slightly different refractive index and appears optically homogeneous both in transmitted and in reflected light. When a nucleus is injured or killed, its protoplasm coagulates and forms a gel permeated with a reticular network and granules.

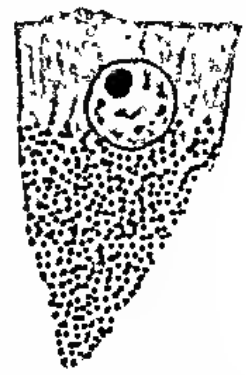


FIG 4.—GRANULES IN AN ACINOUS CELL OF THE HUMAN PANCREAS

Orth fixation, hæmatoxylin and eosin

Granules. The term granules has been given to particles of different sizes and of very diverse nature seen in living cells and in cells after fixation. They are not necessarily solid but may be semi-solid or liquid. Ultra-microscopic granules exist and can be detected by dark-field illumination. Minute granules, if abundant, give the protoplasm a dark color. Often they are absent from the peripheral layer of the protoplasm, or exoplasm, which is then clear, somewhat firmer, and chemically different from the inner endoplasm. Certain granules remain apparently unchanged throughout the life of the cell, as in some cells of the blood; others vary in size or position with different phases of cell activity. Many forms of secretory activity are accompanied by the appearance, growth, and disappearance of specific granules. It is evident, therefore, that the careful observation of protoplasmic granules is of great importance.

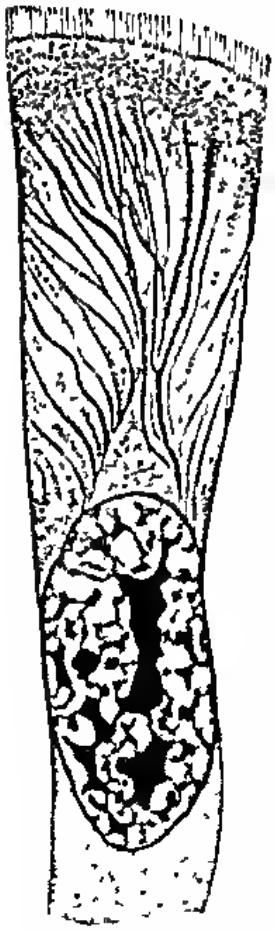


FIG. 5.—FIBRILS IN AN EPITHELIAL CELL FROM THE INTESTINE OF A FROG. (Heidenhain)

Fibrils. Protoplasm, when observed in ordinary histological preparations often appears permeated with a delicate meshwork of fibrils, which are embedded in the clear ground substance or hyaloplasm. The constituent fibrils may form an irregular network or may be parallel, passing from one end of the cell to the other. In oblique and transverse sections of such cells, the fibrils are cut across, so that they appear as short rods, or even as granules. Fibrils may be extremely slender, as in the case of those which radiate through the protoplasm at the time when the cell divides; or they may be quite coarse, like the permanent fibrils characteristic of muscle and nerve cells.

With ordinary microscopes fibrils are usually not resolved in living cells because they have nearly the same index of refraction as the more fluid ground substance. Since protoplasm can be drawn out into threads with a microdissecting needle but retracts on removing the needle, there is indicated some sort of submicroscopic structure. Studies of x-ray dif-

fraction patterns disclose that this substructure is made up of chains of long molecules. Bundles of parallel chains of long, anisotropic, polypeptide molecules on reaching sufficient size become visible microscopically and are called fibrils or fibers. "This parallelism of very long anisotropic molecular chains causes the *double refraction*, the unexpected tensile strength, the stickiness, and the possibility of being spun characteristic of the cytoplasm."[1]

Fig. 6.—Vacuoles in a Young Fat Cell

Vacuoles. Protoplasm often contains large or small drops of clear fluid, fat, or some other substance less highly organized than the surrounding material. In ordinary histological preparations the contents are usually dissolved out, and the spaces which were occupied by these droplets appear clear and empty, and are known as vacuoles. They vary greatly in size, and one, several or many of them may be found in a single cell. When acid dyes, like trypan blue or particulate matter as carbon, carmine, etc. are injected into an animal the particles accumulate in cytoplasmic vacuoles which increase in size as more particles become stored.

A specific type of vacuoles found singly or as an anastomosing canalicular network near the centrosome and characterized by staining in the living cell with the dye neutral red is supposed by some to be related to secretion and called the vacuolar apparatus, or *vacuome*.

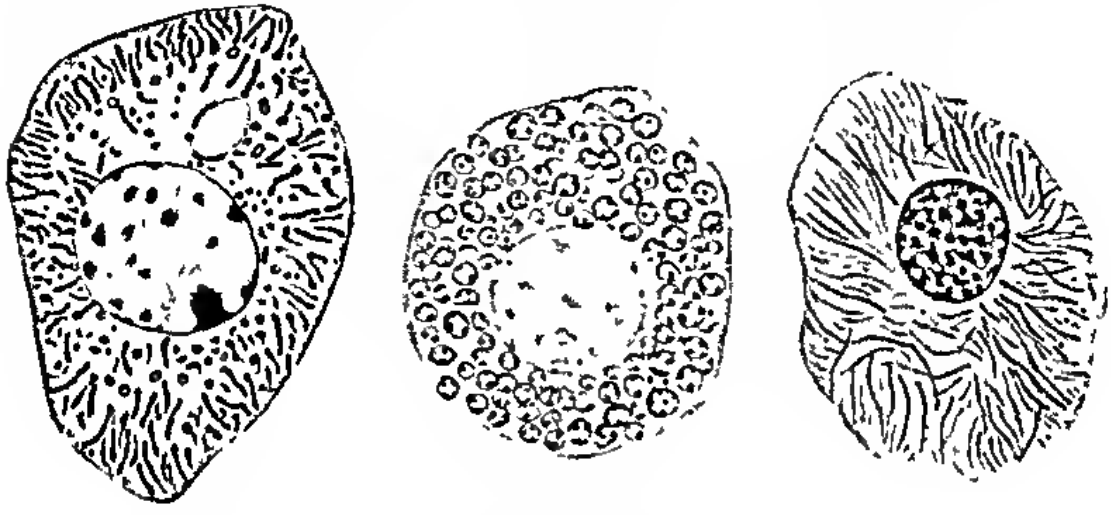
Fig. 7.—Mitochondria in a Hepatic Cell of a Dog

Regaud fixation; Mallory's phosphotungstic acid hæmatoxylin

Mitochondria. Mitochondria[2] are apparently the most constant structures found in the cytoplasm of plant and animal cells. They are small bodies varying in size and form, some appearing as smooth filaments, others as short rods and spherical granules. Frequently they are visible in living cells, but they are more strikingly demonstrated when stained supravitally with very dilute solutions of the dye Janus green B. Through a reduction of the dye they become first a greenish-blue, then red and finally colorless. Cowdry[3] attributes this action to the presence of diethylsafranin in the dye molecule which is thought to be a specific stain for mitochondria. They are very sensitive to cellular injury and to alterations of conditions within a cell—the filamentous and rod-shaped forms become segmented into granules. *Figure 8* illustrates the effect of a hypotonic and hypertonic salt solution on the mitochondria in the cells of an amphibian liver. After proper fixation they may be stained

[1] Frey-Wyssling, 1940. [2] Benda, 1897, 1898 and 1902a. [3] Cowdry, 1916 and 1918.

by iron or phosphotungstic acid hæmatoxylin, various modifications of Altmann's anilin-acid fuchsin and other dyes, making more or less permanent preparations. Beams and King[1] show by centrifuging cells that the mitochondria are heavier than the ground cytoplasm. Although they have long been considered to contain proteins and lipoids, actual detailed chemical analyses were first made on them by Bensley[2] who found that they contain in liver cells proteins and unknowns about 65%,



A B C

FIG. 8.—MITOCHONDRIA IN THE CELLS OF AXOLOTL LIVER

A, normal, B, swelling in hypotonic salt solution, C, shrinkage in hypertonic salt solution. Formaldehyde fixation, iron-hæmatoxylin (Aschkow).

glycerides 29%, lecithin and cephalin 4%, cholesterol 2%. The function of mitochondria is still unknown but through changes in form and distribution they reflect cellular activity. It is possible that they are centers of enzyme action and of oxidation.

Internal Reticular Apparatus. The Italian histologist, Camillo Golgi[3] (1898) quite by accident observed a reticular formation in nerve cells. Since then, this internal reticular apparatus or Golgi apparatus has been seen in all kinds of cells—somatic and sex cells—after special fixation and post-impregnation with silver nitrate or other silver salts, and with osmium tetroxide (osmic acid). In somatic cells, it most frequently forms a network in the cytoplasm near the nucleus, but it may appear as more condensed masses, threads and plates. A Golgi apparatus is not seen in living cells, nor is its presence revealed by microdissection, and no trace of it can be detected in the ash after microincineration. Beams and King[4] centrifuged (400,000 g.) bits of Guinea-pig uterus and found the Golgi apparatus displaced in the glandular cells—a finding considered as suggestive of its being a definite cell organoid. Various interpretations

[1] BEAMS AND KING, 1934a. [2] BENSLEY, 1937. [3] GOLGI, 1898.
[4] BEAMS AND KING, 1934b.

have been given for the nature of the Golgi apparatus—(1) a morphological reticulum; (2) a specific substance called the Golgi substance; (3) a non-specific substance, diffuse lipoids and proteins adsorbed at various interfaces as on the surface of granules, droplets and vacuoles and (4) a region in the cytoplasm containing reducing substances. Ludford[1] wrote: "The Golgi apparatus is that region in the cytoplasm or cells which brings about the reduction of osmium tetroxide at a lower temperature, or in a shorter time than is required to produce a total blackening of the cells" (cf. Owen and Bensley).[2] Parat,[3] and others see an homology between the Golgi apparatus and the vacuome or system of vacuoles within the cytoplasm but this idea is denied by many cytologists. Excellent reviews on the subject are given by Cowdry,[4] Bowen,[5] and Kirkham and Severinghaus.[6]

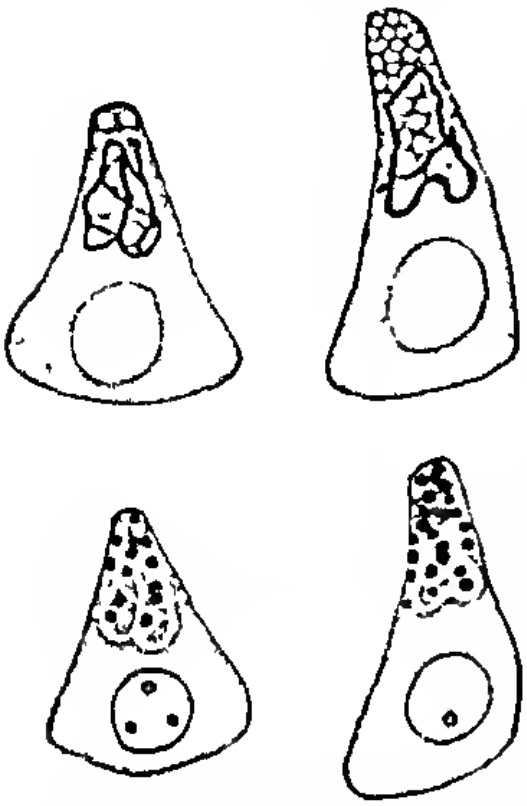


FIG.—9. TWO CELLS FROM PANCREAS OF GUINEA-PIG, STAINED FOR GOLGI APPARATUS (ABOVE), THE SAME TWO CELLS AFTER BLEACHING AND STAINING WITH IRON HAEMATOXYLIN SHOW CLEAR CANALS CORRESPONDING EXACTLY WITH THE BLACKENED NETWORKS. (Redrawn from Cowdry, General Cytology.)

Canaliculi. The cytoplasm of certain cells contains fine canaliculi or spaces which communicate with the outside. These may be simple, branched or arranged in a network and are perhaps only temporary extensions of a system of vacuoles to the surface. Such canaliculi are seen best in special preparations of the salivary glands, in the parietal cells of the stomach and in the liver.

Inclusions. Various foreign bodies as bacteria and cell debris, also food materials—proteins, carbohydrates (glycogen) and fats when seen in cells as visible particles are called inclusions. Particles of carbon, carmine, silica, colloidal dyes, colloidal metals, etc. introduced into the body intentionally or accidently and on becoming stored in cells are also classed as inclusions. Pigment granules and crystals may occur as inclusions whether they are formed in the cells themselves or brought to them.

Pigments. The pigments of the human body may be divided into two groups: (1) the *endogenous pigments* formed by the cells and (2) the *exo-*

[1] LUDFORD, 1924a. [2] OWEN AND BENSLEY, 1929. [3] PARAT, 1928.
[4] COWDRY, 1924. [5] BOWEN, 1926. [6] KIRKHAM AND SEVERINGHAUS, 1938.

genous pigments introduced into the body from without. The endogenous pigments are the hæmatogenous pigments derived from the coloring matter in the red blood corpuscles and the non-hæmatogenous pigments. The hæmatogenous pigments include the iron-containing pigments—hæmoglobin which occur as yellow to yellowish-brown granules, droplets and crystals, and hæmosiderin seen as yellowish-brown to brown granules and clumps. Among the iron-free pigments there are hæmatoidin which occurs as orange to red rhombic plates, granules and needles; the black granular malarial pigment; the bile pigments and the decomposition product of hæmoglobin called hæmatoporphyrin. This

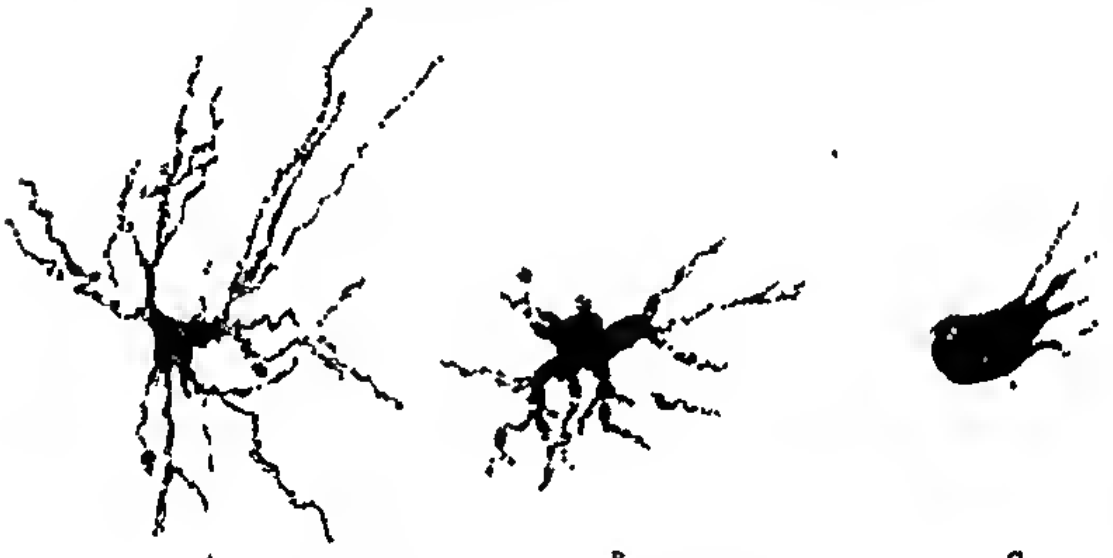


FIG 10.—PIGMENTED CELLS, PERICARDIUM OF A FROG
A, expanded, B and C, contracting. Formaldehyde fixation unstained

latter pigment is red in ultra-violet light. The non-hæmatogenous pigments include melanin which occurs normally in the epidermis, hair, pia mater and the eye, and pathologically in the liver, heart and brain. *Melanin is seen as dark-brown to black, iron and fat-free granules.* Edwards and Duntley[1] made spectrophotometric analyses of the pigments and color of living human skin, ('Spectrophotometry consists of the measurement of the light reflected at each wave-length of the visible spectrum'). They identified melanin in the deeper layers of the epidermis and an allied substance diffused throughout the epidermis which they call 'melanoid.' This hitherto unidentified melanoid is regarded as a degeneration product of melanin. Other non-hæmatogenous pigments are the special fatty pigment lipochrome or lutein and lipofuscin the 'wear and tear' pigment. This wear and tear pigment occurs as yellow to yellowish-brown granules, staining with the fat soluble dye Sudan III.

[1] EDWARDS AND DUNTLEY, 1939.

It may be seen in many different kinds of cells—glandular cells, cardiac muscle cells, ganglion cells, etc. and arises through degenerative changes in the cells.

The exogenous pigments are extremely diverse. They include carotene an orange-yellow fat soluble pigment derived from carrots, squash and certain other yellow vegetables. The yellow color of the fat in the human body is due to carotene. Edwards and Duntley identified it in the stratum corneum of the human epidermis. Other exogenous pigments as carbon, silver, dyes, etc. are deposited in the skin and in the cells of the deeper tissues.

Crystals and Crystalloidal Substances. The most characteristic of these formations are the rod-shaped crystalloids or Reinke's crystals[1] observed in the interstitial cells of the human testis. They are variable both in number and size; in some cells there is found a single long crystalloid with rounded or pointed ends, while other cells contain several shorter ones. Ordinarily they occur only in the cytoplasm, but they have been seen in the nucleus. The origin and composition of these crystalloids is unknown. Less characteristic are the long slender crystalloids with sharp-pointed ends seen singly near the nucleus of the Sertoli cells and the spermatogonia in the human testis. Six-sided prismatic crystals are found in a small percentage of nuclei in the liver and kidney cells of the dog[2] and other Canidæ.[3] Some of these crystals appear as hexagonal plates within a clear area in a nucleus, others are so long that they stretch a nucleus to a length several times the ordinary diameter. In the kidney the crystals are always elongated with the main axis more or less parallel to the lumen of the tubule. The significance of these intranuclear crystals is not known, but there are indications that they are related to uric acid metabolism.[4]

Fig. 11.—Crystalloid in an Interstitial Cell of the Testis, from a 25 Yr. Old Executed Man (Reinke)

Fig. 12.—Intranuclear Crystal in a Hepatic Cell of a Dog.

Orth fixation, Mallory's phosphotungstic acid—hæmatoxylin

With so many constituents and with a very unstable colloid matrix, protoplasm may show a great variety of forms in fixed tissues. It has been described as hyaline, granular, fibrillar, or foam-like, *i.e.* having the structure of an emulsion. These various appearances are indicated in Fig. 3. The different fixatives may coagulate the hyaloplasm in different patterns, perhaps guided by unstained inclusions; but the various forms of coagulation occur with such constancy that their study is of the utmost importance, and differ-

[1] Reinke, 1896. [2] Grandis, 1889. [3] Weatherford, 1939.
[4] Weatherford and Trimble, 1940.

ences noted in fixed cells may be taken to represent actual differences in the living.

To attempt to describe our present conception of the complex activities of cells would lead into the fields of physics and chemistry. Frequently the results of such activities can be recognized histologically by changes in form or content of the cell, and these changes should be carefully studied. These instances constitute another link between the dead material studied and its function in the living.

Nucleus. The nucleus[1] (Latin, *nucleus*, 'the kernel of a nut'; Greek, κάρυον, karyon, 'a nut') is typically a well-defined round body, situated near the center of the cell, appearing denser or more coarsely granular than the surrounding protoplasm (Fig. 3). There are characteristic variations in the shapes of nuclei, in their position within the cells and in their structure.

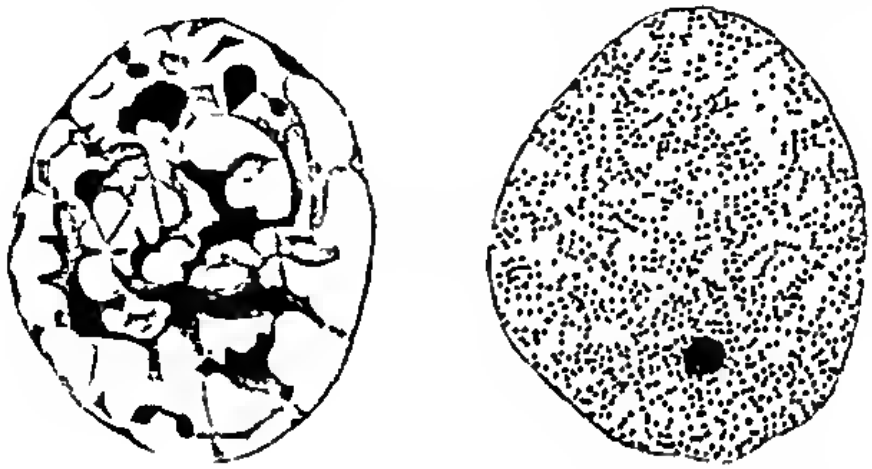
Ordinarily the karyoplasm, or nuclear substance, is sharply marked off from the cytoplasm by the *nuclear membrane*. Sometimes, in preserved tissue, the cytoplasm has shrunken away from the nuclear membrane, so as to leave a narrow space partially encircling it; and in certain living cells the nucleus migrates through cytoplasm, as if it were an independent body. But there are phases of cell-development in which the nuclear membrane disappears and no line can be drawn between karyoplasm and cytoplasm. At all times they have a common structural basis. The ground substance of the nucleus, corresponding with the hyaloplasm, is the *nuclear sap;* and it contains, for spongioplasm, a meshwork of delicate *linin* fibrils. These help to form the nuclear membrane, in which they terminate. The nuclear membrane, nuclear sap, and linin reticulum do not stain deeply, and are therefore grouped together as the achromatic constituents of the nucleus.

The principal chromatic constituent of the nucleus is known as *chromatin*. It stains deeply, since it contains a large amount of nucleic acid, which has a marked affinity for basic dyes. Chromatin occurs in the form of granules, which are bound together in strands or masses by the linin fibers (Fig. 3). The masses, known as chromatin knots, occur

[1] The nucleus is described and named by Robert Brown in a paper, *On the Organs and Mode of Fecundation in Orchidea and Asclepiadea* read before the Linnean Society on November 1 and 15, 1831 and printed in the Trans. Linnean Soc., vol. XVI, pp 685–738, 1833. "In each cell of the epidermis of a great part of this family, especially of those with membranaceous leaves a single circular areola, generally somewhat more opake than the membrane of the cell, is observable . . . This areola, or nucleus of the cell as perhaps it might be termed, is not confined to the epidermis, being also found not only in the pubescence of the surface, particularly when jointed as in Cypripedium, but in many cases in the parenchyma or internal cells of the tissue, especially when these are free from deposition of granular matter," pp. 710–711.

especially at the points of intersection in the linin meshwork. Sometimes they are attached to the nuclear membrane, or so distributed over its surface that it appears to consist of chromatin. It forms morphologically the most important part of the nucleus.

Certain nuclei contain one or more round bodies, which belong with the chromatic elements because of their deep staining, but which are chemically different from chromatin. These bodies, known as *nucleoli*,[1] are stained with acid or neutral dyes. For this reason they are said to be composed of oxychromatin, as opposed to the basichromatin of the



A B

FIG. 13.—TWO NUCLEI FROM THE INTESTINAL EPITHELIUM OF A SALAMANDER. Sublimate fixation, A, Iron hæmatoxylin, B, Vanadium hæmatoxylin. (Heidenhain.)

chromatin knots. They are also said to be composed of paranuclein, whereas chromatin is composed of nuclein. In distilled water the structures formed of nuclein disappear, but those consisting of paranuclein remain. Sometimes a nucleolus, lodged in the nuclear reticulum, is more or less covered with chromatin, but the term should not be applied to irregular knots of chromatin, even when most of the chromatic material within a nucleus is gathered into one or two such bodies. These are the so-called false nucleoli (pseudonucleoli).

Every nucleus, therefore, consists of ground substance or nuclear sap, a network of linin, and granules and masses of chromatin. Usually it is surrounded by a membrane, and sometimes it contains a nucleolus. Most cells contain a single nucleus; but occasionally a single cell contains two nuclei, as is frequent in the liver, or even several nuclei, as in certain cells associated with bone. Non-nucleated bodies, like the mam-

[1] The history of the *nucleolus* named by Valentin (*Repertorium T.* 4, *1839*) is discussed at length by T. H. Montgomery, Jr., 1898, 'Comparative cytological studies, with especial regard to the morphology of the nucleolus,' Jour. Morph., vol. 15, pp. 265–582. A nucleolus is present also in the nucleus of the plant cells, being seen by Schleiden (1838), who describes it as "a small sharply outlined body . . . or thick-walled hollow globule," without giving any name to it

malian red blood corpuscles, and the dead outer cells of the skin, have lost their nuclei in the course of development.

McClung would draw no distinction between linin and chromatin, nor between oxychromatin and basichromatin. 'It is much more reasonable to conclude that observable differences of appearance in the chromatin are due to its varying states rather than to admixtures of substances.' It is 'a semi-fluid colloid, capable of a wide range of intrinsically controlled movements.' It varies with different fixatives and different technical procedures. 'In preparations of a higher order of technical excellence it is possible to see that chromatin is directly continuous with the nuclear matrix. In turn it may be noted that there exists no break between nucleus and cytosome, but that there is direct continuity between the substances of these two cellular regions marked only by a line of greater concentration at the delicate karyotheca' (nuclear membrane).[1] In living cells in tissue cultures the nucleus is glassy, without differentiation, its border marked by a thin white line under the dark-field illumination. 'No linin thread or chromatin granules are to be seen. These are fixation, coagulation, or precipitation products, and do not represent living structures. The nucleoli, one or two in each nucleus, are irregular in outline and change form and position.'[2] Nuclear characteristics are said, however, to be brought out by ultraviolet photography, even in living cells.

Functionally the nucleus is regarded as a center for chemical activities necessary for the life of the cell. It is believed to produce substances which pass out into the cytoplasm, where they may be further elaborated. Evidences of nuclear extrusions into the cytoplasm have been frequently recorded. But the interactions between the nucleus and cytoplasm, of such nature that they cannot be observed under the microscope, are presumably of far greater biological importance.

Fig. 14.—Centrosome (Diplosome) in Cell of Testis of Grasshopper.

Centrosome. The centrosome or *centriole* is typically a minute granule in the center of a small sphere of differentiated protoplasm. Often the term is applied to this entire structure, but it refers particularly to the central granule; the enveloping sphere is known as the *attraction sphere*, and it is composed of *archoplasm*. When a cell is about to divide, delicate fibrils, either rearranged from the protoplasmic reticulum or formed anew, radiate from the archoplasm toward the periphery of the cell. The central granule becomes subdivided into two, which then move apart. In *resting cells*, or those which are not undergoing division, the centrosome may already have divided into a double body or *diplosome* preparatory to the next division of the cell (Figs. 3 and 14).

Centrosomes have been detected in many forms of resting cells, and it is assumed by some authorities that the centrosome is an invariable constituent of the cells of the higher vertebrates. According to this

[1] McClung, 1924. [2] Lewis, W. H. and Lewis, 1924.

opinion the centrosome may become inconspicuous but it never loses its identity. Often they are found very close to the nuclear membrane, which may be indented to accommodate them; and rarely, as in certain cancer cells and in one form of the worm *Ascaris*, they have been reported as within the nucleus. They may occur near the free surface of certain cells, usually in the form of diplosomes. Just above the diplosome, such cells may send out contractile projections of protoplasm (pseudopodia), with the activity of which the diplosome may be in some way associated. Pseudopodia, with an underlying diplosome, have been observed in the columnar cells of the human large intestine. It is believed

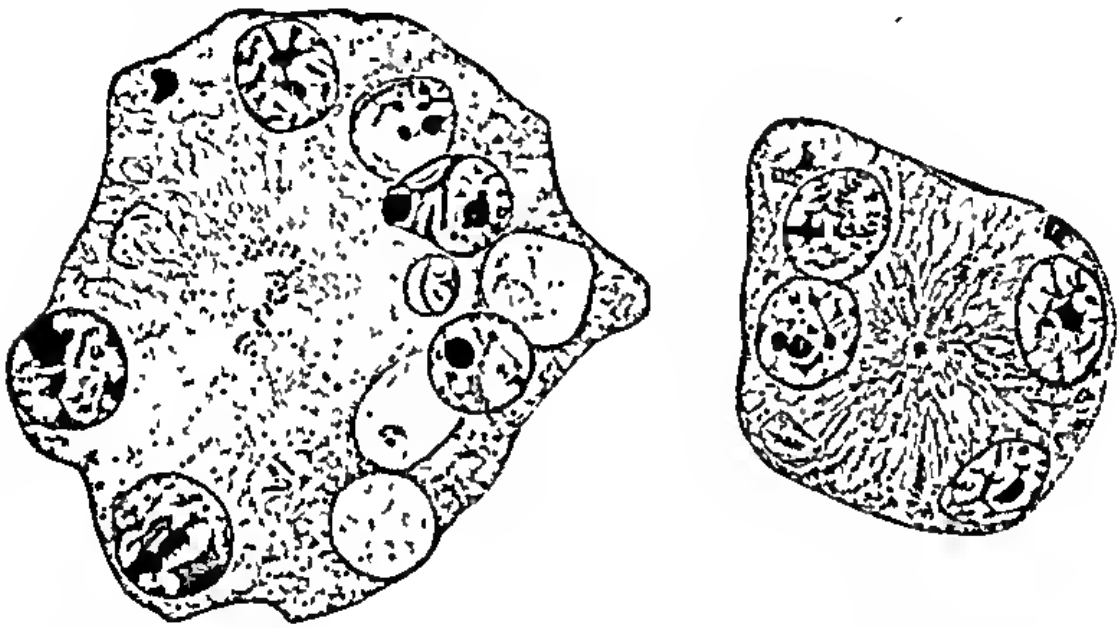


FIG. 15.—MULTINUCLEATE WANDERING CELLS FROM A LYMPH GLAND OF THE RABBIT. NOTE MULTIPLE CENTRIOLES. (Heidenhain.)

that the diplosomes may multiply by fission, and that thus they may give rise to the numerous motile hairs, or *cilia*, which project from certain cells. Of these they form the *basal bodies* (see p. 75). In many gland cells the centrosome lies in the midst of the protoplasm where the secretion accumulates. The discharge of the secretion is accomplished by the contraction of the protoplasmic strands in which the centrosome is lodged. In all these relations the centrosome appears to be a center for motor activities, and it is described as the kinetic or dynamic center of the cell.

Cell Membrane. The protoplasm at the surface of certain cells floating in the blood or lymph forms a thin pellicle, apparently as a result of protoplasmic concentration, or other reaction to the surrounding medium. Cells which line the greater part of the digestive tube, and have only one surface directed toward the intestinal contents, are provided with a thick membrane on the exposed surface. Such a membrane

is called a cuticular border, or *cuticula*. On the other side of these cells, the membrane is much thinner, and on the basal surface it is sometimes lacking. In such cases the protoplasm appears to be continuous with that of the underlying cells. In other cases the entire cell is devoid of any recognizable membrane. A definite cell membrane, therefore, is not an essential part of a cell; if present it ranges from a thin pellicle, on the border line of visibility, to a well-defined wall, which may be formed as a secretion of the underlying protoplasm. If the several surfaces of the cell are in relation to different environments, there is often a corresponding difference in the structure of their walls.

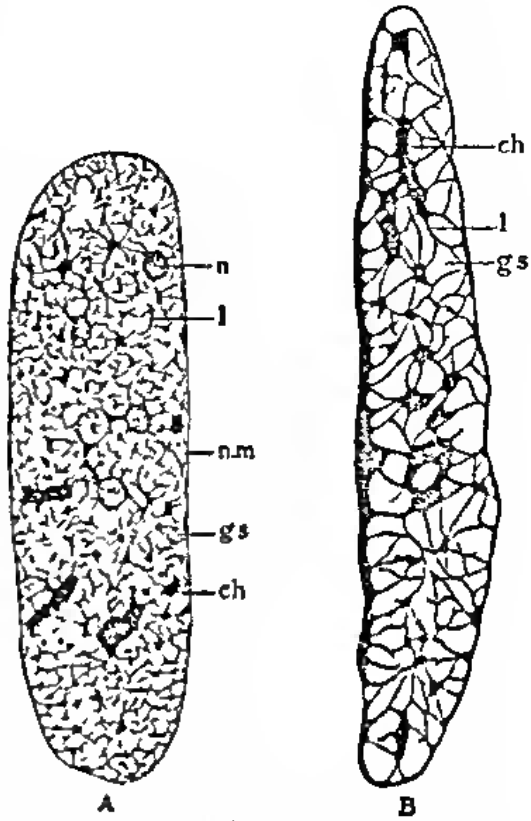


FIG. 16.—NUCLEI OF STRIATED MUSCLE FIBRE FROM YOUNG SALAMANDERS (AMBLYSTOMA) (*Eycleshymer*)
A, from a 7 mm. embryo, B, from one of 26 mm.; ch, chromatin knot, g s, ground substance, l, linin fibril, n, nucleolus, n m, nuclear membrane

In examining a group of cells, it will be important to determine whether they are merely in contact, or actually continuous. Sometimes cells are so completely fused that their nuclei are irregularly distributed through a single mass of protoplasm. Such a formation is a *syncytium* in which the position of the nuclei is the only means of estimating the territory of a single cell. A syncytium may arise from the fusion of cells, or, as in striated muscle fibers, it may be due to the multiplication of nuclei in an undivided mass of protoplasm. Instead of being completely fused, cells are often joined to one another by protoplasmic processes of varying length and width, thus forming cellular networks. Fibrils within such a syncytium may pass continuously from the protoplasm of one cell into that of another.

Although cell membranes are often inconspicuous in animal cells, they cannot be overlooked in plants. Thus cork is a mass of dead cells from which nuclei and protoplasm have disappeared, leaving only the cell walls. In describing cork, Robert Hooke introduced the name 'cell,' in 1665. He wrote: "I took a good clear piece of Cork and with a Pen-knife sharpen'd as keen as a Razor, I cut a piece of it off, and thereby left the surface of it exceeding smooth, then examining it very diligently with a Microscope, me thought I could perceive it to appear a little porous . . . These pores, or cells, were not very deep, but consisted of a great many little Boxes——." In this way one of the briefest and most important of scientific terms was introduced.

FORM AND SIZE OF CELLS

The Shapes of Cells.* Surface tension accounts for the tendency of isolated cells to become spherical—the ovum *par excellence*, fat cells and escaping oil droplets, also vacuoles and soap-bubbles. The shape of such bodies when closely associated in masses has only recently been critically examined.

If spheres of the same size, resting on a flat surface, are arranged in a compact layer, each sphere becomes bounded by six others. A superim-

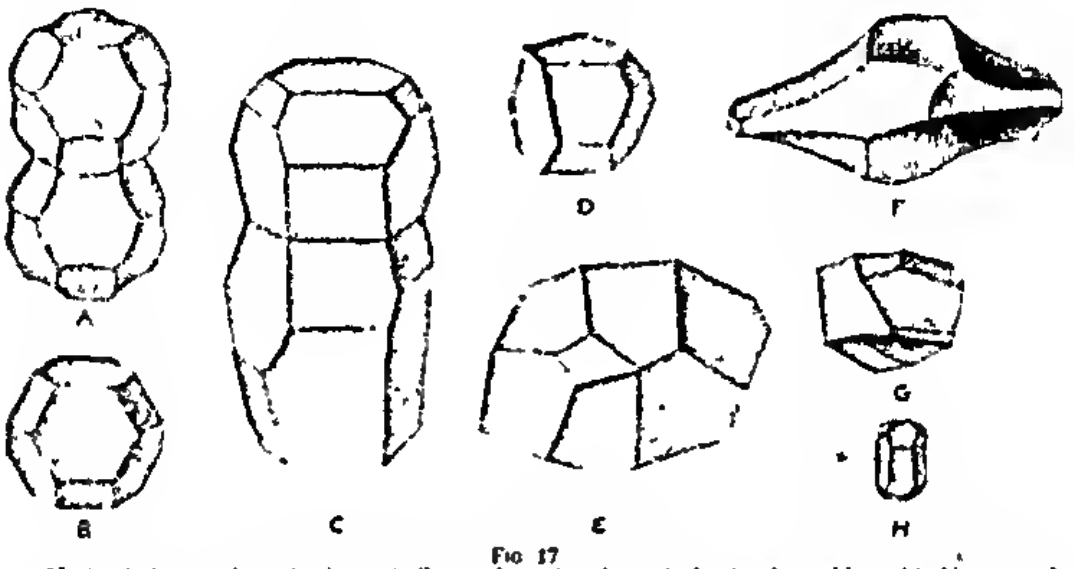


FIG. 17

A, two of Kelvin's "minimal tetrakaidecahedra" (one above the other) which solve the problem of dividing space into uniform bodies of minimal surface (Phil. Mag., 1887, 5th ser., 24 503–514)—here roughly drawn, since "no shading could show satisfactorily the *delicate* curvature of the hexagonal faces." *B*, Kelvin's "orthic 14-hedron," with slightly greater surface area than the minimal; all facets are plane and regular. C–G, wax plate reconstructions of actual cells (H, schematic). C, two successive cells from pith of *Eupatorium*. Above, a 13-hedron (orthoid 14-hedron in the aspect figured but lacking a facet on the hidden surface) below, an irregular 14-hedron. D, an 11-hedral cell of elder pith, as produced by vertical insertion of an orthoid 14-hedron. E, fat-cell from the human panniculus adiposus—15-hedral with many pentagonal facets and an atypical tetrahedral anale. H, G, and F, basal, middle, and outer cells, in average volume as 1 6 18, from the many-layered stratified buccal epithelium of a human embryo of 5 months. Except at the basal and free surfaces, the enlarging cells maintain an average of 14 facets. Basal cells may have a single facet toward underlying tissue, 6 lateral contacts with other basal cells, and an average of 4 with overlying cells in *case the latter have not enlarged*.—11 facets altogether. Approximate magnifications: C, × 170; D, × 150; E, × 300; F, G, H, × 750 diam. (F. T. Lewis.)

posed layer, settling into the depressions of the first layer, provides three additional contacts per sphere, and a similar layer beneath adds three more—12 contacts altogether. Compression of plastic spheres so arranged was assumed to yield dodecahedra, long accepted as the basic shape of cells in masses.

In 1873, after an experimental study of the junctions of soap films (as in foam), Plateau announced two laws which such films obey: (1) Only 3 films meet along an edge, and at angles of 120°, or as near thereto as conditions permit; and (2) Only 4 bubbles meet at a corner. Dodecahedra in aggregates violate the latter law since at each of six corners per dodecahedron, *six of them meet at a mathematical point.*

* The section on the Shapes of Cells was contributed by Dr. F. T. Lewis.

In 1887 Lord Kelvin solved the problem of dividing space, without interstices, into uniform, similarly oriented bodies of minimal surface, in accordance with Plateau's laws. These hypothetical polyhedra have 14 facets, or contacts with neighboring bodies, 8 of which are hexagonal with a specific undulating surface, and 6 are plane quadrilaterals with curved edges (Fig. 17, A). When stacked, three polyhedra meet along every edge at angles of precisely 120°. All corners are the required trihedral angles, where the polyhedron is in contact with only three neighbors. This shape Lord Kelvin named the *minimal* tetrakaidecahedron (or 14-hedron). A close approximation to that ideal pattern, having all surfaces plane, 8 being regular hexagons and 6 true squares, he called the *orthic* 14-hedron (Fig. 17, B). A rough approximation, in which the 8 hexagons and 6 quadrilaterals need be neither regular nor plane, has since been named the orthoid 14-hedron, and that is presumably as close an approach to the geometrical pattern as any cell can produce.

Sir D'Arcy Thompson, in his admirable book "Growth and Form" (1917) remarked that in "ordinary microscopic *section* it would seem practically impossible to distinguish the 14-sided figure from the 12-sided," but he was misled to suppose that "the dodecahedral configuration . . . is generally assumed." Aroused by that statement, reconstructions from serial sections were made, of some 250 cells, in elder pith, adipose tissue, stratified epithelium, and amphibian precartilage. The average number of facets of these cells was 13.97. Then it was demonstrated that compressed lead shot of a given size, unarranged in a cylindrical container and compressed until all interstices had been obliterated, also had an average of close to 14 facets (14.17).[1]

It has been found that a random arrangement, which merely avoids the niceties of adjustment required when 4 cells meet along a line, or when 5 or 6 meet at a mathematical point at certain corners, is sufficient to eliminate the 12-hedral pattern and to explain the prevalence of the 14-hedral average. Surface tension should mold such irregular 14-hedral products of chance into shapes with uniform length of edge and angles of 120°, thus approaching Lord Kelvin's theoretical pattern.

The observed cell shapes with an average of close to 14 facets are almost indescribably diverse. They may have from 6 to 23 facets apiece, and their facets range from triangles to nonagons. The orthoid form (6 quadrilaterals, 8 hexagons) is a rarity, found in less than 1 per cent. of the cells. Pentagonal facets, always common, are sometimes more frequent than any other shape, which is of interest since no uniform polyhedra with one or more pentagonal facets can fill space without

[1] Marvin, 1939*a*.

interstices. A curious geometrical necessity governs these patterns. *With trihedral angles only*, the sum of the sides of the polygons covering a cell (or any other body) must be 12 less than if they were all hexagons. This is true whether the facets be as few as three, or occur in countless numbers, and it has many histological applications.[1] With every tetrahedral angle introduced, two sides more are lost; four sides for a pentahedral angle, etc. The occasional occurrence of cells with one or more tetrahedral angles explains, in part at least, why the observed facets per cell average a decimal short of the full fourteen.[2]

In a mass of cells having an average of 14 facets, cell division occurs without affecting that average, provided that the division plane is on the average hexagonal (*i.e.*, one that encounters and subdivides six facets of the dividing cell). Such an average for the division plane is expected, but has not been fully demonstrated. Small cells with few facets thus become intermingled with large cells of many facets. If, however, division should be prevalent in 3- or 4-sided planes, cutting off corners of cells instead of bisecting them, the average for the mass could be reduced to 13 or less.

Inequality in cell size, with corresponding differences in the number of facets, may arise also from unequal growth. In a mass of 14-hedra, some may enlarge, wedging their way between smaller cells and gaining facets at their expense, without affecting the general average. This "gliding growth," prevalent in certain animal tissues, is so restricted in plants that it has been denied altogether.

The Kelvin patterns require not only that the cells be uniform in size and shape, but that they be strictly oriented. Even soap-bubbles, of as nearly the same size as they can be made, when assembled unoriented in foam, present diverse cell-like shapes and not the ideal figure.[3] The requisite orientation of cells can be provided by a prevailing plane of cell division, whereupon the orthoid pattern is often so closely approached by individual cells that one cell division more or less, or the change in level of a single septum, would produce it (Fig. 17, C). Since no other shape could exist uniformly throughout the mass, and the observed forms are accountable as its derivatives, the 14-hedron is properly regarded as the typical shape of undifferentiated cells in masses, and the minimal 14-hedron as its ideal surface tension refinement.

[1] Lewis, F. T., 1933*a* and 1943.

[2] Marvin (1939*b*), using an isolation method, obtained for 100 cells of vegetable pith, an average as low as 13.36; Hulbary (1940), for 100 cells has reported the highest average, 14.29. From all studies the average appears to be slightly below 14, perhaps 13 8

[3] Matzke, 1940.

Whether smooth muscle fibers are elongated 14-hedra—whether mesenchymal nuclei preside over 14-hedral areas of vacuolated cytoplasm reduced to irregular strands and shreds—are among the many problems of cell shape inviting further study, yet difficult of approach.

The size of cells ranges from that of yolks of birds' eggs—which are single cells, at least shortly before being laid—down to microscopic structures four thousandths of a millimeter in diameter. The thousandth of a millimeter is the unit employed in microscopic measurements.[1] It is called a *micron*[2] (pl. micra or microns) and its symbol is the Greek letter μ. The small cells referred to are therefore four microns (4 μ) in diameter. The size of any structure in a section of human tissue may be roughly estimated by comparing its dimensions with the diameter of a red blood corpuscle found in the same section. These red corpuscles are quite uniformly 7.5 μ in diameter.

CYTOMORPHOSIS

Cytomorphosis is a comprehensive term for the structural modifications which cells, or successive generations of cells, undergo from their origin to their final dissolution.[3] In the course of their transformation, cells divide repeatedly, but the new cells begin development where the parent cells left off. Cell division, therefore, is an unimportant incident in cytomorphosis.

Cytomorphosis is a continuous advance in which four successive stages are recognized—first, the stage in which the cells are undifferentiated; second, the stage of specialization or differentiation; third, the stage of degeneration; and fourth, the stage in which the cells die and are removed. These may be considered in turn.

Undifferentiated cells, as can be seen in sections of young embryos, are characterized by large nuclei and little cytoplasm. They multiply rapidly, but the rate of division declines with the gradual increase of the protoplasm and the consequent functional differentiation of the cell. In the adult, relatively undifferentiated cells are found in many situations, as, for example, in the deepest layer of the epidermis. As the cells at the surface die and are cast off, new ones come up from below to take their

[1] HARTING, P., 'Das Mikroskop. Theorie, Gebrauch, Geschichte und gegenwartiger Zustand desselben,' Braunschweig, 1859 (proposed one thousandth of a millimeter, 'Mikromillimeter,' or 'mmm.,' p. 506).

[2] LISTING, J. B., 1869 (suggested p. 5, 'Mikron' or 'Mikrum' and the symbol μ. English trans., micron). Carl's Repertorium für Experimental-Physik, etc., Bd.V.

[3] The term *cytomorphosis* was introduced by C. S. Minot in 1901 in a lecture entitled 'The Embryological Basis of Pathology' (Science, 1901, vol. 13, p. 494). Cytomorphosis is further discussed by Professor Minot in 'The Problem of Age, Growth, and Death,' published by G. P. Putnam's Sons, 1908.

places. But since the basal cells can produce only epidermal cells, they are themselves partly differentiated. From this point of view the fertilized ovum, which can produce all kinds of cells, must be regarded, in spite of its size and great mass of yolk-laden protoplasm, as the least differentiated cell.

Differentiated cells may preserve a round or cuboidal form, but usually they are elongated, flattened, or stellate. The cytoplasm usually contains coarse granules, fibrils, masses of secretion or other special formations. As a result of their own protoplasmic activity, the cells of many tissues become surrounded by some sort of an *intercellular substance* or *matrix*, which may far exceed in bulk the cells which produced it. Intercellular substances may be solid or fluid. When present in small amount they form thin layers of *cement substance* between closely adjacent cells; in large amount these substances constitute a ground work in which the cells are embedded as, for example, in cartilage and bone.

Although the differentiation of cells is chiefly cytoplasmic, corresponding changes in the nucleus also occur. Thus while the muscle cells of the salamander are elaborating complex fibrils, the nuclei become modified as shown in Fig. 16. Undifferentiated nuclei have little chromatin and one or more nucleoli. Sometimes the fully differentiated nucleus is characteristic of the type of adult cell, as in the nerve cell or the plasma cell, but since the nucleus often changes with the functional activity of the cell and may also appear differently after different fixatives, it is not usually a safe diagnostic guide.

Degeneration is the manifestation of the approaching death of the cell. In nerve cells this process normally takes place very slowly. These cells remain active throughout life, and if destroyed, they can never be replaced. In many glands, in the blood and in the skin, however, the cells are constantly dying and new ones are being differentiated. In a few organs the cells perish, but no new ones form, so that the organ to which they belong atrophies. Thus a large part of the mesonephros (Wolffian body) disappears during embryonic life; and the ovary in later years loses its chief function through the degeneration of its cells.

The optical effects of degeneration cannot at present be properly classified. In a characteristic form, known as 'cloudy swelling,' the cell enlarges, becoming pale and opaque. In another form the cell shrinks and stains deeply, becoming either irregularly granular or homogeneous and hyaline. The nucleus may disappear as if in solution (karyolysis, chromatolysis); or it may become densely shrunken or *pycnotic*, and finally break into fragments and be scattered through the protoplasm (karyorrhexis). If the process of degeneration is slow, the cell may divide. It may be able to receive nutriment which it cannot assimilate, and thus its

protoplasm may be infiltrated with fat and appear vacuolated. It may form abnormal intercellular substances, for example, amyloid; or the existing intercellular substances may become changed to mucoid masses, or have lime salts deposited in them. Thus an impairment or perversion of function is often associated with optical changes in the cell substance. In tissue cultures W. H. Lewis[1] recognizes a granulation of the cell ('death granules') as the first evidence of approaching death.

The removal of dead cells is accomplished in several ways. Those near the external or internal surfaces of the body are usually shed or desquamated, and such cells may be found in the saliva and urine. Those which are within the body may be dissolved by chemical action or devoured by phagocytes.

Every specimen of human tissue exhibits some phase of cytomorphosis. In some sections a series of cells may be observed leading from those but slightly differentiated to those which are dead and in process of removal. Because of the similarity and possible identity of this normal 'physiological' regression with that found in diseased tissues, such specimens should be studied with particular care.

The first differentiation of a cell is not necessarily permanent, for at some stage of its differentiation it may exceptionally change its type. Cells already differentiated as connective tissue cells are, under certain circumstances, apparently converted into cartilage or bone cells. On the other hand, in tissue cultures the new cells are sometimes of a more embryonic type than their parents, and in the growth of glands it is probable that the active, highly specialized secreting cells of one period become the simple duct cells of the next. This change to a simpler form is called dedifferentiation. Probably the type of cytomorphosis and the possibility of dedifferentiation depend largely on the surroundings of a cell.

VITAL PHENOMENA

The vital properties of cells are fully treated in text-books of physiology. They include the phenomena of irritability, metabolism, contractility, conductivity, and reproduction. Under irritability may be grouped the response of cells to stimuli of various sorts, such as heat, light, electricity, chemical reagents, the nervous impulse, or mechanical interference. Metabolism, in a wide sense, includes the ingestion and assimilation of food, the elaboration and secretion of desirable products, together with the elimination of waste products. Contractility may be manifest in the locomotion of the entire cell, in the vibratile action of slender hair-like processes, the *cilia*, or in contraction of the cell body. Conductivity is the power of conveying impulses from one part of the cell to another. Reproduction is seen in the process of cell division. Many phases of these activities are observed in microscopic sections and as

[1] Lewis, W. H., 1923.

such they will be referred to in later chapters. A few which are of general occurrence will be described presently.

Amœboid Motion. The unicellular animal, Amœba, exhibits a type of motility known as *amœboid*, which has been observed in many sorts of cells in the vertebrate body. In marked cases, as in certain white blood corpuscles (the leucocytes), the cell protoplasm sends out fine or coarse processes which divide or fuse with one another, causing the cell to assume a great variety of forms. The processes may be retracted, or they may become attached somewhere and draw the remainder of the cell body after them, the result of which is locomotion or the so-called *wandering* of the cell. Such wandering cells play an important part in the economy of the animal body. Their processes can flow around granules or cells and thus enclose them in protoplasm. Some of these ingested bodies may be assimilated by the cell as a result of complex chemical and osmotic reactions. Cells which feed on foreign particles and can alter or digest them are known as *phagocytes*. Amœboid movements take place very slowly. In preparations from warm-blooded animals they may be accelerated by gently heating the object.

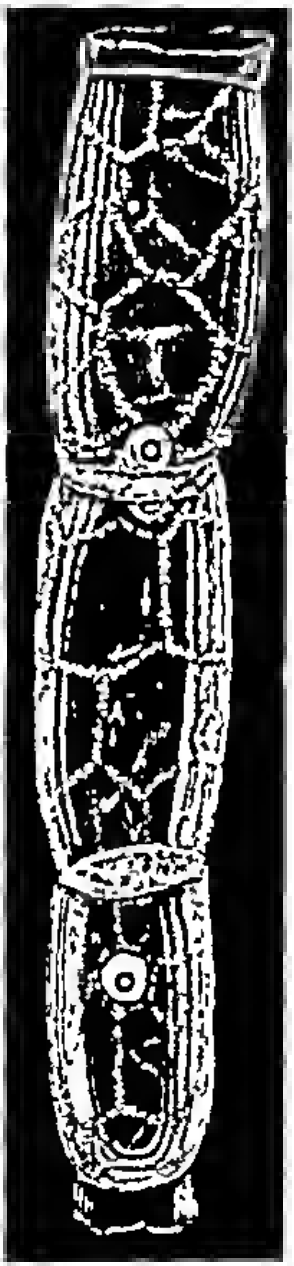


FIG. 18.—THREE CELLS FROM AN ARTICULATED HAIR OF POTATO, WITH A RETIFORM CURRENT OF MUCUS UPON THE WALLS.

The arrows indicate the direction of diffusion currents. Slightly enlarged after Schleiden, 1838.

Another form of motion is that which occurs within the protoplasm of fresh cells, whether living or dead, and consists in a rapid oscillation of minute granules, due to diffusion currents. Although these movements were first observed within protoplasm, it was soon shown that they occurred when various inert particles were suspended in a liquid. Robert Brown described the motion in 1828, in an essay entitled 'On the General Existence of Active Molecules in Organic and Inorganic Bodies,' and the phenomenon is called the molecular or Brownian movement. It may often be seen in salivary corpuscles.

FORMATION AND REPRODUCTION OF CELLS

In the past, two sorts of cell formation have been recognized, namely the spontaneous generation of cells, and the origin of cells through the division of pre-existing cells. According to the theory of spontaneous

generation it was once thought that animals as highly organized as intestinal worms came into existence from the fermentation of the intestinal contents. After this had been disproved, it was still thought that unicellular animals arose spontaneously and that cells might be formed directly from a suitable fluid, the cytoblastema. Something of the sort may have occurred when life began, and it is the expectation of certain investigators that conditions may yet be produced which shall lead to the formation of organic bodies capable of growth and reproduction. At present, however, only one source of cells is recognized—the division of existing cells—*omnis cellula e cellula.*[1] A nucleus likewise can arise only by the division of an existing nucleus; it cannot be formed from non-nucleated protoplasm.

The term growth is used either for an increase in volume or an increase in weight. Growth has been considered as synonymous with cell division. This is not correct, although cell division is usually one of the most important factors. An increase in volume must eventually be correlated with an increase in dry substance, but for limited periods an increase in volume may result from an intake of water only. An increase in volume of a tissue is the result of three positive and three negative factors.[2] The positive factors are: (1) cell multiplication, (2) cell enlargement, (3) centrifugal migration. The negative factors are: (1) cell destruction, (2) shrinkage of cells, (3) centripetal migration. The formation of intercellular spaces and of intercellular substances are secondary to the above. These factors may be closely linked or independent. Cell division alone does not lead to an increase in volume, because the two daughter cells are not larger than the mother cell. For example, in the cleavage of a frog's egg there is no increase in volume, but in cell number.

Amitosis. Amitosis, also called direct cell division, rarely occurs. The name implies that the process takes place without the formation of 'threads' which accompany the more common form of cell division. The nucleus merely becomes increasingly constricted until it is divided in two. Preceding the division of the nucleus the nucleolus, if present, may divide also and supply each half of the nucleus with a nucleolus. The centrosome, when seen at all, remains passive, usually between the nuclear halves. It has been suggested that amitotic division is resorted to when the centrosome, as such, is not present in the cell; *i.e.* certain ciliated cells, in which the centrosome is represented by the basal bodies (see p. 75). But Kindred[3] finds that in ciliated cells enough of the centrosome may remain after the formation of the basal bodies to permit

[1] Virchow, R., 1855. Arch. f. pathol. Anat. u. Physiol. u f. klin. Med., Bd. 8., p. 23.
[2] Mayer, 1933. [3] Kindred, 1927.

mitosis, and that in the same region the two types of cell division may coexist.

Conklin[1] does not consider this form of nuclear division a true cell division. He explains the apparent amitosis as an attempt to increase the nuclear surface within a cell, for metabolic purposes, as in the lobulated nuclei of some blood cells. After injurious treatment of cells during the usual form of division he found occasionally a scattering of nuclear material, and he therefore considered the various forms seen in amitosis as the result of modified mitosis. These atypical mitoses have been called *endomitoses*, *pseudoamitoses*, or *reaction-amitoses*.

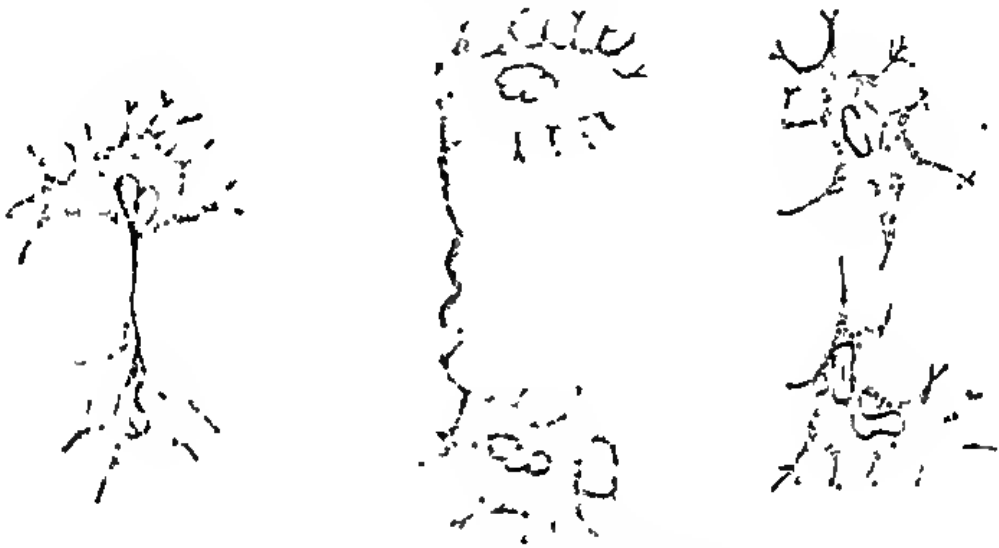


Fig. 19.—Amitosis. Wandering Cell of a Frog (Arnold).

Binucleate cells or those with bipartite nucleus are not sufficient proof of cell division. The inactivity of the chromatin and the almost inevitably unequal distribution of the nuclear contents which would result from a simple division of the nucleus, when compared with the careful regulation in these respects to be presently described, make it extremely doubtful if amitosis can result in normal daughter cells.

Mitosis. Mitosis,[2] also called indirect division and karyokinesis, is the ordinary mode of cell division. It is an elaborate process apparently designed to insure the equal division of the essential portions of the nucleus between the daughter cells. This is accompanied by the production of certain filaments, and the name *mitosis* (Greek, *μίτος*, a thread) indicates this feature. In direct division or *amitosis* these filaments are not developed. Although mitosis is a continuous process, it has been conveniently divided into four successive phases—the *prophase*, *metaphase*, *anaphase*, and *telophase*. During the prophase the chromatic material of the nucleus prepares for division and collects in the center of the cell.

[1] Conklin, 1917. [2] Flemming, 1882.

It is divided in halves in the metaphase, and the two halves move apart during the anaphase. The chromatic material becomes reconstructed into resting nuclei during the telophase. The various patterns which the chromatic material and protoplasmic fibrils present during these phases are known as *mitotic figures*.

Mitotic figures are found in all rapidly growing tissues, but especially favorable for preliminary study are the large cells in the root tips of plants. In longitudinal sections of root tips, the cells are cut at right angles to the plane of cell division, which is desirable; and often in a single section 5 mm. long, all the fundamental stages may be quickly located. The following general description of mitosis is based upon such easily obtained preparations, and the plant selected is the spiderwort (*Tradescantia virginiana*) following the article by Farmer and Shove.[1]

The cells to be described are found in the interior of the root tip, just back of the protecting cap of cells which covers its extremity. They are oblong in shape and their long axis corresponds with that of the root. The walls are very distinct, and the cells consist of granular vacuolated protoplasm, which in preserved specimens is generally irregularly shrunken.

The resting cells (Fig. 20, A) contain large round nuclei in which the chromatin is in the form of fine granules evenly distributed throughout the nucleus. A nucleus usually contains from two to five round nucleoli, each of which, when in focus, is seen to be surrounded by a clear zone. The nuclear membrane is distinct.

Prophase. The first indication of approaching division is a change in the chromatin, which becomes gathered into fewer and coarser granules and takes a deeper stain. Portions of the linin network break down, so that the chromatin granules come to be arranged in long convoluted threads. Such threads are developing in the cell, Fig. 20, B, but are more perfectly formed in C. It is possible that at a certain stage the nucleus contains only a single continuous thread, but this condition cannot be demonstrated in Tradescantia. The stage of nuclear division in which the chromatic material appears to be arranged in a coiled thread or skein is called the spireme stage. The 'close spireme' (B) is succeeded by the 'loose spireme' (C). Successive stages in the development of the spireme in animal cells are seen in Fig. 29, D, E, and F.

As the spireme develops, the nuclear membrane becomes less distinct, and the clear zones disappear from around the nucleoli. The nucleoli become apparently less regular in outline, and forms which suggest that two of them have fused (Fig. 20, B) are perhaps more frequently seen than in resting cells. Usually it is stated that the nucleoli break up into

[1] FARMER AND SHOVE, 1905.

smaller bodies toward the time of their dissolution, and that some of these escape into the cytoplasm after the disappearance of the nuclear

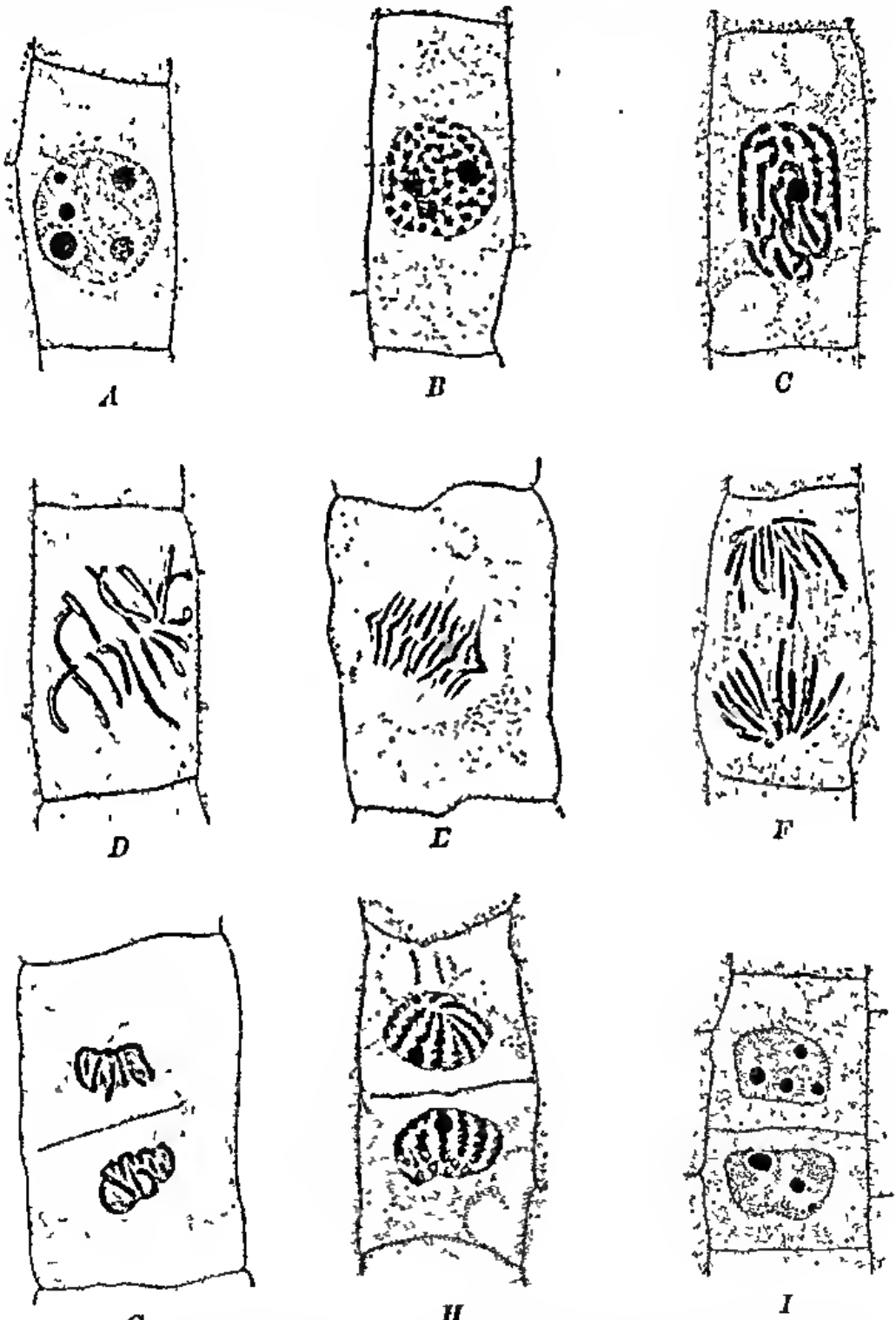


FIG. 20.—MITOTIC CELL DIVISION IN THE ROOT TIP OF *Tradescantia virginiana* × 125 diam. A, resting cell, B, C, D, prophase, E, metaphase, F, anaphase, G, H, I, telophase.

membrane. If one considers, with McClung, that nucleoli, linin, and nuclear membrane are all chromatin in different states, their disappearance merely denotes an altered condition and the accumulation of all

the nuclear material in the coiled thread. The thread appears beaded, as certain masses of its chromatin are larger than others, and those beads soon divide longitudinally to form pairs, so that the thread appears double. Certain of the larger masses fuse and become smoother, separating from other parts of the thread, which is thus segmented into a number of individual bodies, the *chromosomes.*[1]

In the stage shown in Fig. 20, D, which may be regarded as the end of the prophase, the chromosomes are present. They are straight or curved rods of different lengths. Sometimes they appear as bent V-shaped bodies, but these often represent two chromosomes with their ends together. J-shaped forms, with one long and one short arm, have been described in various plants. These shapes are apparently not the result of chance, but are constant in their individual variations. Every cell of any one type of plant or animal shows, during mitosis, a certain number of chromosomes, every one recognizable by its individual shape and character, and alike in all cells. Every chromosome, since it is formed by the coalescence of longitudinally divided 'beads' is itself formed of two closely apposed halves. Usually this is hard to demonstrate, but occasionally the ends of the chromosomes are seen to be slightly cleft.

The chromosomes become so arranged that one end of the rods, or the apex of the V is situated in the equatorial plane, which extends transversely across the middle of the cell. Often it is temporarily tilted (as in D and E) as if the mitotic apparatus had shifted to a position in which it obtained more space. It may do this mechanically if the contents of the cell are under pressure. When the chromosomes are gathered at or in the equatorial plane, they constitute collectively the *equatorial plate.* Because of their stellate arrangement at this stage, which is best seen in transverse sections of the cell, this mitotic figure is known as the *aster.*

Metaphase. In the metaphase (Fig. 20, E), the two longitudinal halves of each chromosome are being drawn apart toward the opposite poles of the cell. If the chromosome is V-shaped, the separation of the two halves begins at the apex of the V.

At this stage an achromatic figure, known as the *spindle,* is evident in plant cells, and is more sharply defined in animal cells. As seen in Fig. 20, it consists of fibrils which pass from the equatorial plate toward either pole, where, in animal cells, there is a well-defined granule, the centrosome. Around each centrosome there are radiating protoplasmic fibrils, forming the *polar radiation.* The polar radiation is also called an aster, and the two asters connected by the spindle are known as the *amphiaster.* Some of the spindle fibers are attached to the chromo-

[1] WALDEYER, W., 1888. Arch. f. mikr. Anat., Bd. 32, gives name 'chromosome,' p. 27.

somes and appear to pull their halves apart; others pass from pole to pole without connecting with the chromosomes. In animal cells the spindle arises as the two centrosomes, lying beside the nucleus, move apart (Fig. 29, A). As they pass to the opposite poles of the nucleus, the spindle forms between them.

In the cells of root tips, a condensation of protoplasm forms a cap at the poles of the nucleus at the time when the nuclear membrane and

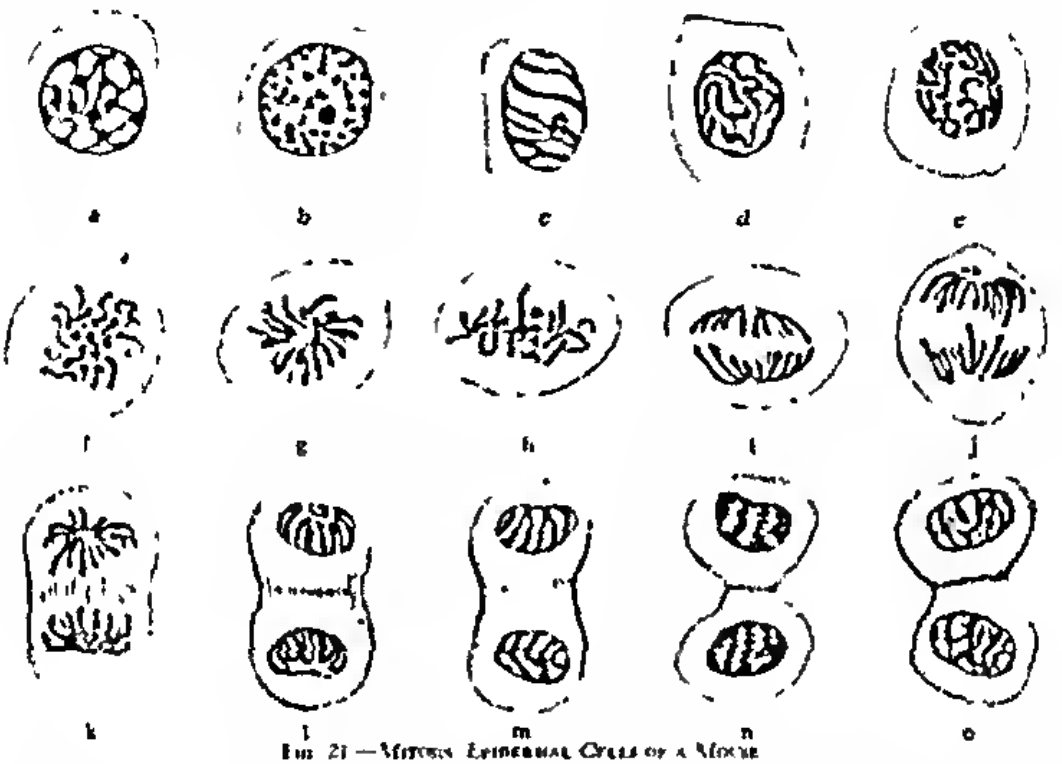


Fig. 21.—Mitosis in Epidermal Cells of a Mouse

Bouin fixation. Iron hæmatoxylin (Ohno & Picón). a–b, resting; c–d–e, prophase; f–g–h, metaphase; i–j–k, anaphase; l–m–n, telophase; o, resting.

nucleoli are disappearing. From the 'polar cap,' spindle fibers develop which invade the nucleus, and also radiations which have been traced even to the cell walls. A definite centrosome, however, probably does not exist in the higher plants.

According to Chambers[1] microdissection of the dividing cell shows that the spindle, though giving in fixed material the appearance of colorless fibrils, is in the living cell a hyaline body with no visible structure, but varying in viscosity from the surrounding cytoplasm. In the plant cell it is of a jelly-like consistency, while in the insect cell it is more fluid; but in both it can be separated from the cytoplasm and still retain its shape. The chromosomes are held in the viscid mass. The fibrils seen in fixed cells merely represent the coagulation of the colloid material, but are nevertheless important indications of lines of stress or local variations in viscosity. Somewhat similar figures can be seen in fields between two magnetic poles, but it is not necessary to conclude that mitosis is the result of magnetic energy. Gurwitsch[2] gives reasons for concluding that the source of the energy which causes mitosis is the production of emanations similar to ultra-violet rays by the decomposition of glucose in the blood, in the yolk, and in certain tissues.

[1] Chambers, 1924. [2] Gurwitsch, 1928.

Anaphase. In the anaphase the halves of each chromosome move to the opposite poles (Fig. 20, F). The figure thus produced is known as a double star or *diaster*. Since each chromosome has divided into two, the original number of chromosomes is preserved, and an equal number will be found in either star. They cannot all be brought into focus together, and because of overlapping, they are hard to count. Sometimes one chromosome, longer than the others, remains for a time as a continuous bar from one aster to the other. Between the asters there are always straight spindle fibers, but they vary in distinctness. (The anaphase in an animal cell is well shown in Fig. 21, I-J-K., and Fig. 30, D.)

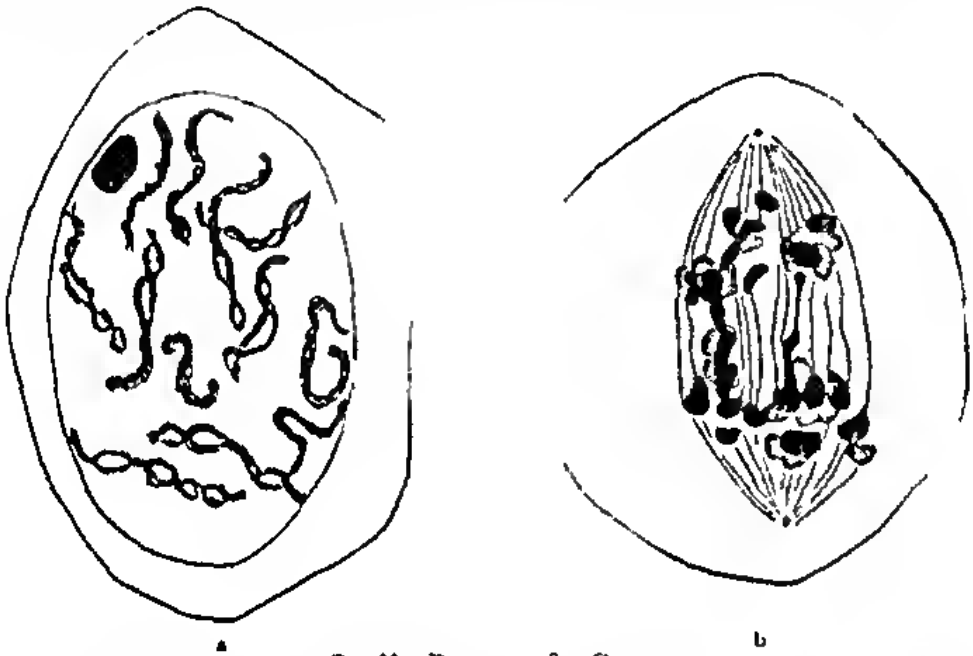


FIG. 22.—*Division of Sex Cells*

a, large cell (diakinesis) showing spiral twisting of chromosomes. Heterochromosome present. b, anaphase of primary spermatocyte with x–y chromosome not yet divided. Forty year old man. (Evans and Swezy.)

Telophase. After the chromosomes have reached the opposite poles, they form two dense masses. They are generally said to unite end to end, thus forming a spireme thread. Immediately after the anaphase the chromosomes form a very compact mass, easily overstained so that it appears solid. Subsequently the mass enlarges, and the chromosomes become coarsely granular, taking the form of wide bands. Every chromosome is thought by some authors to swell to a vesicle (see lower nucleus in H), and the closer approximation or fusion of these vesicles so disguises their individuality that they cannot later be recognized. The resting nucleus would thus contain all the individual chromosomes, but in disguised form. Rabl long ago suggested that the chromosomes persist in the resting nucleus, although disguised by lateral branches or in diffuse granular form. Others consider that they are formed anew at each cell division, and lose their individuality again in the telophase, not being present as chromosomes in any form in the resting nucleus. What-

ever the correct interpretation, the spireme dissolves into the scattered chromatin granules. The nucleoli reappear, usually at first at the edge of the nucleus. Finally the nuclear membrane reappears.

In animal cells a constriction of the wall occurs at the equator between the two daughter nuclei. This leads ultimately to the separation into two cells. But in plants, probably because of the stiffness of the cell walls, the new wall arises as a series of thickenings of the interzonal spindle fibers, which at this stage form a barrel-shaped bundle (Fig. 20, G). The thickenings coalesce to form a membrane which does not at first reach the sides of the cell. While this wall is developing the nuclei are in a condition resembling the spireme stage of the prophase. The

FIG. 23.—FIGURE ASSUMED BY DROPS OF A SUSPENSION OF INDIA INK IN SALT SOLUTION WHEN PLACED IN THE MIDDLE OF LARGER DROPS OF SALT SOLUTION OF GREATER DENSITY (Leduc)

entire mitotic figure is therefore called the double spireme or *dispireme*. The cell wall is soon completed and the nuclei return to the resting condition (Fig. 20, I).

The time required for mitotic cell division varies from half an hour (in man) to five hours (in amphibia). After death, if the tissues are not hardened by cold or reagents, it is thought that mitoses go on to completion. Forty-eight hours may elapse before they entirely disappear from the human body.

Pluri-polar Mitosis. Under abnormal conditions, as in the cancer cells, spindles may develop simultaneously in connection with three or four centrosomes. Similar pluri-polar spindles have been produced experimentally, by treating cells with various poisonous solutions, and by radiation. An unequal distribution of chromatin may occur under such conditions, and this may happen also with bipolar spindles.

Number and Individuality of the Chromosomes. It is now generally believed that every species of plant or animal has a fixed and characteristic number of chromosomes, which regularly recurs in the division of all its cells, with the exception of the germ cells, in which the number is reduced. In certain species, however, the two sexes regularly differ from

one another in the number of their chromosomes, and one sex may contain an odd number. Usually the number of chromosomes is believed to be even.

There is considerable difficulty in counting the chromosomes. Generally it is possible that some have been cut away in the process of sectioning, so that, if the number is believed to be invariable, the highest number found in any cell is assumed to occur regularly. Another source of error lies in the fact that a bent chromosome may be counted as two, or rods with their ends overlapping may appear as one. In the frog the number of chromosomes is reported as twenty-six in the female, twenty-five in the male. In the grasshopper the numbers are given as twenty-

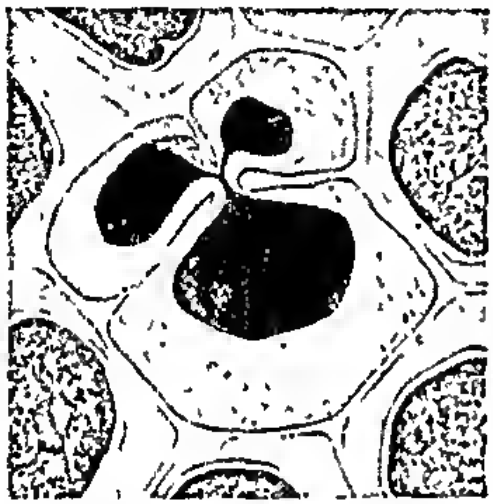


FIG. 24.—ASYMMETRICAL TRIPOLAR PSEUDOAMITOSIS. INFLUENCE OF X-RAYS ON CORNEAL EPITHELIUM, URODELA LARVA. (Alberti and Politzer.)

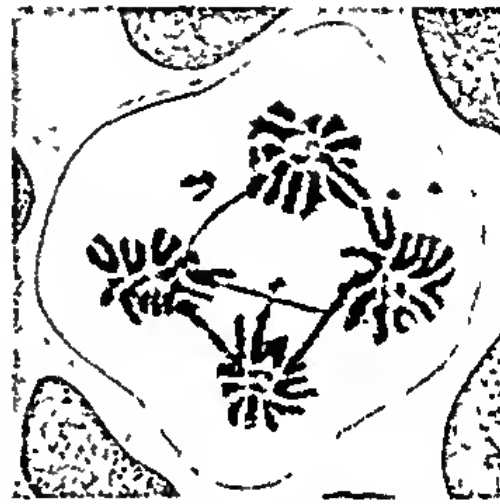


FIG. 25.—CELL DIVISION CORNEAL EPITHELIUM URODELA LARVA. INFLUENCE OF X RAYS. TETRASPIREME WITH BRIDGES. (Alberti and Politzer.)

four and twenty-three. In man Painter[1] places the number at forty-eight. In the worm Ascaris only two chromosomes are present. There is some indication that the number increases with the complexity of the animal.

Among the lizards Painter[2] recognizes macrochromosomes and microchromosomes (large and small) and finds that all of the Iguanidæ have twelve macrochromosomes, of which at least three pairs are strikingly alike in all species of the genus, whereas the number of microchromosomes varies in the different species. In some closely allied varieties of plants the number of chromosomes seems to run in numerical sequence, seven in one variety, fourteen and twenty-one in two other varieties. In two species of minnow the chromosomes vary in size, and can be recognized in hybrids by this characteristic.

Not only is the number of chromosomes in each cell of a given species believed to be constant, but they appear to form a definite series, the

[1] PAINTER, 1923. [2] PAINTER, 1921.

members of which differ somewhat in shape and size. Moreover, they occur in pairs of similar size and shape, but the two members of a pair need not lie side by side. The examination of the minnow hybrids referred to above shows that one chromosome of each pair is derived from each parent. More detailed analysis of the individual chromosomes reveals a further subdivision into 'chromomeres,' swellings of the chromatic material linearly arranged along the chromosome in certain stages of its development (compare Fig. 30). These have been identified in thirteen generations of one species of grasshopper. The chromomeres are supposed to carry individual characteristics, which may be transmitted to the offspring. This 'chromosomal theory of heredity' has long been discussed, and is ably given by McClung.[1]

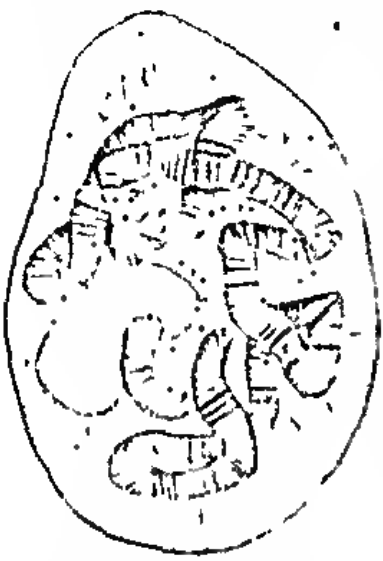


Fig. 26.—Nuclei of a Salivary Gland Cell of the Larva of Chironomus. Permanent Specimen. Iron Alum Stain, Methyl Green (Preparation).

In addition to a definite number of pairs of chromosomes an extra body is often noticed. The difference in the number of chromosomes recorded for the male and female of some species is due to the presence of this 'accessory chromosome,' which, according to McClung's hypothesis, now well established, is the bearer of those qualities which determine sex. It is now known that an unpaired chromosome is not present in all species, a dissimilar pair serving the same purpose; the 'sex chromosome' may be considered as attached to one of the pair, but the chromosome number would be alike in both sexes. The sex chromosomes and the special type of mitosis occurring in spermatogenesis will be discussed later.

This difference is indicated by the formula X-O, where the accessory chromosome (X) has no mate, and X-Y, where both the accessory and a dissimilar mate (Y) are present. In man the sex chromosomes are of the X-Y type, and the number forty-eight for both sexes. Although in the opossum Painter could establish the fact that the smaller chromosome of the pair denotes the female, he is not sure whether this is also true in man.

Recently Riddle[2] has discussed sex reversal in the bird. Testicular tissue, according to this author, develops in birds from which the left ovary has been removed. Among lower animals the presence of male and female gonads in one individual is recognized. It is interesting to speculate on the relation of the sex chromosome in these instances. The student may be referred to a discussion of the subject in Jordan and Kindred.[3]

The great importance of accurate knowledge of the chromosomes is shown by the following considerations. As a result of mitotic cell division,

[1] McClung, 1924. [2] Riddle, 1925. [3] Jordan and Kindred, 1926.

it is evident that every new cell regularly receives one-half of each chromosome found in the parent cell, and thus the number of chromosomes remains constant. But in the germ cells the number is invariably reduced, and in some animals it becomes exactly one-half of the number found elsewhere in the body. In such a case, when the male sexual cell, or *spermatozoon*, unites with the female sexual cell, or *mature ovum*, in the process of fertilization, the original number is restored. Each parent thus contributes one-half of the chromosomes found in the cell which gives rise to a new individual; and since each of these divides with every subsequent cell division, it is evident that one-half of the chromatin in every cell of the adult body is of maternal origin and one-half of paternal origin. The process by which the sexual cells acquire the reduced number of chromosomes and become ready for fertilization is known as *maturation*. The production of the sexual cells in the male is called *spermatogenesis* and in the female *oogenesis*.

Spermatogenesis. The material for the study of mammalian spermatogenesis is ordinarily difficult to obtain in quantities sufficient for students' use, and unsatisfactory in other ways. Reference should be made to the excellent monograph on spermatogenesis in man by Evans and Swezy,[1] who have succeeded in elucidating the number and forms of human chromosomes, and to the more recent paper by Gatenby and Beams.[2] In its essential features, the process of spermatogenesis in insects corresponds with that in mammals, and very favorable material can be obtained in abundance from grasshoppers of various genera.

As seen in sections, each lobe of the testis of the grasshopper contains a considerable number of closed sacs or cysts, which are filled with sexual cells; and all the cells within a cyst are in approximately the same stage of development. Usually the testis is sectioned as a whole, and the specimen consists of a group of lobes cut transversely or obliquely. Cross sections from the apical portion, furthest from the outlet, will contain younger stages than the sections lower down in the lobe, since the cysts form at the apex and gradually move downward. At the apex, according to Davis,[3] there is an *apical cell* which is surrounded by young sexual cells known as *spermatogonia*. The spermatogonia move away from the apical cell, and each becomes enclosed in a cyst-wall derived from the surrounding tissue. Within the cysts thus formed, the spermatogonia multiply, and the cysts in the upper part of the lobe are filled with spermatogonia. After repeated divisions the spermatogonia pass through a period of growth, accompanied by a rearrangement of their nuclear contents. The large cells with characteristic nuclei which are thus produced are known as *primary spermatocytes*. They fill the cysts further down in the

[1] Evans and Swezy, 1929. [2] Gatenby and Beams, 1935. [3] Davis, 1908.

lobe. Each primary spermatocyte divides into two *secondary spermatocytes*, and each of these divides into two *spermatids*, after which no further cell division is possible until fertilization takes place. But each spermatid becomes transformed from a round cell into a linear body with a whip-like tail, and is then capable of independent motion. Since in this form these cells were once thought to be parasitic animals living in the

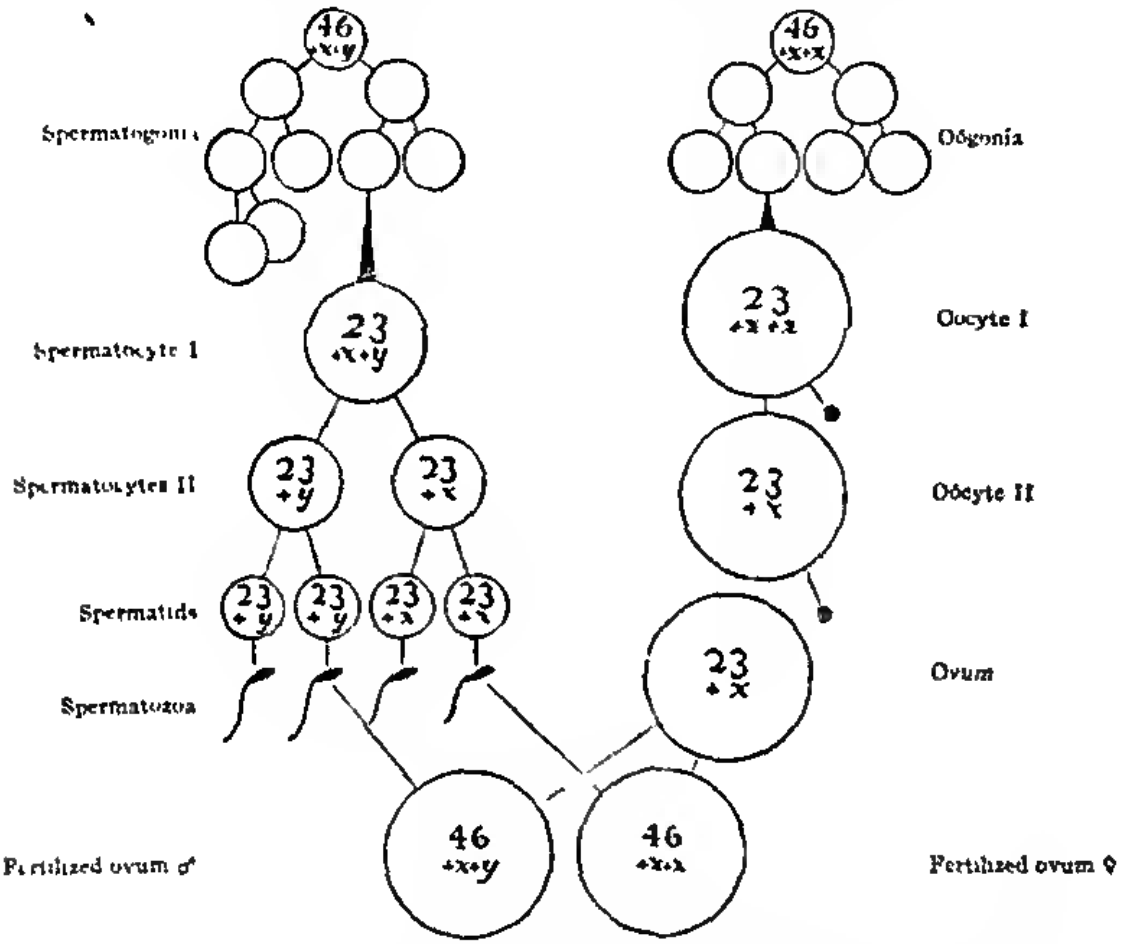


FIG. 27.—DIAGRAM OF SPERMATOGENESIS, OÖGENESIS AND FERTILIZATION. THE CHROMOSOME NUMBERS ARE THOSE FOR MAN.

spermatic fluid, they received the name *spermatozoa*, which they still retain.[1] Cysts containing spermatozoa occur near the outlet of the lobe, or if the grasshoppers are collected late in the season, they may be found throughout most of the testis.

The succession of cell divisions described in the preceding paragraph is shown in tabular form in the left half of Fig. 27. The number of chromosomes indicated is that accepted for man, but otherwise the diagram is quite as applicable to the grasshopper. In this figure, how-

[1] It has been proposed to substitute the term *spermium* for *spermatozoon;* and consequently *spermiocyte* and *spermid*, for spermatocyte and spermatid. The secondary spermatocytes are sometimes called præspermatids or præspermids; but these changes in name are of questionable value.

ever, only two spermatogonial divisions have been included. The number of times which the spermatogonia may divide before becoming spermatocytes is considerable and presumably indefinite. As seen in sections, the spermatogonia, spermatocytes, and spermatids may be described as follows, using for illustrations Davis's figures of a common grasshopper—*Dissosteira carolina*.

Spermatogonia. The nucleus of each spermatogonium contains the full number of chromosomes, which in most of the grasshoppers (Acrididæ) is 23. With every spermatogonial division, each chromosome is split lengthwise. In this and other respects the mitotic figures are quite like those occurring elsewhere in the body. They are shown in Fig. 29, A, B, and C. When the twenty-three chromosomes have formed the equatorial plate, it is sometimes possible to see all of them in a single trans-

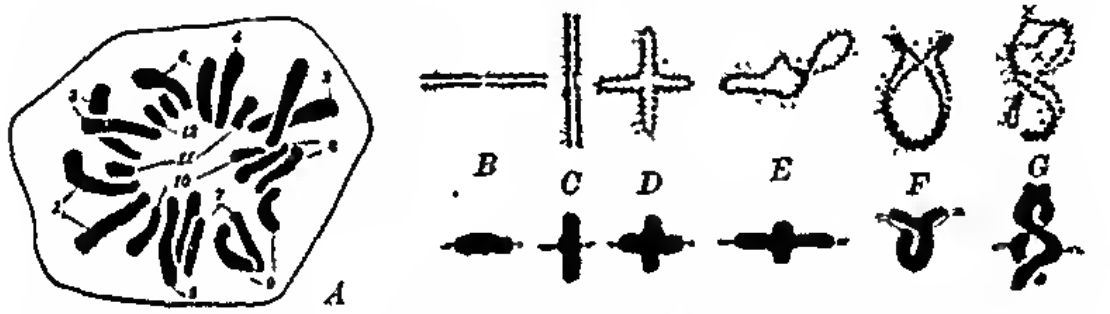


Fig. 28.—A, Polar View of the Metaphase of a Spermatogonial Division in *Dissosteira carolina*. × 1450 (After Davis.)

The pairs of chromosomes have been numbered. B-G, various forms of tetrads, from primary spermatocytes (Davis and Robertson.)

verse section of the cell (Fig. 28, A), and it is at this time that the chromosomal pairs and sex chromosomes are most readily recognized.

Primary Spermatocytes. After frequent spermatogonial divisions, certain of the cells become primary spermatocytes by passing through a 'growth period.' They are shown in Fig. 29, D, E, and F. All of the chromosomes, except the accessory chromosome, have become filamentous, but the accessory chromosome remains as a compact, darkly staining body close to the nuclear membrane. It resembles a nucleolus, for which in fact it has been mistaken. True nucleoli may occur in these cells, together with the accessory chromosome, but they stain differently.

As the primary spermatocytes prepare for the next division, the spireme becomes resolved into *eleven* segments, each of which represents the two members of a pair of chromosomes joined end to end, instead of into twenty-two segments as in ordinary divisions. In man the number would be 23 + x + y, instead of 46 + x + y. The granules embedded in the spireme thread divide longitudinally as usual, so that each segment contains a double row of granules (Fig. 29, F). The rows may pull apart, except at the ends of the segments, so that loops are formed, containing the material of four half chromosomes, *i.e.* the two linear halves of each

member of a pair. The loops contract in various ways to form eleven chromosomes, which, because of their four parts, are known as *tetrads*. The structure of the tetrads is shown in Fig. 28, B–G. The filaments seen in the upper row of drawings contract into corresponding solid forms of chromosomes seen in the lower row, in which the place of attachment to the spindle fibers has been indicated.

Each tetrad represents two chromosomes joined end to end and split lengthwise. The simplest forms are shown in Fig. 28, B and C, which illustrate respectively two ways in which the tetrad may later divide. The two component chromosomes may simply be pulled apart, as indicated in B, in which the spindle fibers are attached to the ends of the rod. If this takes place, each secondary spermatocyte will receive one member of every pair of chromosomes which occurred in the spermatogonium, but no part of the other member. Such a division, which eliminates one-half of the chromosomes from the daughter cell, is known as a *reductional division*. The other form of chromosome division is known as *equational*. When it takes place, every chromosome divides lengthwise, and the daughter cells receive one-half of every chromosome in the parent cell. This occurs in ordinary cell division, and also in the division of the tetrads provided that the spindle fibers are attached to the place where the two component chromosomes come together (Fig. 28, C). As a stage in the separation of the two halves of a rod-shaped tetrad, cross-shaped forms are produced (Fig. 28, D). If the separation is almost complete, such shapes are seen as in E. The arms of the tetrad which are not attached to the spindle fibers may bend toward one another and unite, so as to form rings (F), or they may twist about like a figure 8, as shown in G. If the spindle fibers are attached to the points xx in the upper figure in G, the division will be equational; if as shown in the lower figure it will be reductional.

Usually it is considered that the division of the tetrads into double bodies, or *dyads*, is equational, and that the division of the dyads, which takes place when the secondary spermatocytes divide, is reductional. According to Davis, however, the first division of the tetrads is reductional and the second division is equational. In either case the end-result is the same. Each spermatid will contain one of the four parts of each tetrad, and thus one member of every pair of chromosomes will be eliminated from any given spermatid. The peculiar divisions leading to the reduction of the number of chromosomes are known as 'mitoses of maturation,' or 'meiotic divisions.'

Since in the testis tetrads occur only in the primary spermatocytes, the cells shown in Fig. 29, G–J, are easily identified. These are successive stages in the division of the primary spermatocyte. In G the accessory

chromosome is seen as a rod-shaped body above and to the right; in H it is below and to the right. In J it is obliquely placed just above the equa-

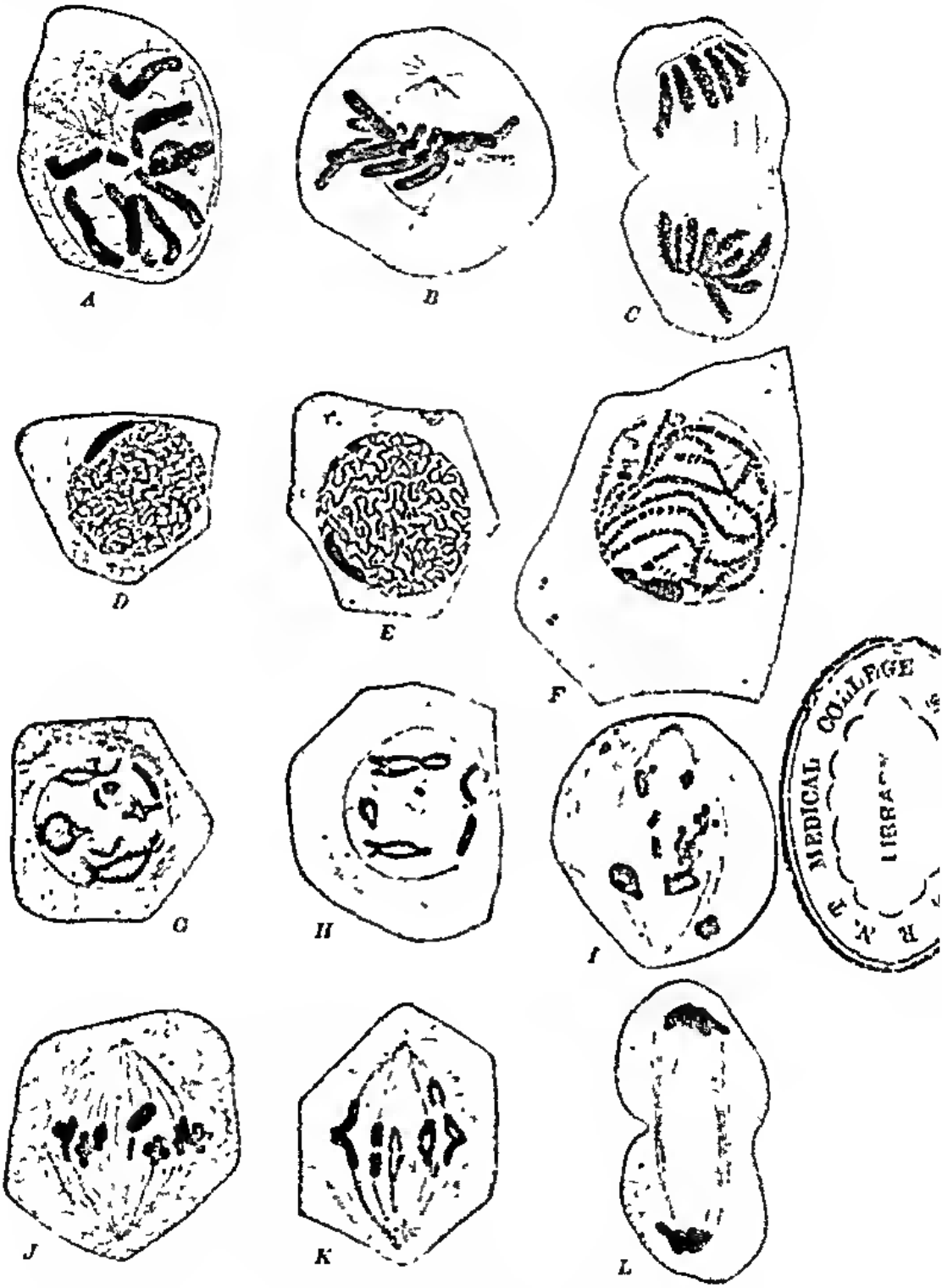


FIG. 29.—SPERMATOGENESIS IN *Dasytes cæruleus*. A–F × 1450, G–L × 900. (DAVIS.)
A, B, C, prophase, metaphase, and telophase of a spermatogonial division. D–L, successive stages in the division of a primary spermatocyte into secondary spermatocytes.

torial plate and in K it is passing to the upper pole of the spindle. In the spermatogonial divisions the accessory chromosome always divides with

the others; but in the division of the primary spermatocyte it passes undivided into one of the daughter cells. Thus one secondary spermatocyte will receive eleven chromosomes (dyads) and the other will receive twelve (eleven dyads, and the accessory chromosome). In the late anaphase shown in Fig. 29, L, the accessory chromosome cannot be recognized. In animals with the X-Y type of accessory chromosomes, one of the unmatched pair passes to each daughter cell, and the number is equal in both.

Secondary Spermatocytes. The secondary spermatocytes pass rapidly from the condition shown in Fig. 29, L, to that of Fig. 30, A. A nuclear membrane has developed, and the dyads have become somewhat filamentous. Without passing through a complete resting stage they proceed to divide as shown in Fig. 30, B–F. The dyads separate into their component halves. In those secondary spermatocytes which received the accessory chromosome, that body will be seen dividing with the dyads, and each spermatid will receive one-half of it. It has been questioned whether the division of the accessory chromosome is longitudinal and therefore equational, or transverse and reductional. Many cytologists consider that if a chromosome splits lengthwise, all of its parts will be represented in the resulting halves, but if it divides transversely, essential elements will be lost. This conception lends importance to the question of transverse or longitudinal division of the accessory chromosome. By the division of this chromosome it comes about that one-half of the spermatids contain twelve chromosomes, and one-half contain eleven, as indicated in the diagram. The spermatids shown in Fig. 30, F, contain the accessory chromosome.

Spermatids and Spermatozoa. In forming spermatozoa, the spermatids become elongated, (Fig. 30, F and G). The chromatin within the nucleus is distributed in fine granules throughout the linin reticulum. Close to the nuclear membrane a small dark body has appeared, from which a slender filament has grown out. This body is usually described as the centrosome. A condensation within the cytoplasm, seen also in F, is known as the *paranucleus* It is of uncertain origin, but may proceed from the cytoplasmic structures called mitochondria. The paranucleus forms a sheath about the axial filament.

Successively later stages are shown in Fig. 30, H, I, and J. The chromatin within the nucleus becomes homogeneous and very dense; at the same time the nucleus elongates and forms the *head* of the spermatozoon. This is enveloped by the cell membrane, but there is no appreciable layer of protoplasm around it. The centrosome elongates and forms the *middle piece* of the spermatozoon; and the axial filament, with a covering derived from the paranucleus and cytoplasm, constitutes the *tail*. Only a portion

of the tail is included in the figure. The human spermatozoon likewise consists of a head, which is essentially the nucleus, a middle piece con-

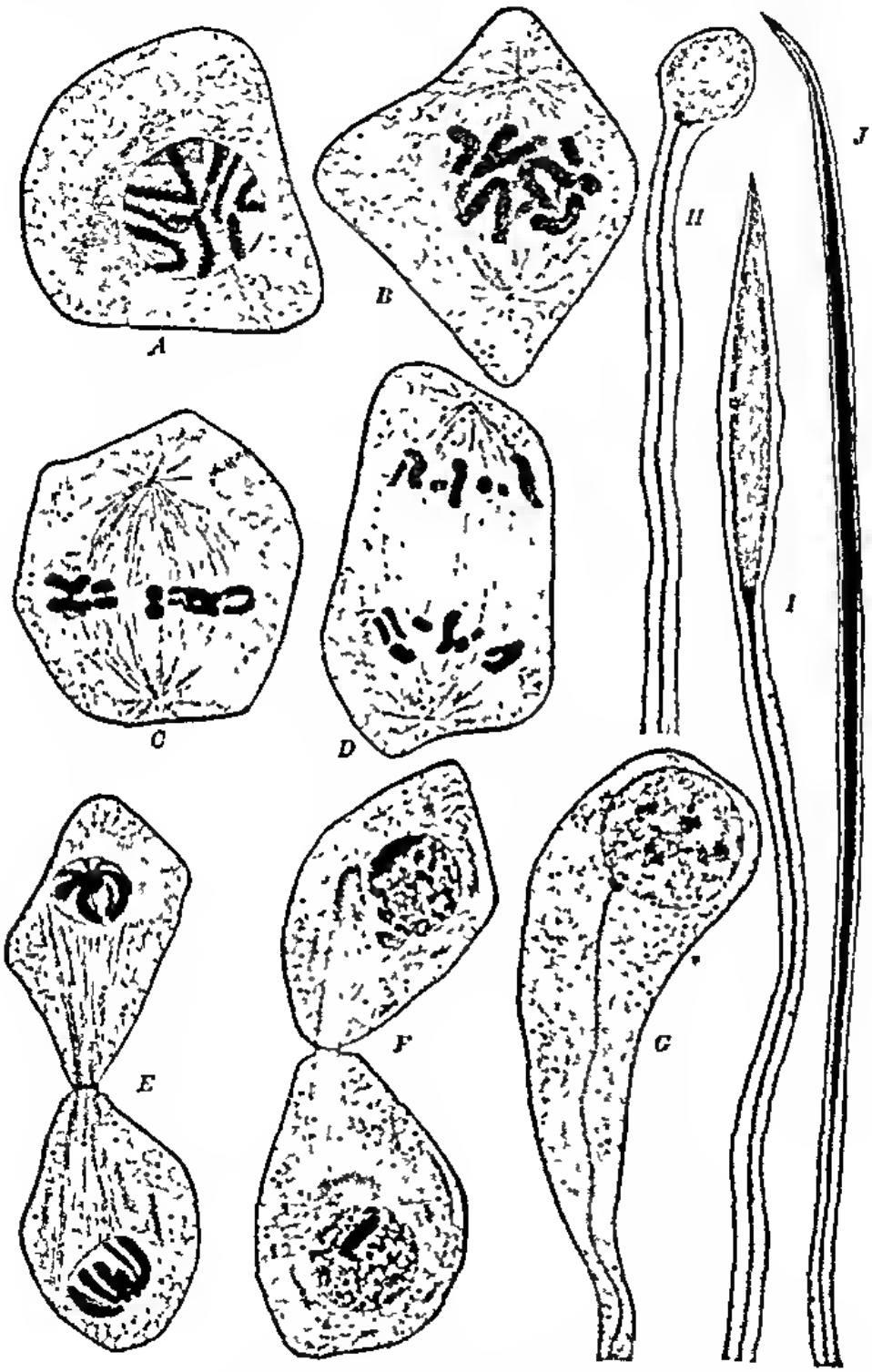


FIG. 30.—SPERMATOGENESIS IN *Diemictylus viridescens*. × 1450. (DAVIS.)
A–F, successive stages in the division of a secondary spermatocyte into spermatids. G–J, successive stages in the transformation of spermatids into spermatozoa.

taining the centrosome, and a tail; but the form of the head is very different from that in the grasshopper. It will be described in a later chapter.

Although the spermatozoa of the grasshopper appear alike, it has been shown that one-half of them contain eleven chromosomes, and one-half contain twelve. The mature ova all contain twelve chromosomes. If a spermatozoon with eleven chromosomes unites with an ovum with twelve, a male animal will be produced, in every cell of which there will be twenty-three chromosomes. But if the spermatozoon contains twelve chromosomes, a female animal is formed, containing twenty-four chromosomes in every cell. Thus sex appears to be determined by the presence or absence of a chromosome within the spermatozoon, or by the difference in one chromosome of the X-Y type, as in man.

Oögenesis. Mature ova result from a succession of cell divisions closely comparable with those which produce spermatozoa. The primitive female sexual cells correspond with the spermatogonia, and are called *oogonia*. They are provided with the full number of chromosomes, and divide an indefinite number of times. After a period of growth they become *primary oocytes*, in which the number of chromosomes is reduced one-half. The primary oöcytes divide to form *secondary oöcytes;* and these again divide to produce the *mature ova*, which are incapable of further division unless fertilization takes place. (The term *ovum* is ordinarily loosely applied, so that it includes not only the mature cells, but also oöcytes, and the clusters of cells resulting from the division of the fertilized ovum.)

Although the mature ovum and the spermatozoon are closely similar in their nuclear constitution, they differ radically as to form, size, and cytoplasmic structure. The ova are very large cells, stored with nutriment for the embryo which each one may later produce. In the higher vertebrates they are formed in relatively small numbers. Probably only five or six hundred, ready for fertilization, are produced by the human female in a lifetime, but this does not include a vastly greater number which degenerate before becoming mature. The male produces some 340 billion spermatozoa. A large number must be produced, since many will fail to traverse the uterus and tube so as to find the ovum at the time of its discharge from the ovary. The ova of lower vertebrates, which are fertilized and develop outside of the body, are discharged in great numbers; in certain fishes from three to four million are produced annually.

The multiplication of oogonia in the human ovary takes place before birth, and about fifty thousand are produced. At birth, or shortly thereafter, all the oögonia have become primary oöcytes. At first the oöcytes are small, but they enlarge at varying rates, and the largest are indistinguishable from mature ova except by their nuclear contents. Since some grow more rapidly than others, the ovary in childhood contains

primary oöcytes of many sizes. Each oöcyte becomes enclosed in a cyst or follicle. The way in which these follicles develop, and the manner in which the oöcyte escapes into the uterine tube by the rupture of these follicles, will be described in connection with the ovary. Between the cells of the follicle and the oöcyte, there is a broad, radially striated membrane, known as the *zona pellucida* or zona radiata. This zona has sometimes been regarded as a cell membrane, but the oöcyte divides within it as if enclosed in a capsule. The radial striations have been interpreted as slender canals containing processes of the follicular cells, and the zona has been considered as a product of these cells. In certain cases a perivitelline space has been described as encircling the oöcyte and thus separating it from the zona, but this space has been considered as artificial, or as a refractive line wrongly interpreted as a space.

The cytoplasm of the oöcyte becomes charged with yolk granules or spherules. They constitute the *deuteroplasm* (or deutoplasm), but this term is equally applicable to fat droplets and other secondary products of the protoplasm. In the human oöcyte the granules are centrally placed, and they are so transparent, when fresh, as to cause only a slight opacity. In the eggs of many animals the yolk is more highly developed, and it may be evenly distributed or gathered at one pole. Within the cytoplasm of the developing oöcyte, a large dark body of radiate structure is sometimes conspicuous. It is inappropriately known as the *yolk nucleus*, and is probably a derivative of the centrosome and surrounding archoplasm. Other 'vitelline bodies,' of uncertain origin and significance, have been described. Some have been considered as nuclear extrusions.

The nucleus of the oöcyte is very large and vesicular. The chromatin occurs chiefly along the nuclear membrane and about the nucleolus. The nucleolus is also very large, and Nagel stated that in the fresh condition it exhibits amœboid movements, but this observation has not been verified. The nuclei of the oöcytes ordinarily show no signs of mitosis, and they may remain in the resting condition for thirty years or more and then divide. Many of them, however, will degenerate without division.

The cell divisions which give rise to the secondary oöcyte and the mature ovum respectively have seldom been observed in man,[1] (cf. Fig. 465). Some of the cells within the ovary may be secondary oöcytes. From what is known of other mammals, however, it may confidently be assumed that the cell divisions take place as shown in the diagram, Fig. 27, on the right side.

When the primary oöcyte divides, the chromosomes, reduced in number, also divide and are equally distributed to the daughter cells;

[1] Hoadley and Sikes, 1928.

but the great mass of cytoplasm remains with one of these cells, namely, the secondary oöcyte. The other cell, which is relatively very small, is known as the *first polar body*, or polar cell. It has the same nuclear contents as the secondary oöcyte, and may divide into two other polar bodies, equivalent to mature ova. More often it degenerates without division. When the secondary oöcyte divides, it likewise produces one large cell, the *mature ovum*, and one small cell, the *second polar body*. The latter is said to be capable of fertilization, but to what extent it may develop is unknown. Functionally the production of polar bodies serves to prevent the subdivision and distribution of the nutritive material elaborated within the primary oöcyte. One mature ovum with abundant yolk is provided at the expense of three ova (polar bodies) which degenerate.

Although the maturation of the ovum is not well known in man, nor even the presence of definite polar bodies, the entire process has been carefully studied in other mammals, notably in the mouse.[1] It has been shown that the maturation of the ovum of the mouse takes place rapidly, both of the oöcyte divisions being accomplished within from four to fifteen hours. The first polar body usually forms before the oöcyte is discharged from the ovarian follicle—in other words, before *ovulation* takes place. The second polar body is usually formed in the uterine tube, after the spermatozoon has entered the oöcyte. Long and Mark[2] have found that the chromosomes of the primary oöcyte are tetrads, or bodies showing transverse and longitudinal divisions; and that those of the secondary oöcyte are dyads. They believe that the first division is transverse or reductional, and that the second is equational.

The difficulty of counting chromosomes is apparent from the varying numbers which have been reported in the mouse. After reduction the number has been placed at 8, 12, 16, 18 and 20 by different observers.

The polar bodies in the mouse are relatively large. In the upper part of Fig. 31, A, a polar body is about to be formed, and it is completely cut off from the oöcyte in C. In D and G, two polar bodies are shown.

Fertilization. In the mouse, from six to ten hours after coitus, spermatozoa have made their way to the distal end of the uterine tube, where fertilization takes place. According to Long and Mark, the maturation of ova usually occurs at some time during the period from '13¾ to 28½ hours' after the mouse has given birth to a litter; and during the process of their maturation, the oöcytes are discharged from the ovary and enter the distal end of the tube. Here, if fertilization takes place, a single spermatozoon penetrates the zona pellucida. In a section obtained by Sobot-

[1] Sobotta, 1895. [2] Long and Mark, 1911.

ta, the entrance of the spermatozoon has been partially accomplished (Fig. 31, B). Its tail lies outside of the zona, and appears to have become thickened. In another specimen Sobotta found the head, middle piece and a part of the tail within the cytoplasm of the oöcyte. The tail had broken as it crossed the zona, and the portion remaining outside had

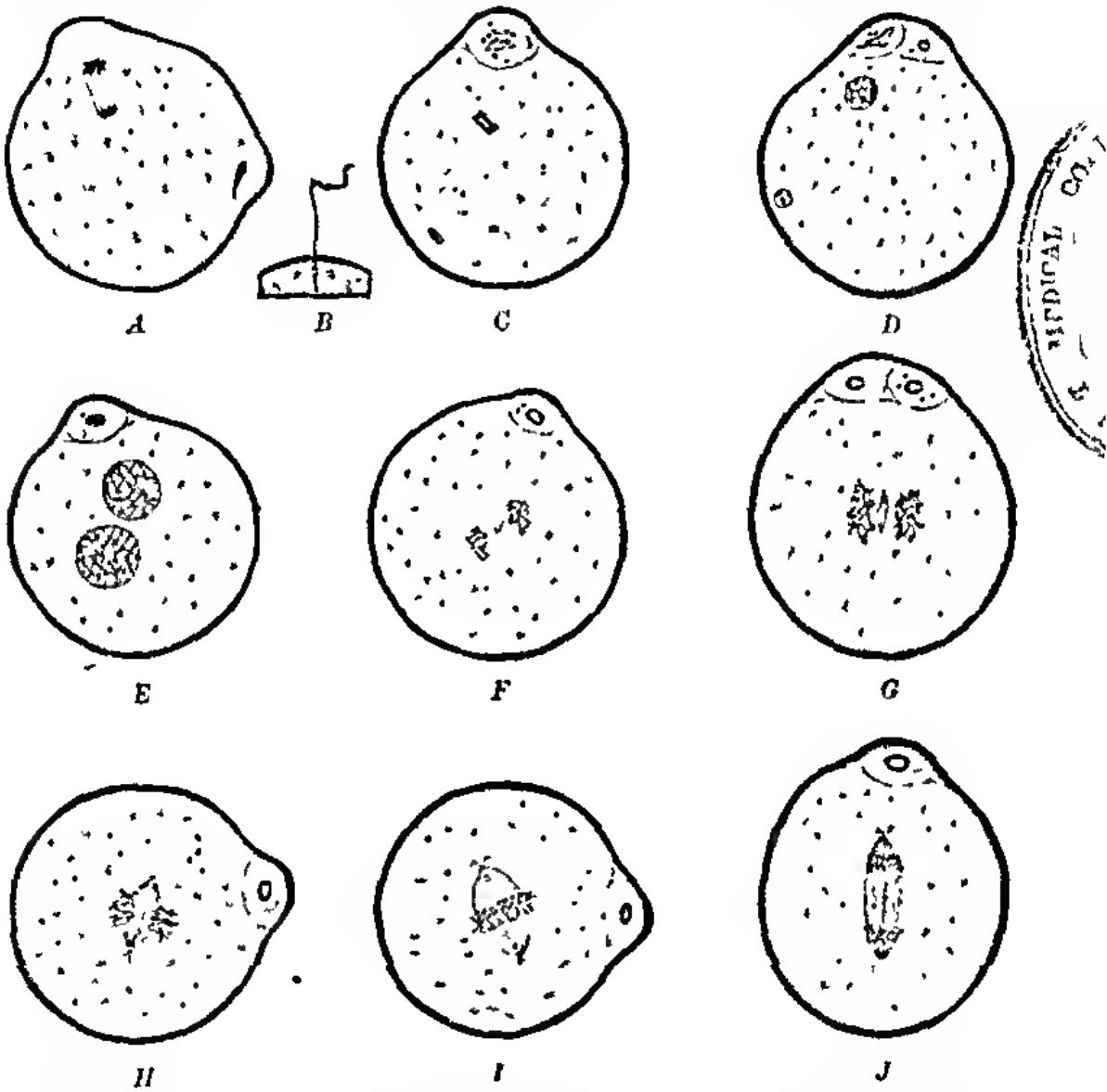


FIG. 31.—MATURATION AND FERTILIZATION OF THE OVUM OF THE MOUSE. A, C-J, × 500. B, × 750. (Sobotta.) A–C, entrance of the spermatozoon and formation of the second polar body. D–E, development of the pronuclei. F–J, successive stages in the first division of the fertilized ovum.

drawn together and was disintegrating. In some animals it is said that the entire spermatozoon enters the ovum, but in others only the head and middle piece. In any case the tail appears to be a propelling apparatus which becomes functionless after the head and middle piece have passed through the zona. It has entirely disappeared in the stage shown in Fig. 31, A, in which the head of the spermatozoon is seen within the oöcyte on the right side of the figure. Meanwhile the oöcyte is becoming

a mature ovum by undergoing divisions and producing the second polar body; and the anaphase of this division is shown in Fig. 31, A. Sobotta stated that no centrosomes occur in connection with the spindles of the maturation divisions, and Long and Mark have likewise failed to find any 'typical centrosomes.'

In Fig. 31, C, the second polar body has become a separate cell. The chromosomes of the ovum, which is now mature, have formed a compact mass. They next become resolved into a chromatic reticulum, and a resting nucleus is produced, provided with a nuclear wall and distinct nucleoli (D and E). This nucleus, which becomes large and moves toward the center of the cell, is known as the *female pronucleus*. Meanwhile the head of the spermatozoon has enlarged and formed the *male pronucleus*, as shown in C, D, and E.

The two pronuclei, which are very similar, develop rapidly, 'probably within a few minutes after the entrance of the spermatozoon.' Simultaneously they prepare for division, and the chromatic reticulum of each becomes resolved into the reduced number of chromosomes which it received during maturation (Fig. 31, F). A centrosome with astral radiations, is now seen between the two groups. In G, it has divided in two, and the spindle has developed. There has been much discussion as to the origin of these centrosomes. Since in this case they arise by the division of a single body, the possibility that one comes from the spermatozoon and one from the ovum has been eliminated. Moreover, in the mouse they cannot be derived from the surviving centrosome of the last maturation division of the ovum, for that division takes place without centrosomes. Therefore the centrosome must either be brought in by the spermatozoon as a constituent of its middle piece, or it must be a new formation. Sobotta accepted the former alternative, and he observed a centrosome in connection with the head of the spermatozoon in certain stages (Fig. 31, C) but not in all. It is probable, according to Conklin, that 'the source of the cleavage centrosomes may differ in different animals, or even in the same animal under different conditions.'

Later stages in the division or 'cleavage' of the fertilized ovum into two cells are shown in H–J. The groups of chromosomes come together upon the spindle so that the full number, characteristic of the species, is restored. Each chromosome then divides lengthwise, and thus each daughter cell receives one-half of its chromosomes from the male parent and one-half from the female parent. This is strikingly evident when the eggs of the fish *Fundulus*, which have long rod-shaped chromosomes, are fertilized with the sperm of *Menidia*, which has shorter rods. Moenkhaus,[1] who performed this experiment, states that the two kinds of

[1] Moenkhaus, 1904.

chromosomes remain grouped and bilaterally distributed on the spindles during the first and second divisions of the fertilized ovum, but that later they become gradually mingled.

Important information in regard to the nature of fertilization has been obtained by experiments upon unfertilized eggs. Changes in the concentration or composition of the sea water in which the eggs of marine animals have been placed, mechanical agitation, or, in the case of frogs' eggs, puncturing the outer layer with a needle, have led to repeated cell divisions. In this way embryos or larvæ have been produced from unfertilized eggs, and, in a few instances, adult animals. Loeb, who has been a foremost investigator in this field, concludes that the spermatozoon causes the development of the egg by carrying a substance into it which liquefies the cortical layer of the egg, and thereby causes the formation of a membrane. 'This membrane formation, or rather the modification of the surface of the egg which underlies the membrane formation, starts the development.' At the same time there is an acceleration of the oxidations in the egg. 'What remains unknown at present is the way in which the destruction of the cortical layer of the egg accelerates the oxidations.'

For the physicist and chemist, the production of mitotic figures and the process of fertilization have been subjects of great interest, and discussions of their significance will be found in various text-books of physiology and biological chemistry.

General Histology

HISTOGENESIS

SEGMENTATION AND THE FORMATION OF THE GERM LAYERS

The body is composed of groups of similarly differentiated cells, similar therefore in form and function. Such groups are known as *tissues*. Histology (Greek, *ἱστός*, 'a textile fabric') is the science of tissues, and histogenesis deals with their origin. There are as many tissues in the body

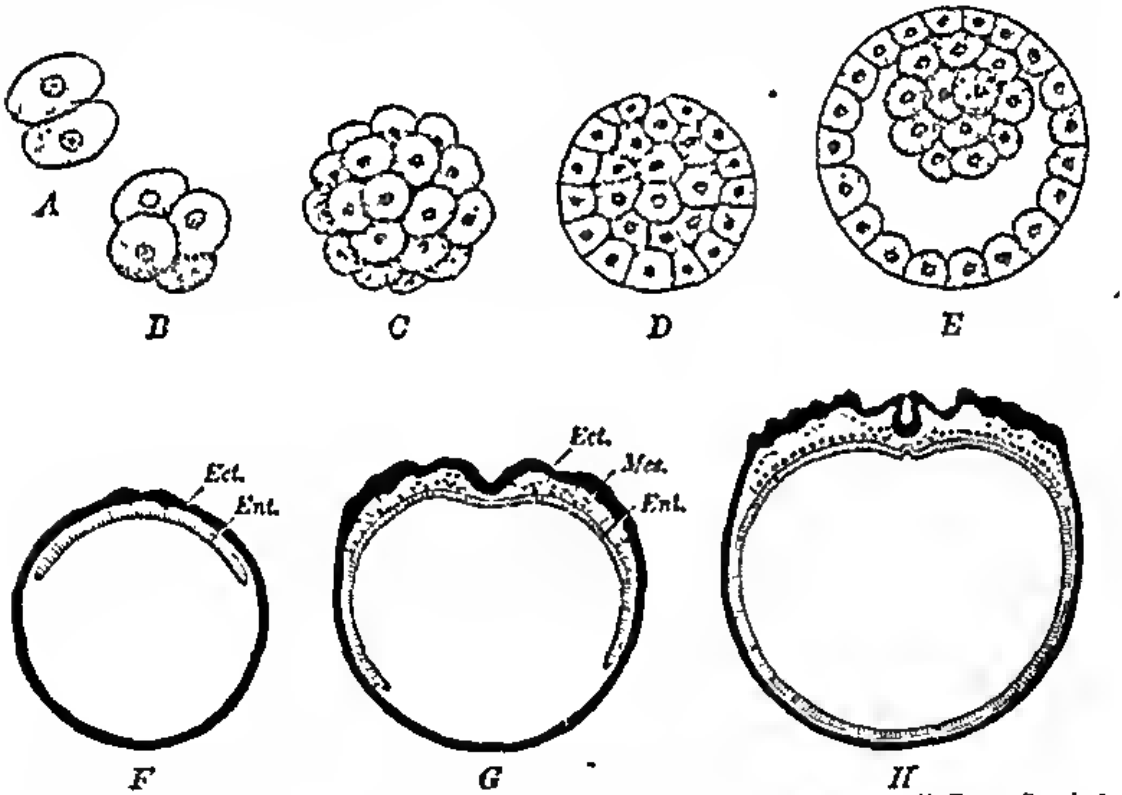


FIG. 32.—SEGMENTATION OF THE OVUM AND FORMATION OF THE GERM LAYERS IN THE RABBIT (A-E, van Beneden; F H, Duval)

A-C represent surface views of the two-cell stage, four-cell stage and morula respectively. D H are vertical sections In D and E the inner cell mass is heavily shaded Ect., ectoderm Ent., entoderm Mes., mesoderm.

as there are 'sorts of substance'; thus the liver consists essentially of hepatic tissue, and the bones of osseous tissue. All of these, however, are modifications of a small number of fundamental tissues, the development of which may now be considered.

It has already been stated that a new individual begins existence as a single cell, the fertilized ovum. This cell then divides by mitosis into a pair of cells, Fig. 32, A; and these again divide, making a group of four,

B. By repeated mitosis a mass of cells is produced, which because of its resemblance to a mulberry, is called a *morula*, C. Development to this point is known as the *segmentation* of the ovum.

A section through the morula of the rabbit is shown in Fig. 32, D. An outer layer of cells surrounds the *inner cell mass*. Soon a cup-shaped cleft, crescentic in vertical section, forms between the outer and inner cells as shown in E, and this cleft enlarges until the entire structure becomes a thin-walled vesicle, within and attached to one pole of which is the inner cell mass, F. Cells from this mass gradually spread beneath the outer layer until they form a complete lining for the vesicle. The slightly different stages in primates which, however, lead to the same results will be described later (p. 541). The inner layer is called *entoderm*, and the outer layer *ectoderm*.

Before the entoderm has encircled the vesicle, a third layer has appeared between the other two. This middle layer is the *mesoderm* (Fig. 32, G). It arises from the place where the ectoderm and entoderm blend with one another. The layers may be separated and floated apart except at this spot where they are 'tied together.' This place is therefore called the *primitive knot*. The mesoderm also spreads laterally from a longitudinal thickening of the ectoderm, which extends backward from the primitive knot and marks out the future longitudinal axis of the embryo. This thickening is the *primitive streak*. Arising from the primitive knot and primitive streak, the mesoderm spreads out rapidly between the ectoderm and entoderm, and splits laterally into two layers (Fig. 32, H). One of them (the *somatic* layer) is closely applied to the ectoderm, and the other (the *splanchnic* layer) to the entoderm. Between them is a cavity, known as the *body cavity* or *cœlom*, which in the adult becomes subdivided into the peritoneal, pleural, and pericardial cavities. The ectoderm and the somatic mesoderm together form the body wall or *somatopleure;* the entoderm and the splanchnic mesoderm together form the intestinal wall or *splanchnopleure*.

Reviewing the preceding paragraphs it is seen that the fertilized ovum, through segmentation, forms a morula, which later becomes a vesicle composed of three *germ layers*, the outer or ectoderm, inner or entoderm, and middle or mesoderm. By the folding of these layers the body as a whole acquires its form; and by their growth and differentiation all the organs and tissues are produced, together with the *fetal membranes* which surround the embryo. Omitting for the present all reference to the membranes, the fundamental changes which the germ layers undergo may be briefly considered, as follows:

Ectoderm. A portion of the ectoderm forms a layer of cells covering the body of the embryo. In the adult this becomes the outer layer of

the skin, or the *epidermis*, and from it, hairs, nails and the mammary, sebaceous and sweat glands develop. It is reflected under the eyelids and over the front of the eye, and forms the lacrimal glands. It extends into the external auditory opening and there forms the ceruminous glands; and into the nasal, oral, anal and urogenital apertures. Within the mouth it forms the salivary glands, the enamel of the teeth, and the cells associated with the sense of taste. Thus it extends far back toward the pharynx, and dorsally, in its deepest part, it produces the anterior lobe of the hypophysis, which will be described in a later section. In the nose it also extends far inward, so that it lines the accessory cavities which push out from the nasal cavity into certain bones of the head, and it forms the olfactory cells. An inpocketing of the ectoderm produces the lining of the deep portion of the ear, including the auditory cells, and, as will be seen, the ectoderm gives rise to the lens and retina of the eye. Thus the ectoderm not only forms the outer covering of the body, with extensions into the several apertures, but it produces various sensory cells which are stimulated from external sources.

The second great derivative of the ectoderm is the nervous system. It arises in young embryos as the *medullary groove*. This is a longitudinal groove or furrow, situated in front of the primitive knot and appearing in cross section as a median dorsal depression (Fig. 32, G and H). Later the groove becomes a tube by the coalescence of its dorsal edges, which are about to unite in H. The tube then becomes completely separated from the epidermal layer of ectoderm, as in Fig. 35.

The closure of the medullary groove to form a tube begins near the anterior end of the embryo and proceeds backward. Thus for a time the tube opens to the exterior both anteriorly, at the *anterior neuropore*, and posteriorly, at the *posterior neuropore*. Eventually the neuropores become closed over, and the tube is then wholly detached from the epidermal layer. The form of the tube is shown in Fig. 33, which represents a dissected reconstruction of a chick embryo. In this dissection the epidermal layer, which covers the upper or dorsal surface of the embryo, has been almost all removed. A portion of it which forms a fold under the head and around the anterior neuropore has been left in place, and also a portion around the *rhomboidal sinus*, which may be regarded as an expanded posterior neuropore. By removing the epidermal layer, the medullary tube has been exposed. Anteriorly it shows a succession of expansions which are to form the brain, and also a pair of lateral outpocketings, or *optic vesicles*, each of which will become the retina of an eye. Posteriorly the tube is slender, and this part becomes the spinal cord. The brain and spinal cord, which are derived directly from the medullary tube, constitute the *central* nervous system. The *peripheral* nervous system

consists of bundles of nerve fibers which ramify throughout the body, together with masses of nerve cells associated with these fibers. The

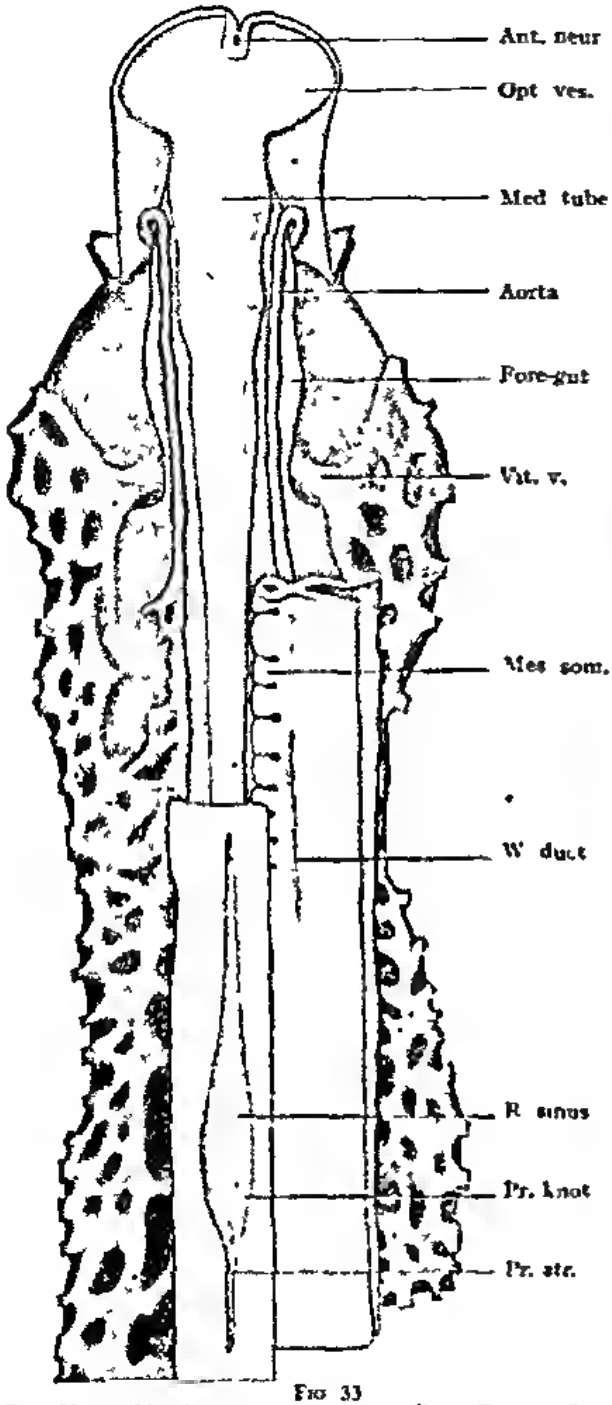


Fig 33

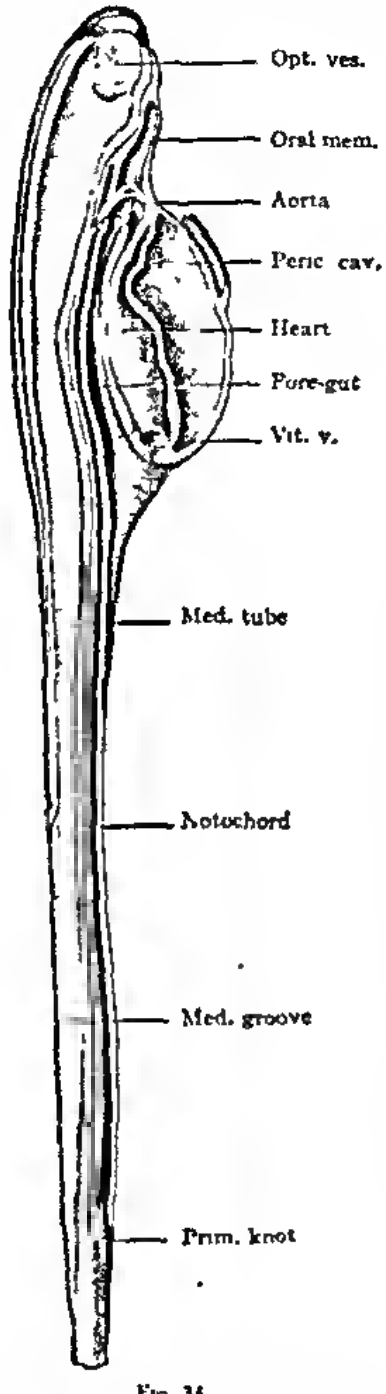


Fig. 34.

FIGS. 33 AND 34.—RECONSTRUCTIONS OF A CHICK EMBRYO, INCUBATED APPROXIMATELY 30 HOURS. × 30

Fig 33 represents a dorsal view. The ectoderm has been removed except around the rhomboidal sinus and under the head. On the left side, all the mesoderm except the blood vessels has also been removed, a portion including nine somites remains on the right side. The lowest layer beneath the vessels, is the entoderm. Fig. 34 is a median sagittal section, except that the entire heart has been included. *Ant. neur.*, anterior neuropore; *Med. groove*, medullary groove, *Med. tube*, medullary tube, *Mes. som.*, mesodermic somite; *Opt. ves.*, optic vesicle, *Oral mem.*, oral membrane, *Peric. cav.*, pericardial cavity; *Pr. knot*, primitive knot, *Pr. str.*, primitive streak; *R. sinus*, rhomboidal sinus; *Vit. v.* vitelline vein, *W. duct*, Wolffian duct.

nerve cells are detached ectodermal cells, arising chiefly from the dorsal part of the medullary groove, and the fibers are protoplasmic outgrowths of these detached cells and of others which remain in the wall of the

medullary tube. Thus the entire nervous system, central and peripheral, is ectodermal in origin.

Entoderm. In young mammalian embryos the entire entoderm forms the lining of a spherical sac, known as the *yolk-sac* (Fig. 32, H). In birds the mass of yolk, which may be regarded as lodged in the thickened ventral wall of the yolk-sac, is so extensive that the cavity of the sac is merely a flattened dorsal cleft. The yolk-sac gives rise to the entire intestinal tube, together with all its outgrowths. They are therefore lined with entoderm, and they develop as follows.

A flattened finger-like extension of the yolk-sac projects forward into the head, under the notochord (Figs. 33 and 34). This outpocketing is the *fore-gut*, which gives rise to the pharynx, œsophagus, stomach, and anterior part of the small intestine. Near its anterior extremity it comes in contact with the ectoderm and fuses with it, thus forming the *oral membrane* or oral plate. By the rupture of this membrane, an opening from the exterior into the pharynx is produced.

Similarly the *hind-gut* develops as a pocket from the posterior part of the yolk-sac. It gives rise to the lower portion of the small intestine and the entire large intestine, and fuses with the ectoderm, forming the *cloacal membrane*. In later stages the ventral part of the posterior end of the hind-gut becomes cut off from the dorsal part; the ventral subdivision forms the bladder, and the dorsal subdivision becomes the lowest part of the rectum. At the same time the cloacal membrane is correspondingly subdivided into the urogenital membrane which closes the outlet of the bladder, and the anal membrane which closes the rectum. Later these membranes rupture, and the line of demarcation between ectoderm and entoderm is then difficult to determine.

In addition to forming the lining of the pharynx and entire digestive tube, together with most of the bladder and its outlet, the entoderm lines the following important organs, which arise as outgrowths of the pharynx and digestive tube: the auditory tube, extending from the pharynx to the ear; the entire respiratory tract, including the larynx, trachea and lungs; and it forms the chief part of the liver, the pancreas, the thyroid gland and certain constituents of the thymus.

Mesoderm. The mesoderm, growing from the primitive streak after the axial structures, medullary groove and notochord (see below) have been formed, spreads forward on either side of these as well as laterally and caudally, lying between the ectoderm and entoderm. Near the medullary groove it is greatly thickened (Fig. 35 corresponding to the upper part of Fig. 32, H), and the thickened part becomes cut into block-like masses by a series of transverse clefts. The masses are called *mesodermic somites,* and a pair of them occurs in each transverse segment

of the body. They increase in number as new ones become cut off from the unsegmented mesoderm in the posterior part of the embryo. At first each somite may contain a cavity, which is an extension of the cœlom, but the cavity is soon obliterated by a plug of cells. In dorsal view some of the somites are shown on the right side of Fig. 33; the rest have been cut away. Except in the region of the somites the mesoderm splits into two sheets, as has already been described, the splanchnic and somatic layers, enclosing the cœlom.

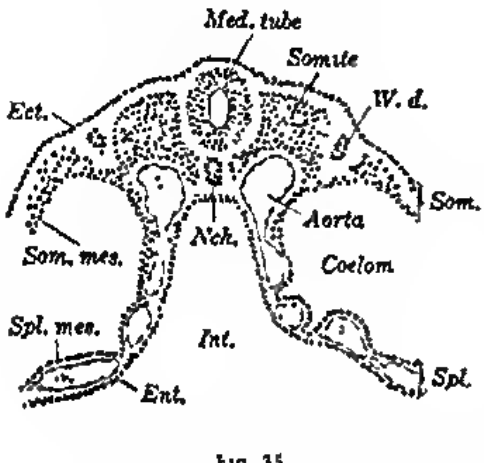


Fig. 35

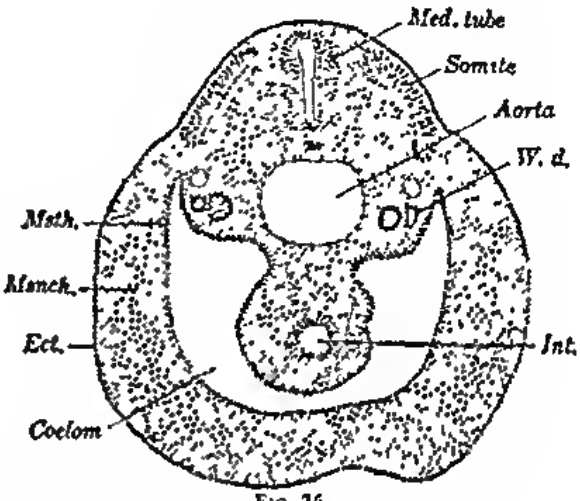


Fig. 36

Fig. 35.—Transverse Section of a Rabbit Embryo Measuring 4·4 mm. (9½ days) × 60
Fig. 36.—Transverse Section of a Rabbit Embryo Measuring 5 mm. (11 days) × 40

Ect., *ectoderm*, Ent., *entoderm*, Int., *intestine*, Med. tube, *medullary tube*, Msnch., *mesenchyma*, Msth., *mesodermal epithelium*, Nch., notochord, Som., somatopleure, Som. mes., somatic mesoderm, Spl., splanchnopleure, Spl. mes., splanchnic mesoderm, W. d., Wolffian duct

In later stages each somite gives rise to a stream of cells which spreads around the medullary tube, notochord and aorta. After these cells have been given off, the somite appears as a plate-like structure (Fig. 36), known as the *dermo-myotome*. The principal derivative of the dermo-myotome is the voluntary musculature of the body. In producing the various voluntary or skeletal muscles, certain cells of the dermo-myotome become transformed into muscle fibers. These are at first arranged in segmental masses, but the masses become subdivided into groups representing the individual muscles. The groups become separated from one another and shift to their final positions. Subsequently they acquire their connections with the bones, which develop later than the muscles. The remainder of the dermo-myotome breaks up into cells which form the deep portion of the skin.

Connecting the somites with the lateral somatic and splanchnic layers of the mesoderm, there is a narrow neck of cells (as seen in cross section, Fig. 35) which is known as the intermediate cell mass, or *nephrotome*. The nephrotomes at first are not segmentally divided, but form the floor of a longitudinal groove in the mesoderm, lateral to the somites. The nephrotomes give rise dorsally to a longitudinal cord of cells, which

later becomes a tube, and is known as the *Wolffian duct*. It lies in the groove above the nephrotomes. The duct grows posteriorly and acquires an opening into the entodermal bladder. The nephrotomes then become separated from the somites and from the lateral layers of the mesoderm, and their cells become arranged so as to form coiled tubes, which empty into the Wolffian duct. In this way the mesoderm gives rise to the renal system, which consists essentially of coiled mesodermal tubes, receiving urinary products from the blood and conveying them through the Wolffian duct to the bladder. Later, parts of the urinary system lose their primary function and become the ducts of the genital system.

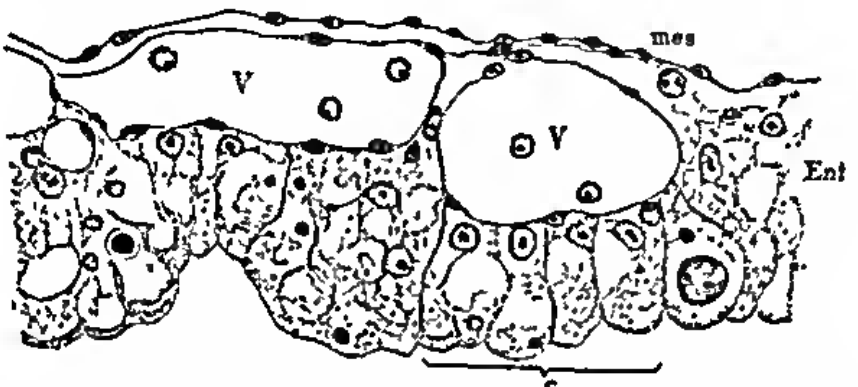


FIG. 37.—WALL OF THE YOLK-SAC FROM A CHICK OF THE SECOND DAY OF INCUBATION. (Minot.)
Mes., Splanchnic mesoderm, Ent., entoderm, four distinct cells of which are shown at c; V, V, blood vessels containing a few young blood cells

The lateral somatic and splanchnic layers of the mesoderm produce the lining of the pleural, pericardial, and peritoneal subdivisions of the cœlom, as already stated. They give rise also to an important tissue known as *mesenchyma*. With the production of mesenchyma the tissues of the embryo may be divided into two sorts, namely, *epithelium* which covers an external or an internal surface of the body, and *mesenchyma* which fills the space between two layers of epithelium. These relations are clearly shown in the cross section of the abdomen (Fig. 36). The body wall consists of a layer of ectodermal epithelium externally, and of mesodermal epithelium internally, with a thick layer of mesenchyma between the two. Similarly the intestinal wall consists of mesodermal epithelium toward the cœlom, and entodermal epithelium toward the intestine, with mesenchyma between them. Epithelium is thus produced by all the germ layers, but mesenchyma is almost exclusively the product of the mesoderm. It is formed not only from the lateral splanchnic and somatic layers of the mesoderm, but also from the somites. The tissue which has been described as spreading from the somites around the medullary tube, notochord and blood vessels, and into the deep portion of the skin, is mesenchyma. It also surrounds the tubules derived from the nephrotome.

Under higher magnification, it is seen that epithelium is a layer of closely compacted cells, but that mesenchyma is a protoplasmic network, the meshes of which are filled with a fluid intercellular substance. If this substance is abundant, the nuclei of the mesenchyma are widely separated; but if it is scanty they are quite close together. Mesenchyma gives rise to a great variety of tissues, including involuntary muscle, adipose tissue, cartilage, and bone. Both the cells and the intercellular substance may become variously modified. The most widespread derivative of mesenchyma is *connective tissue*, which invests the nerves, vessels, muscles and epithelial structures, binding them together in organs, and filling the interstices of the body.

The origin of the blood and blood vessels remains to be considered. In very early stages the vessels appear as cellular strands, some of which contain a lumen, situated between the mesoderm and entoderm. Associated with these strands, but further out on the yolk-sac, there are clusters or 'islands' of blood cells, surrounded by a thin layer of flattened cells known as *endothelium*. The entire system soon forms a network of distinct vessels situated in the splanchnopleure. Generally the vessels and the corpuscles within them are considered to be mesodermal, but some authorities have regarded them as entodermal, and others have proposed to describe them as forming a separate germ layer or 'angioblast' (more appropriately *angioderm*).

In the chick embryo shown in Figs. 33 and 34, the network of vessels in the splanchnopleure has formed a complete circulatory system. By a process of folding, portions of the net have been brought together under the fore-gut, where the vessels from the two sides have fused and formed a single median tube, the heart. The two large trunks, derived from the network, which convey the blood from the yolk-sac to the heart are known as *vitelline veins*. The heart divides anteriorly into two vessels (the *aortæ*) which pass from the under side of the fore-gut to the upper side, and then extend posteriorly. They finally connect by branches with the network over the yolk, from which they have been derived. Through this system, nutriment taken from the yolk is brought to the heart by the vitelline veins, and distributed throughout the body by the aortæ.

In mammals also, a complete system of vessels is established early in development. Whether all later vessels arise as branches of this primary endothelial network, or whether they are formed by the coalescence of mesenchymal spaces, or by transformation of mesenchymal cells into endothelial cells is discussed later. There is a very close connection between the endothelium and the surrounding mesenchyma.

The histogenesis of the blood is likewise very difficult to follow. The simplest interpretation is that all forms of blood corpuscles are descend-

ants of the cells found in the blood islands of the yolk-sac. According to this hypothesis these cells multiply in certain places to which they have been carried by the circulating blood, for example, in the liver in later embryonic life and in the bone marrow of the adult; and they differentiate into the red and white corpuscles of various kinds. The difficulties which this hypothesis encounters will be discussed in later sections.

The *notochord* is a longitudinal rod extending forward from the primitive knot to the under side of the brain. It has been described as coming from every one of the germ layers. It arises from the primitive knot, a region where all layers are blended, and as it grows forward may fuse with one or other in different classes of embryos. In the diagram, Fig. 32,

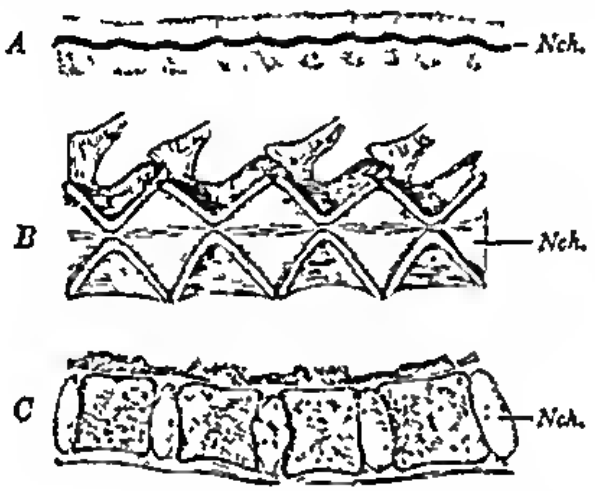


FIG. 38.—THE NOTOCHORD

A, in a sheep embryo of 14·6 mm (after Minot). B, in a cod fish. C, in man (Dwight).

H, it is shown as an elevation of the entoderm; in Fig. 35, it appears as a group of cells completely detached. Later the rod becomes surrounded by mesodermal cells, which develop into the bodies (or centra) of the vertebræ together with the intervertebral ligaments between them. These are shown in Fig. 38, A, as alternating light and dark areas respectively. The notochord in passing through them shows 'segmental flexures.' In the vertebral column *of a fish (Fig. 38, B) the central notochordal rod has* expanded between the bodies of the vertebræ so as to form large lenticular masses of gelatinous pulp. These retain a very slender connection with one another. In the human adult, the notochord is represented by the series of detached expansions, or *nuclei pulposi*, one of which occurs in each intervertebral ligament (Fig. 38, C). These nuclei are composed of a peculiar tissue, the development of which has been described by Williams.[1] The notochord is very rarely the source of tumors. Occasionally, owing to its connection with the entoderm, which is retained longest anteriorly, it gives rise to a pharyngeal recess.[2]

[1] WILLIAMS, L.W., 1908. [2] HUBER, 1912.

THE FUNDAMENTAL TISSUES

From the foregoing outline of embryological development, it is clear that all the organs of the body are derived from a relatively small number of fundamental tissues. After the fertilized egg has segmented, it gives rise to layers of cells, of which the ectoderm and entoderm are epithelial from the beginning. The mesoderm very early divides into two tissues—epithelium, which lines the body cavity, and mesenchyma, which forms the internal substance of the body wall and intestinal wall. Thus *epithelium* and *mesenchyma* may be regarded as the primary tissues of the body. The groups of blood corpuscles, which are probably derived from the mesenchyma, and the endothelium which surrounds them, also arise very early, and these may be set apart as *vascular tissue*.

The nervous system develops from epithelium, but its cells, singly or in groups, become inbedded in strands and masses of nerve fibers which these same cells send out as processes. Thus little remains in the adult to suggest that the brain or peripheral nerves come from a layer of cells covering a surface, and the nervous system is therefore described as consisting of *nervous tissue*.

The voluntary muscles are formed from cells derived from the epithelium of the mesodermic somites, but they develop as the somite breaks up and its epithelial character is lost. The involuntary muscles are produced by a transformation of mesenchymal cells into elongated muscle cells. For physiological reasons these two kinds of muscle, which are of diverse origin and structure, are classed together as *muscular tissue*.

The relation of the germ layers to the five fundamental tissues which have now been recognized is shown in the following summary.

ORIGIN OF THE TISSUES FROM THE GERM LAYERS

The ectoderm produces:

1. Epithelium of the following organs:—the skin (epidermis) including the cutaneous glands, hair and nails; the cornea and the lens; the external and internal ear; the nasal and oral cavities, including the salivary glands, the enamel of the teeth and anterior lobe of the hypophysis; the anus; the cavernous part of the male urethra; together with that epithelium of the chorion which is toward the uterus and of the amnion which is toward the embryo.

2. Nervous tissue *forming the entire nervous system, central, peripheral and* sympathetic.

3. Muscular tissue, rarely, as of the sweat glands, skin and iris.

The mesoderm produces:

1. Epithelium of the following three sorts: (1) epithelium of the urogenital organs (except most of the bladder and the urethra) and the epithelioid cords of cells in the suprarenal gland; (2) epithelium of the pericardium, pleuræ, and peritoneum and the continuation of this layer over the contiguous surfaces of amnion and chorion; (3) epithelium lining the heart, blood vessels and lymphatic vessels.

2. Muscular tissue, striated (voluntary), cardiac, and smooth (involuntary).

3. Mesenchyma, an embryonic tissue, which forms, in the adult, connective and adipose tissue, bone (including the teeth except their enamel), cartilage, tendon, and various special cells.

4. Vascular tissue, the cells of the blood and lymph, consequently the essential elements of the lymph glands, red bone marrow and spleen.

The entoderm produces:

1. Epithelium of the following organs:—the pharynx, including the auditory tube and middle ear, thyroid gland and thymus; the respiratory tract, including larynx, trachea, and lungs; the digestive tract, including the œsophagus, stomach, small and large intestine, rectum, liver, pancreas, and the yolk-sac; and part of the urinary organs, namely most of the bladder, most of the female urethra, and part of the male urethra (including the prostate).

In the following pages the fundamental tissues will be considered in turn. In connection with them, certain *organs* will be examined. An organ is a more or less independent portion of the body, having a connective tissue framework, and a special blood, lymph, and nerve supply, in addition to its characteristic essential cells. The essential cellular substance of an organ, in distinction from the accessory tissues, is often called its *parenchyma*, the accessory supporting tissues constitute the *stroma* (Gr. *στρῶμα*, bed), in which the parenchyma is embedded.

Such structures as the pancreas and liver are obviously organs. An individual muscle or a particular bone, which has a connective tissue covering or framework, and a supply of vessels and nerves, besides its essential substance, may also be regarded as an organ. The organs which are of widespread occurrence, such as the bones, muscles, tendons and large vessels, will be described with the tissues. The more complex organs are reserved for a later section, entitled 'Special Histology.'

EPITHELIUM

The Dutch anatomist, Frederik Ruysch, recognized that the covering of the margin of the lips is not identical with the epidermis. 'Therefore,' he wrote, 'I shall call that covering the *epithelis*, or papillary integument of the lips.'[1] It is an unfortunate name (*ἐπί*, epi, upon, *θηλή*, thele, Latin *papilla*, the nipple) since it does not refer to the layer upon the nipple, but to that which covers a great number of nipple-like elevations of the underlying tissue. Such elevations or *papillæ* are indeed abundant in the lips, but they occur also under the epidermis. Ruysch substituted *epithelia* for *epithelis* in other sections of his work, and Haller,[2] writing some years later, used the neuter *epithelium*, so that epithelia thus became a plural.

[1] Ruysch, 1703. Thes. anat. III, No. 23, and Tab. 4, fig. 1. 'epithelis'; Thes. anat. VI. No. 115, 'epithelia.'

[2] Haller, 1763. *Elementa physiologiæ*, T. 5, p. 10, 'epithelium.'

As the term epithelium is now used, it includes the epidermis and the lining of the various internal tubes and cavities. It has been defined as a layer of closely compacted cells, covering an external or internal surface of the body, having one of its surfaces therefore free, and the other resting on underlying tissue. But the term is also correctly applied to solid outgrowths from such layers, either in the form of cords or masses of cells. Usually these outgrowths subsequently acquire a cavity, or lumen, around which the cells become arranged in a layer.

The epithelia which cover the skin and line the digestive tube and urogenital organs are thick, as compared with those which line the body cavity, the heart, the blood and lymphatic vessels. For these thin layers, His (1865) introduced the term *endothelium*. He wrote as follows:

> We are accustomed to designate the layers of cells which cover the serous and vascular cavities as epithelia. But all the layers of cells which line the cavities within the middle germ layer have so much in common, and from the time of their first appearance differ so materially from those derived from the two peripheral germ layers, that it would be well to distinguish them by a special term—either to contrast them, as false epithelia, with the true, or to name them *endothelia*, thus expressing their relation to the inner surfaces of the body.

The name endothelium, etymologically absurd because it implies 'within the nipple,' has become generally accepted for the lining of the heart, blood vessels and lymphatic vessels. For the other forms of epithelium which it was intended to include, special names have been proposed.

Minot[1] (1890) introduced *mesothelium* to designate the layer of mesodermal cells which bounds the body cavity. Thus mesothelium does not include the endothelium of the vessels, but it does include the cells of the nephrotome, through which the body cavity may extend, and also the epithelium which bounds the somites in early stages. Minot applied the term also to the thick epithelium of the renal organs, which is derived from the cells of the nephrotome.

The lining of the vessels closely resembles that which lines the body cavities, and to a certain extent this justifies the use of the term endothelium for both layers as proposed by His. But it has been shown embryologically that the vessels and body cavity are of different origin, and are distinct even in the earliest stages. In a third special group is included the linings of the subdural and subarachnoid spaces, the perilymphatic space of the internal ear and the chambers of the eye which arise relatively late in development by the confluence of intercellular spaces in mesenchyma.

[1] Minot, C. S., 1890, Amer. Nat., vol. 24, p. 877–898. "Two large cavities appear in this mesoderm on either side of the median axial line. The mesodermic cells which bound these two cavities assume an epithelial arrangement and are designated *mesothelium*."

These spaces are bounded by a continuous layer of flattened cells derived from mesenchyma, to which is applied the term mesenchymal epithelium (secondary or false epithelium). Some histologists extend the term mesenchymal epithelium to include the linings of joints, bursæ and tendon sheaths, although the majority regard these as lined with flattened, separated, connective tissue cells.

In accordance with these embryological facts, the following use of terms is here proposed:

Endothelium should be restricted to the lining of the heart, blood vessels and lymphatic vessels.

Mesothelium, except in young embryos, should be restricted to the lining of the body cavity and its subdivisions.

Mesenchymal epithelium (or false epithelium) should be applied to the lining of the subdural and subarachnoid spaces, the perilymphatic space and the chambers of the eye.

All of these special forms of epithelium are primarily thin and are derived from the mesoderm. Each form, however, is functionally different. Endothelium while originating from mesenchyma becomes in later stages of embryonic development and in the adult what appears to be a highly differentiated tissue which on proliferation produces cells of its own kind. On occasion, as in inflammation and in tissue cultures, endothelium is stated to revert to a fibroblastic-like state. Mesothelium appears more like a typical flat or squamous epithelium; on its free surface is a condensation or crust (*crusta*), sometimes having the character of a brush border or possessing flagellæ. Mesothelium may become thickened and the cells appear as cuboidal or even columnar, often leading to cysts and tumor formation. Mesenchymal epithelium remains structurally undifferentiated and under the influence of stimulation the cells may react by budding off as free cells. Thick epithelium may be ectodermal, entodermal or mesodermal in origin.

Epithelia differ from one another, not only in origin, but also in the shape of their cells, the number of layers of which they are composed, and the differentiation of their cells. These features should be examined in every specimen studied, and something under each heading should be recorded in any complete description of an epithelium.

Syncytium. In embryonic and other rapidly developing tissues mitotic division may proceed so rapidly that the last step in the process, the formation of new cell walls, may be incomplete when the next nuclear division is initiated. The nuclei continue to divide, but the cytoplasm remains undivided. The result is called a syncytium, 'cells run together.' Occasionally a syncytium may result from the coalescence of older cells also. It may be an irregular mass, or in the form of sheets of tissue or

epithelia. The epithelia of young embryos are frequently syncytial. The spacing and shape of the nuclei indicate the forms which the cells would have taken, if completed by visible cell boundaries. In older embryos and in the adult epithelial cells are usually distinct entities and of definite shape and arrangement.

SHAPES OF EPITHELIAL CELLS AND THE NUMBER OF LAYERS

An epithelium which consists of but one layer of cells is called a *simple epithelium*, and its cells may be *flat*, *cuboidal* or *columnar*. These terms refer to the appearance of the cells when cut in a plane perpendicular to

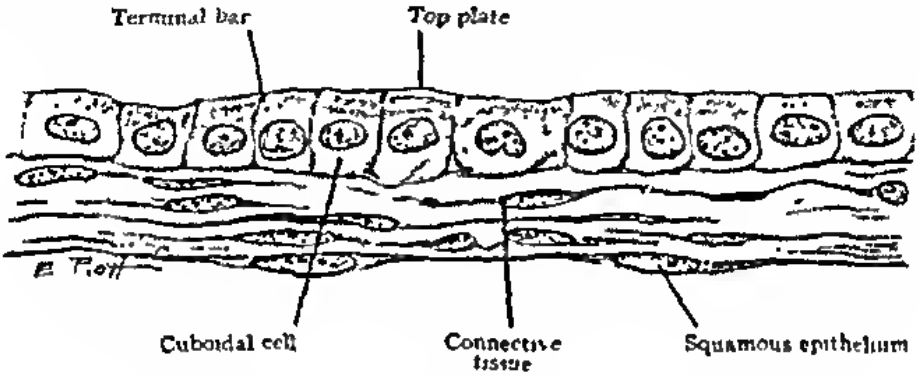


Fig. 39.—Portion of Membranes Surrounding a Pig Embryo Measuring 60 mm. (Allantois above and amnion below) Zenker fixation, hæmatoxylin and eosin

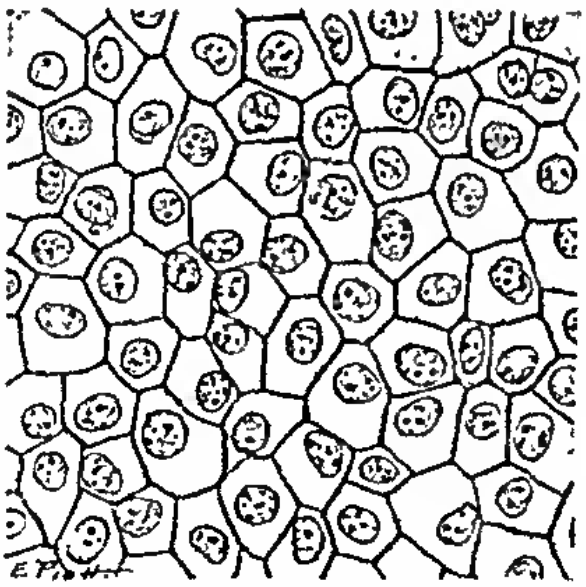


Fig. 40.—Surface View of Allantois from a Pig Embryo of 60 mm. Zenker fixation; hæmatoxylin and eosin

the free surface. If in such a section the outlines of the cells are approximately square, as along the upper surface in Fig. 39, the epithelium is cuboidal; if they are stretched out in a thin layer so that they appear linear, as along the lower surface in Fig. 39, the epithelium is flat. Endothelium is an extremely flat epithelium, in which the cells are so thin that

the nuclei cause local bulgings of the cell membrane. If the epithelial cells are laterally compressed, so that tall forms result, the epithelium is columnar. Such epithelium is less accurately called cylindrical and both cuboidal and flat epithelia are sometimes referred to as pavement epithelium. Intermediate forms, which are described as low columnar or low cuboidal, frequently occur. When lining a small tube, as in some glands, the individual cells of a simple epithelium have the shape of a truncated pyramid. The base of a cell is usually hexagonal, while the free surface, depending upon whether it is smooth or projects into the lumen of the tube is hexagonal or dome-shaped. The cells of certain epithelia change their shape temporarily as in the bladder during disten-

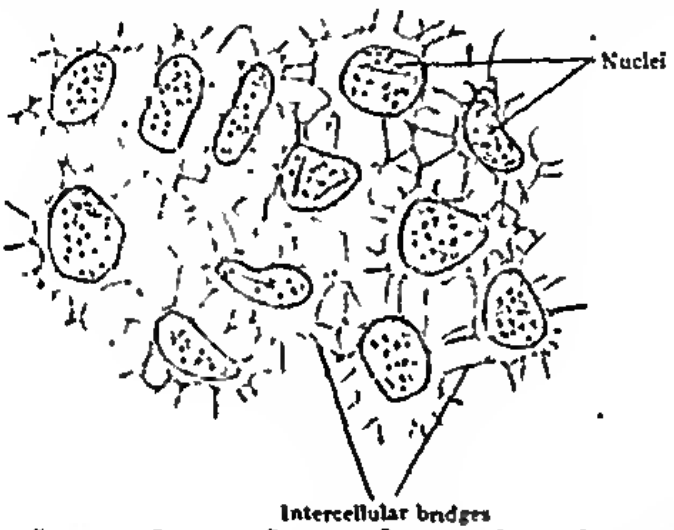


FIG. 41.—FLAT EPITHELIAL CELLS FROM BRANCHIAL PLATE OF A LARVAL SALAMANDER. × 300

sion and in the arteries with every pulsation. During the repair of a denuded surface, the epithelial cells may change from one form to another. A number of investigators have shown that after irritation the simple epithelium lining the trachea and bronchi may become stratified. Wolbach and Howe[1] recorded that the earliest demonstrable effect of vitamin A deficiency is upon epithelium. Rats kept on an A deficient diet for sixty days and longer showed a keratinizing metaplasia of the epithelium in the eye and associated glands, salivary glands, respiratory and urogenital tracts. Upon giving vitamin A the metaplastic epithelia returned to the normal type.

On surface view or when sectioned parallel to the free surface, the epithelial cells of all types appear polygonal, and usually six-sided (*Fig.* 40). Geometrically a circle would come in contact with six surrounding circles of equal diameter, and a cell is usually in contact with six surrounding cells. The cells, however, vary in diameter, and are often surrounded by five or seven cells and occasionally by four or eight. The

[1] WOLBACH AND HOWE, 1933.

mathematical rules governing the shapes of cells in a single sheet, as they cover protuberances or line minute tubes and their branches, and as they multiply by division are given by F. T. Lewis.[1]

The vertical shape which an epithelial cell is to assume, as distinct from the surface mosaic pattern, depends on opposing forces—(1) the tendency to be spherical, as is shown when a young cell, or one without definite exoplasmic differentiation, is allowed to float freely (amœbæ or white blood cells); (2) the tendency of a fluid drop to flatten along a surface, like a drop of oil as opposed to a drop of mercury; and (3) the pres-

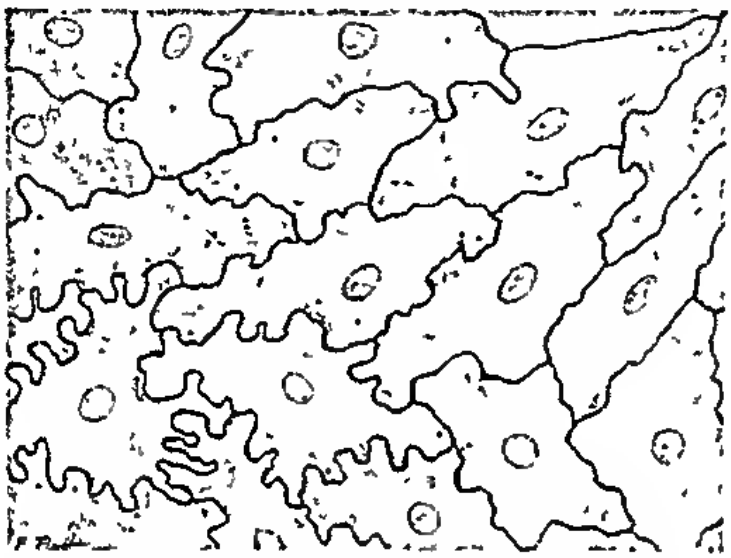


FIG. 42.—MESOTHELIAL CELLS, MESENTERY OF A CAT
At left cell outlines are serrated, at right cells are stretched and outlines more regular. Silver nitrate impregnation

sure of neighboring cells, frequently due to their rapid growth and multiplication. In the surface epithelium of a chick embryo of 96 hours incubation (Figs. 43 and 44) the results of all of these forces can be studied. In certain regions of the surface, the epithelium of the skin is composed of a single layer of flat, or squamous cells, few in number and each covering a large area; in other parts the epithelial cells are cuboidal. The cells are often only partially separated by membranes; *i.e.*, they form a syncytium. This fact, and the frequent mitotic figures seen, show that the tissue is growing rapidly. More cells cover a given area, and each cell can assume a shape more nearly spherical. Further rapid increase of cells must lead by compression to columnar forms, or if the cuboidal cells resist compression, some of their number must be forced upward out of line, making a second layer. Here the cells at first spread out flat on the new surface, but as more and more of them are crowded out of line the new surface cells become cuboidal or columnar, and a third layer may be formed of cells forced out as before. In older embryos

[1] LEWIS, F. T., 1928 and 1933a.

and in the adult many layers may be present, as shown in Fig. 46. Such an epithelium is called stratified. The basal layer alone rests on the underlying tissue. The cells show the cytomorphosis which they undergo in their journey from the basal layer to the surface. In the intermediate region they enlarge and become polygonal. Their actual shape has been

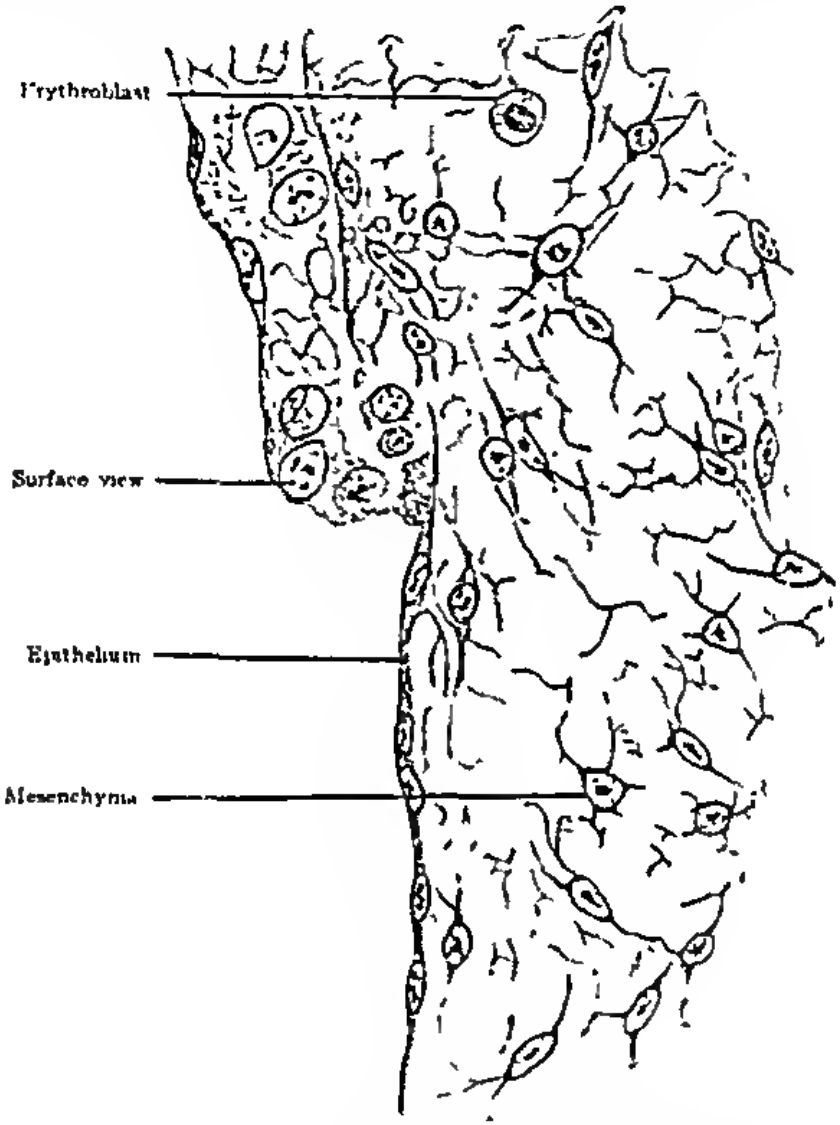


Fig. 43.—Skin and Underlying Mesenchyma of a Chick Embryo of 96 Hours Incubation
The epithelium is simple squamous, and a wrinkle brings part of it into the plane of section, so that it is seen in surface view

studied by F. T. Lewis[1] who finds them fourteen-sided, a shape which could be predicted mathematically as giving the smallest surface area in closely crowded bodies. Toward the surface the cells become squamous, and may be more or less cornified, in which condition they are cast off. In positions in the body where there is much friction, as on the skin and in the œsophagus, and where, therefore, there is a great loss of surface cells, the basal layer rests on projections or papillæ of the underlying

[1] Lewis, F. T., 1925.

tissue, which greatly increase the under surface of the epithelium, and so provide for more basal cells to divide and renew the upper layers. Mitotic figures are not confined to the basal layers, however, and new cells are being added at other levels.

As is pointed out on p. 31, increased cell size usually leads to cell division. In an epithelium, therefore, the large cells are the ones which

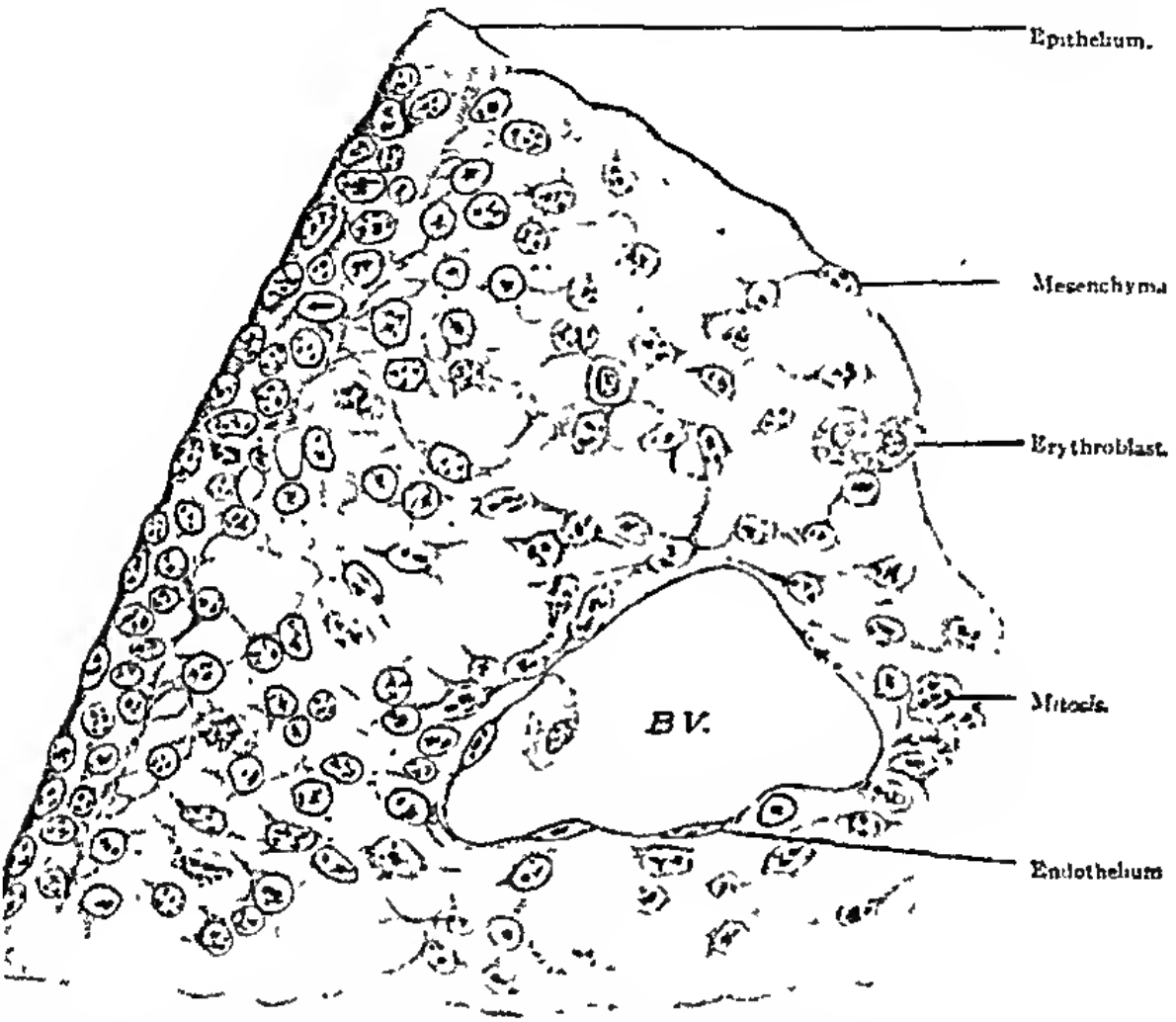


Fig. 44.—Another Part of the Chick Embryo Shown in Fig. 43.
The epithelium has become two or three layered, but still syncytial. The top cells tend to flatten out on the surface.

divide, and in general the large ones are those with many sides. If, in sections, the average number of sides of resting cells is 6, the average number of sides of the dividing cells is 7; and the volume of the resting cells (in the epidermal epithelium of the cucumber) as compared with that of the dividing cells has been found to be as two to three. Stratified epithelium is found in the oral cavity, pharynx, œsophagus and vagina; and in its most complex form with some layers peculiarly modified, it constitutes the epidermis.

In certain organs, and especially in embryos, simpler forms of stratified epithelium occur, which are described as two-layered four-layered,

or *six layered*. Such *epithelia are seen in parts of the excretory ducts of* the testis, the ducts of some glands, fornix conjunctivæ and cornea. The

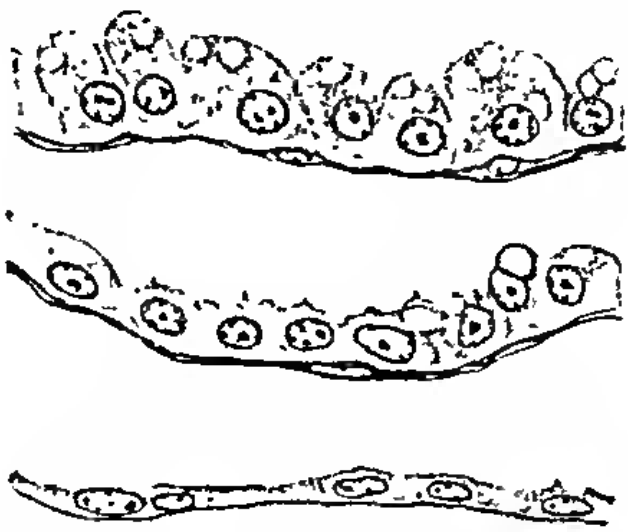


Fig. 45.—Epithelial Cells, Mammary Gland of a Dog.
Three different heights of the cells depending upon stretching of the alveoli by accumulation of milk. Susa fixation. Azan.

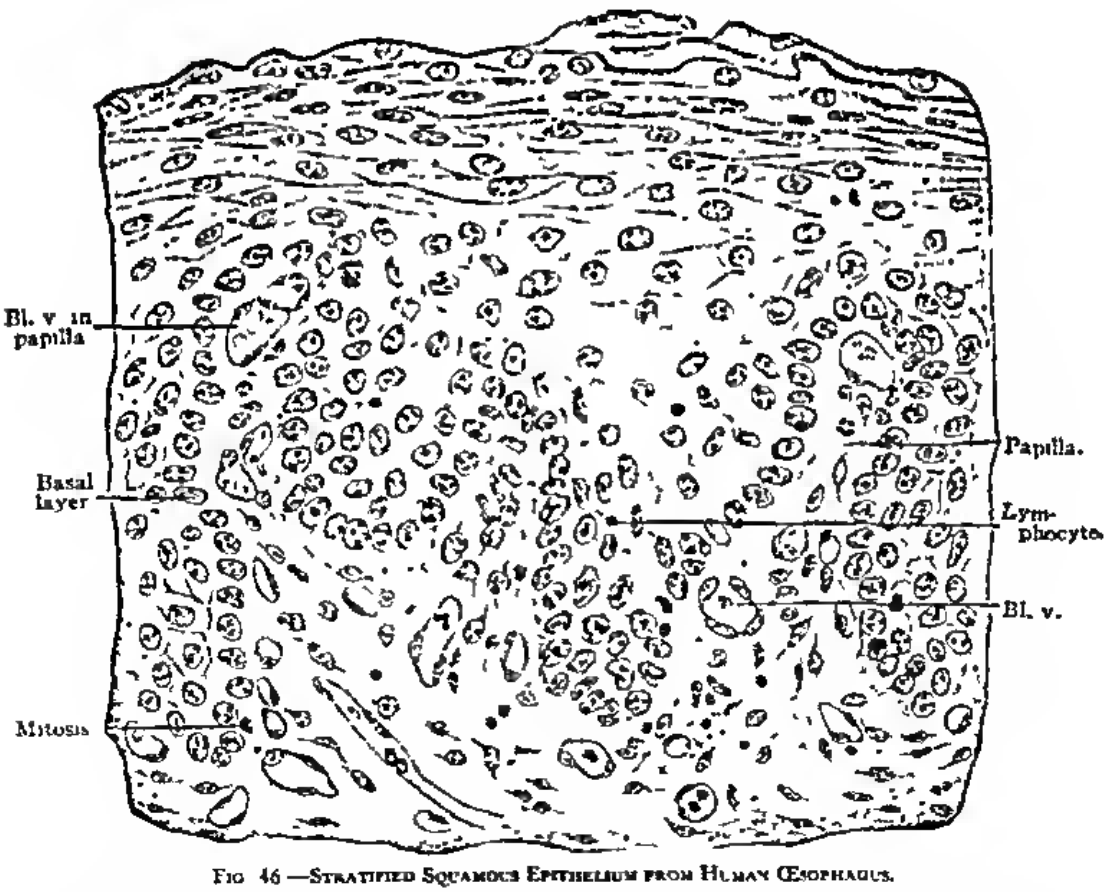


Fig. 46.—Stratified Squamous Epithelium from Human Œsophagus.

superficial cells may be flat, cuboidal or columnar. On the third eyelid of the cat, Koch (1904) described a four-layered epithelium with mucous cells in the second layer under the name 'mixed epithelium.'[1] A some-

[1] Krause's term, 1842.

what similar epithelium occurs on the caruncula lacrimalis of man. A characteristic epithelium with dome-shaped outer cells and tall basal cells, found in the bladder and ureter, is known as 'transitional epithelium.' This term, introduced by Henle (Allg. Anat., 1841) as a designation for epithelia which are intermediate between stratified squamous and simple columnar, is now generally restricted to the

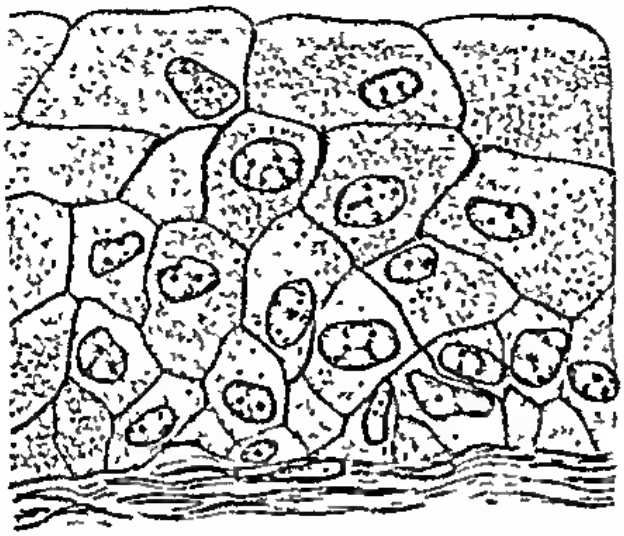


FIG 47.—CONTRACTED BLADDER EPITHELIUM OF DOG
Zenker fixation, hæmatoxylin and congo red, × 750 (Harvey—courtesy of Wistar Institute)

peculiar epithelium of the bladder, ureter and renal pelvis. Transitional epithelium seems specially adaptable to changes due to stretching. When the bladder contracts the cells are heaped up in several layers, but when distended the number may be reduced even to two.

If the cell outlines are indistinct and the sections are thick or oblique, the number of layers in an epithelium may be very difficult to determine.

FIG 48.—DISTENDED BLADDER EPITHELIUM OF DOG.
Zenker fixation, hæmatoxylin and congo red, × 750 (Harvey—courtesy of Wistar Institute)

Thus in a simple epithelium the nuclei may be at different levels, and if the section is not vertical it will show several layers, approaching the condition of the tangential section. In a form known as *pseudostratified*, all the cells reach the underlying connective tissue, but only a limited number extend to the free surface. Its origin as the result of lateral pressure on simple cuboidal epithelium can be readily imagined. Pseudostratified epithelium occurs in the upper part of the respiratory tract, including the trachea and larger bronchi, parts of the ducts of the parotid and submaxillary glands, and the male and female urethra.

PERIPHERAL DIFFERENTIATION OF EPITHELIAL CELLS

Free Surface. The free surface of epithelial cells is often provided with a thickening of varying dimensions, as surface films (crustæ), cuticulæ, striated or brush borders. Under high magnification the cuticular border of the columnar cells in the intestine is seen to be vertically striated, and these striations have been interpreted as minute canals through which protoplasmic processes may be sent out beyond the free surface. In some cases, however, the striated cuticula appears to consist merely of short, parallel protoplasmic rods. In certain cells of the kidney, the rods may become somewhat divergent, giving rise to what is known as the 'brush border' (see p. 464). Longer processes, which are vibratile but not retractile, are called *cilia* (the Latin term for eyelashes). They project from the free surface of certain epithelial cells in the trachea and bronchi (Fig. 49), in the uterus and uterine tube, and in the nasal part of the pharynx together with the auditory tube and naso-lacrimal duct which open into it. Single cilia, or flagella, have been described in certain cells of the thyroid gland in the dog-fish (Cowdry[1]) and in the squamous cells lining the body cavities (Walter[2]), but their significance has not been determined. In the living condition the motion of cilia may be observed in various unicellular animals. It may be studied advantageously in fragments from the margin of the gills of a clam, or in epithelium from the roof of the mouth of a frog. The cilia are numerous, and in the snail Heidenhain counted 110 arising from a single cell. They do not act together, but rapidly succeeding waves, due to the bending of the cilia, pass over the entire surface. By bending sharply downward, each cilium creates a forward current in the overlying fluid, and passes the particles above it to the cilium in front. No sooner does a cilium begin to bend than the next in front takes up the movement and thus the ciliary waves are propagated. In some animals, however, the wave

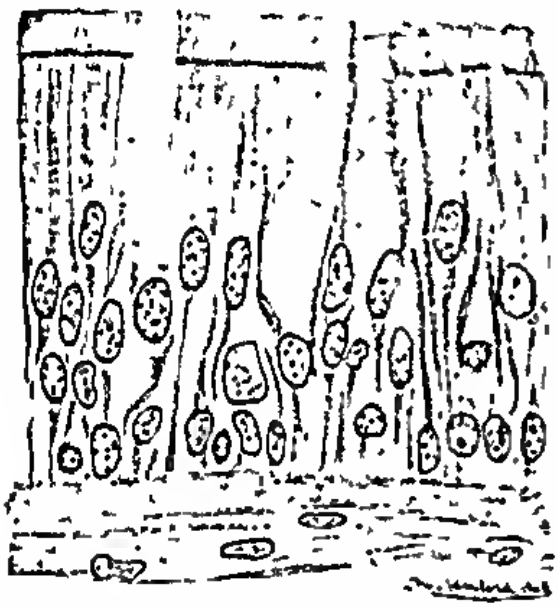


Fig. 49.—Pseudostratified Columnar Ciliated Epithelium, Human Trachea.

Three or more goblet cells are present. The cell walls are indistinct but at the left one cell is cut full length, from the dense basement membrane to the ciliated surface. Basal cells which do not extend to the surface may be recognized.

[1] Cowdry, 1921.

[2] Walter, 1926*a*

proceeds in a direction opposite to that of the effective stroke. The cilia in man produce currents toward the outlets of the body.

Many cilia, however, appear to contain more or less solid axial rods, which generally proceed from round basal bodies resembling centrosomes, and said to be derived from them by fission (see p. 22). The kinetic activity of the centrosome would thus be directed toward the movement of the cilia. As a corollary to this theory Kindred (see p. 31) finds that ciliated cells may divide either by amitosis or by mitosis, but in the latter case the cilia usually disappear. The inference is that the basal bodies have been withdrawn to act as centrosomes in initiating mitosis. Sometimes the bodies are vertically double, and fibrillar extensions from them downward into the cytoplasm may occasionally be observed.

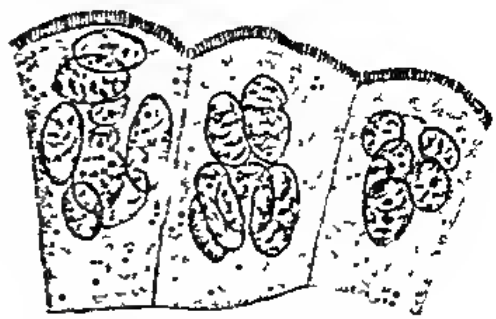


Fig 50.—Multinucleate Cells and Brush Border.
Decidua of Rabbit. (Heidenhain.)

The whip-like processes, or *flagella*, which form the tails of spermatozoa, may be compared with single cilia. Each springs from a body resembling a centrosome, and consists of an axial filament with a surrounding sheath, but whether the filament or the sheath contains the contractile substance is still uncertain.

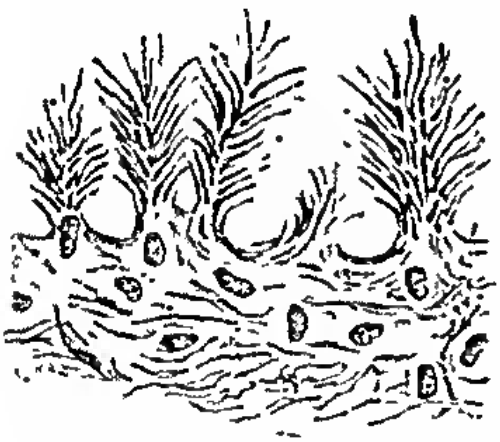


Fig 51.—"Feathered" Epithelium Nictitating Membrane of Pigeon.
Maximow fixation, Bielschowsky × 1200 (Siebert)

Non-motile projections, somewhat resembling cilia, are found in the cells of the epididymis, and of the ductus deferens. They have no basal bodies, and lack the distinctness of true cilia. Generally they appear in conical clumps, which have been compared to the hairs of a wet paint brush. They may be concerned with the discharge of secretion. Other non-motile processes of epithelial cells are the tapering projections of the sensory cells, apparently designed to receive stimuli. The lining of the central cavity of the spinal cord and ventricles of the brain is also provided with short projections, which may be degenerating cilia. It is questionable whether these are motile.

Lateral Surface. The lateral surfaces of epithelial cells may be in close contact with one another, sometimes without intervening cell outlines; or they may be separated by a thin layer of intercellular substance, which is generally fluid. In certain types of epithelia this layer is bounded

at the free surface by an inconspicuous surface film, but in epithelia with prominent cuticulæ definite *terminal bars* close the intercellular spaces, making a network with hexagonal meshes around the individual epithelial cells, just below the cuticula. The terminal bars, which are recognizable by their differential staining, may be the indication of a specially modified surface film where intercellular substance comes in contact with the contents of a tubular organ, as in the intestine; or they may represent the a posed flanges or collar of adjacent cells. The latter is suggested by the fact that perforations have been described in the bars, sometimes so numerous as to leave mere intercellular bridges of the material.[1] In sections perpendicular to the epithelial surface terminal bars may appear either as heavy dots between the cells just below the cuticulæ (Fig. 55, C), or, if the flat surface of a cell is in the optical plane, as a dark line in the same position, visible only at a certain optical focus. They have been found in many epithelia, especially in mucous membranes and glands.

Fig. 52.—Mesothelial Cells of Serous Membrane Seen in Vertical View, Showing Protoplasmic Intercellular Bridges. (Heidenhain)

Between the cells of an epithelium is a fluid or viscous intercellular substance. v. Recklinghausen (1862) gave the name 'cement substance' to it from a study of silver impregnations of flat epithelia. While the term cement substance is still often used, some histologists have doubted its existence. Merkel (1908) referred to it as a 'mythical substance.' He believed that in impregnations of the intercellular spaces, silver nitrate acted upon proteins and became precipitated between the cells.

In the lower layers of the epidermis and the thick oral epithelium, the intercellular substance is clearly seen, and here it is bridged by spiny processes from the adjacent cells. These *intercellular bridges* occur in endothelium and many forms of epithelium, but they are most readily observed in the deep layers of the thick stratified epithelia. Within the bridges, fibrils pass from cell to cell. Chambers[2] doubts the existence of such bridges in living tissues, regarding them as 'either artefacts or indications of a fibrous structure of the intercellular cement or a combination of both.' But F. T. Lewis[3] has shown that they may be the result of the formation of tiny vacuoles between the adjoining cells, which by their partial coalescence leave protoplasmic bridges. In Fig. 53 these may be seen in profile view, and in cross section, when the cell is cut along one of its surfaces. In the intercellular spaces, nutrient fluid makes its way to the outer layers. Whatever nutriment they receive must be

[1] Chlopkow, 1928. [2] Chambers, 1924. [3] Lewis, F. T., 1933b.

derived from the intercellular fluid or through the bodies of the underlying cells. Usually epithelium lacks blood vessels, but their presence has been established in certain epithelia. The classical example of blood vessels in epithelia is in the stria vascularis ductus cochlearis of man described by Kölliker in 1852. A number of histologists have reported blood vessels in the epithelium of the skin and oral mucous membrane in some amphibians, the enamel organ of mammalian teeth, nasal cavity

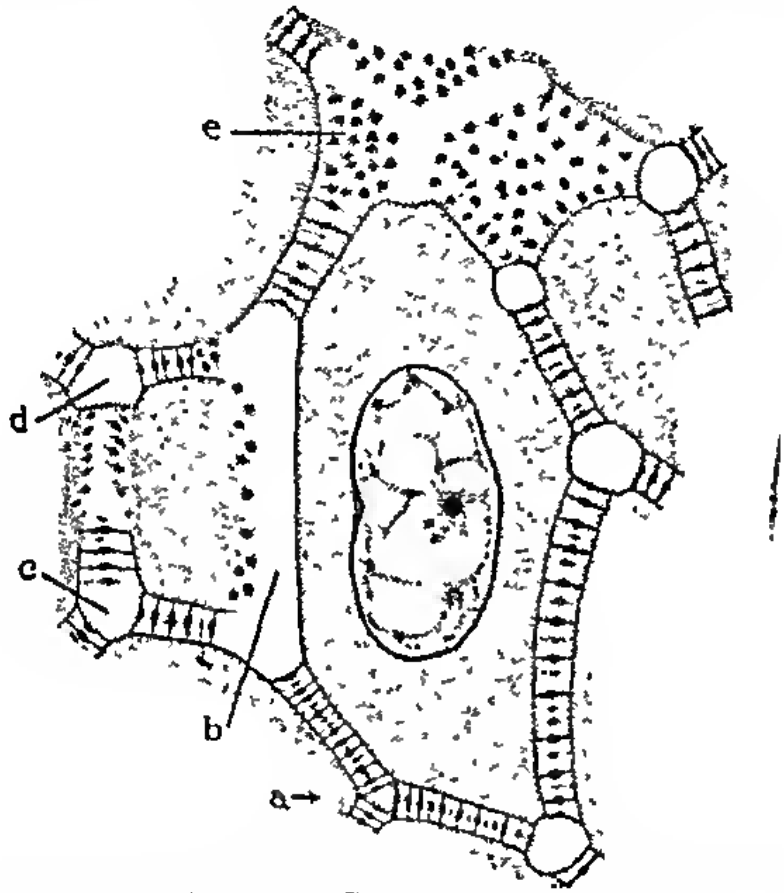


FIG 53.—INTERCELLULAR EDGE-SPACES FOLLOWING THE EDGES OF THE CELL FACETS, a, c, d AND b (CUT LENGTHWISE), AND INTERFACIAL SPACES CROSSED BY BRIDGES (CUT ACROSS AT e). EPITHELIUM OF THE CERVIX UTERI, 3 μ SECTION, MALLORY'S PHOSPHOTUNGSTIC ACID HÆMATOXYLIN (FROM F. T. LEWIS, 1933.)

and parts of the urinary tract. It is likely that some of these reports are based upon observations of sections giving the appearance of vessels in the epithelia while in reality they lie in diverticulæ or the subepithelial connective tissue papillæ. Hæmorrhage and inflammation should always be taken into account in studying the relations between epithelium and blood vessels. Nerve fibers extend among the basal cells of the epidermis and other epithelia, and ramify in contact with these cells, but special methods are required to demonstrate them.

Basal Surface. Epithelium is connected to the underlying tissue in several ways. Some epithelia have minute processes or rootlets extending from the basal surface of the cells anchoring them, while in other epithelia fine connective tissue threads pass from the underlying tissue

into little grooves in the cells. The lower surface is usually well defined and the epithelium is thought to be bound down by a 'cement substance.' Ordinarily the connections between epithelium and underlying tissue is such that the two do not become separated easily, but by various means as maceration in water and different solutions, or on boiling they may become separated and the cells isolated. Many epithelia rest upon a thin basement membrane or *membrana propria* which is usually homogeneous and contains few nuclei. Certain basement membranes have been considered as derivatives of the epithelium, but generally they are clearly of mesenchymal origin.

REGENERATION OF EPITHELIUM

Epithelial cells are constantly dying or being lost. This loss may be replaced by regeneration from the surviving cells. Regeneration occurs in all types of epithelium—simple, pseudostratified, transitional and stratified, but not at the same rate in all epithelia of the same or of different type. In some epithelia mitoses are seldom seen under normal conditions yet when injured some of these, as in the liver, exhibit an extraordinary regenerative capacity. Mitoses are rare in the epithelium lining the follicles of the thyroid gland and the mucosa of the stomach. But when part of a thyroid gland is excised, a certain amount of regeneration takes place from the remaining portion and the same occurs in the epithelium of the gastric mucosa. In those parts of the body subject to abrasion as the skin, oral cavity, pharynx and œsophagus, the outermost cells of the stratified epithelium are constantly being desquamated and their places taken by cells pushing up from below. Mitoses occur in the deeper layers only. Thus there may be said to be a two directional regeneration—in simple epithelium, laterally and in stratified epithelium, vertically. Some epithelia have been considered to proliferate by amitosis but the transformation is probably from multinucleate cells arising from some aberation of mitosis. Such conditions are seen in the superficial cells of the urinary bladder and in mesothelial cells covering the pericardium.

THE NATURE AND CLASSIFICATION OF GLANDS

Many epithelial cells elaborate and discharge substances which do not become parts of the tissue. Such cells are called *gland cells*, and their products are either utilized by the body (secretions) or eliminated as waste products (excretions). A number of such cells grouped in definite formation and for a specific purpose is called a *gland*, which may consist of a few cells only or of many thousands, as in the liver.

The simplest form of gland is merely a single secreting cell situated apart by itself in an epithelium. Such *unicellular glands* are abundant in invertebrates and are represented in man by scattered goblet cells. In the higher animals the secreting cells usually occur in groups, and they are generally found in tubular or saccular outpocketings of the epithelium. This method of outpocketing increases the number of cells in the surface layer of a given area, as may be understood by imagining the number required to cover a flat surface between the right and left limits of the epithelium at the top of Fig. 59, and comparing this with the

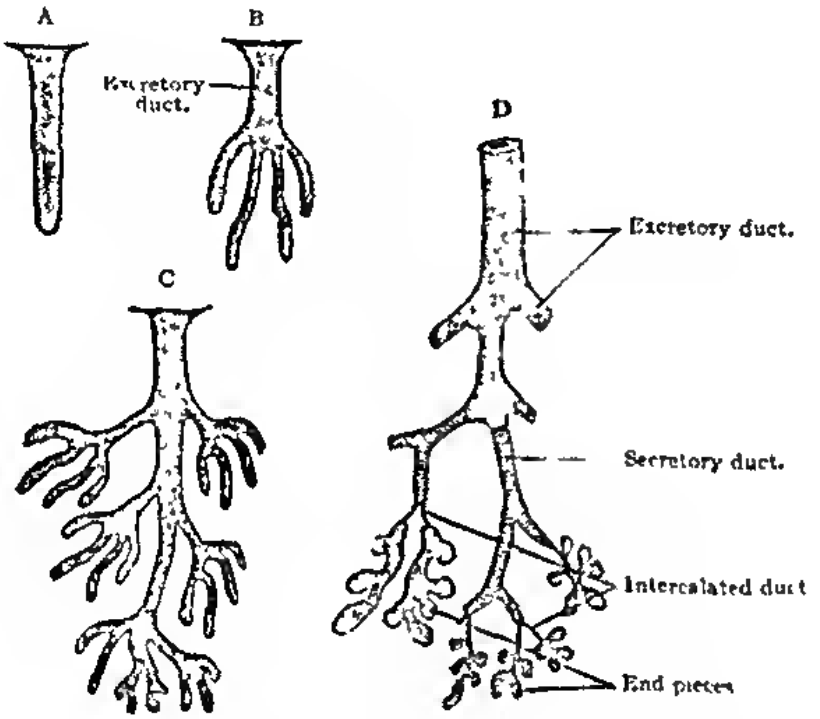


FIG. 54.—DIAGRAM OF VARIOUS FORMS OF GLANDS
The arrangement of ducts in D is that of the human submaxillary gland

actual number to be counted by following the contour of the gland. This is an unbranched tubular gland in vertical section, and in diagram a similar one is shown in Fig. 54, A. Since the tubule ends blindly, all the secretion made by the component cells must pass through the cavity, or *lumen*, to the general surface. The secreting cells may be distributed throughout the tube, or they may be limited to the lower part. In such cases the upper part forms the *duct* of the gland. Sweat glands are unbranched tubes, with a coiled secreting portion in the deeper part of the skin, and a relatively long duct which conveys the secretion to the surface. Many glands are branched, as in Fig. 54, B. This further increases the number of cells bordering on the lumen, without changing the area of the original surface. The main stem becomes the duct, and the characteristic secretion is formed in saccular or tubular 'end pieces.'

Such glands as have been described, either branched or unbranched, occur in great numbers as constituent parts of some organs, and they are classed as *simple glands*. The sebaceous and sweat glands of the skin, intestinal glands, and uterine glands are examples of this class. Many glands are much larger than these, owing to the fact that the epithelial outgrowth has branched repeatedly. It becomes invested with a connective tissue capsule, which sends partitions, or *septa*, among the ramifications of the epithelial tube, thus dividing the gland into lobes and lobules. A lobule usually contains a terminal branch of the duct together with the cluster of end pieces which empty into it. The large glands not only have a connective tissue framework, but also a special supply of

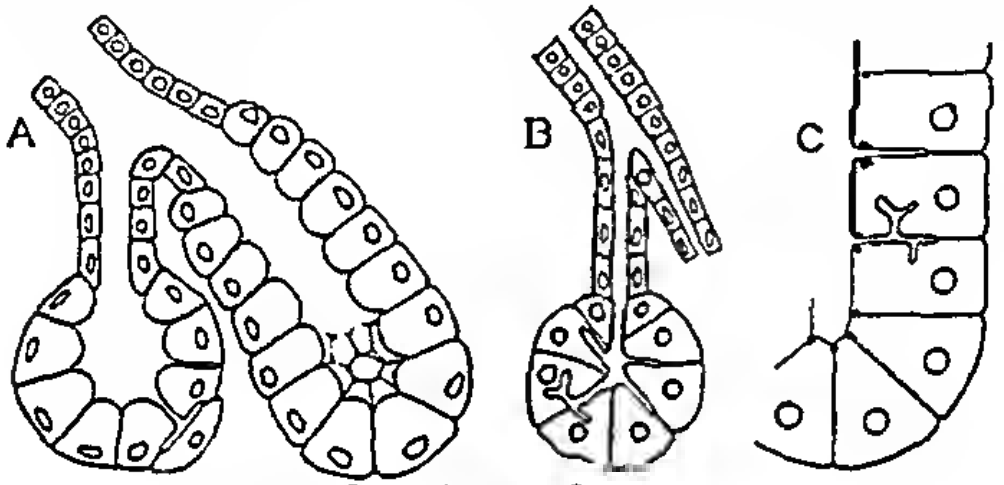


FIG. 55.—DIAGRAM OF GLANDS.

A, alveolus, tubule, B, acinus, C, top plate, terminal bars, inter- and intracellular secretory capillaries.

nerves, blood vessels and lymphatic vessels. Thus they form independent organs, and they are classed as *compound glands*. They include the liver, which discharges its secretion through a single duct; the pancreas, which is formed by the fusion of two glands and therefore has primarily two ducts; and many smaller organs, like the prostate, which is a compact group of glands each of which has a separate duct.

All the glands thus far considered are alike in being outpocketings of epithelium. Most of them develop as masses or cords of epithelial cells which later acquire a central cavity or *lumen*. The secreting cells are usually arranged in a single layer around the lumen. They may discharge their products from their free surfaces directly into the lumen, or into minute canals, either between the cells or within the cells. In some glands the cells are so numerous that certain of them are crowded away from the lumen; from these cells the secretion may find its way to the lumen through minute canals between the neighboring cells.

Such *intercellular secretory canals* (or capillaries) are found in the serous glands of the tongue and in the serous portions of the salivary glands; they occur also in the liver, the gastric and pyloric glands, sweat

glands, lacrimal gland and bulbo-urethral gland. Various forms are shown in the end pieces of the diagrams, Fig. 55, A, B, and C. They occur where two or more cells come together and consequently they are in relation with two or more terminal bars. In longitudinal sections the bars may be seen to extend downward along the canals. *Intracellular secretory canals*, shown in the left half of Fig. 55, B, are similar minute structures within the cells; they are less definite in outline, and are never in relation with terminal bars. They may be transient vacuoles opening at the surface. Sometimes they anastomose and form a network of canals within the cell. They have been observed, together with intercellular canals, in the sweat glands, the liver, and the gastric glands. There are apparently no secretory canals in any purely mucous gland, and they have not been found in the duodenal, intestinal, uterine and thyroid glands, the kidney or the hypophysis.

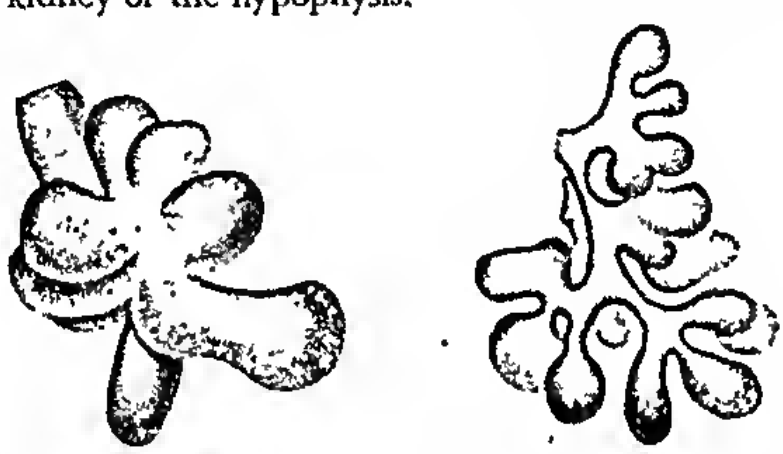


Fig. 56.—Mucous and Serous Alveoli from a Human Submaxillary Gland. Teased Preparations (Pelser.)

The ducts have a clear-cut lumen and are typically lined with a very regular epithelium, showing distinct cell boundaries. The cells usually do not contain the rods, granules or vacuoles characteristic of secreting protoplasm, and the nuclei are not crowded to the base of the cells. In some cases, however, the ducts contain mucous cells, and in the salivary glands a specialized portion of the ducts called the *secretory duct* is believed to discharge salts into the secretion as it passes through them, because it is lined with columnar epithelium, having basal rows of granules. In such glands (Fig. 54, D) the end pieces empty into small ducts, consisting of simple flat or low cuboidal epithelium, the *intercalated ducts*, which lead to the secretory ducts, and they in turn join larger *excretory ducts* of simple or stratified nongranular epithelium.

The end pieces of the glands, as already noted, vary in shape from saccular to tubular. Usually a minute dissection or a reconstruction is necessary to determine what the shape may be. A round termination is called an *acinus* (Latin, a grape or berry) or an *alveolus* (Latin, a trough or tray). These terms are often used interchangeably, but it is better to

restrict the use of the term acinus to imply a rounded shape with narrow lumen, as opposed to the wide, flask-shaped lumen of an alveolus. The elongated forms of end piece are called tubular (Fig. 54, C) and shorter forms or combinations of long and short (D, at left) may be designated tubuloalveolar or tubuloacinar.

During the development of the thyroid gland the duct becomes obliterated, so that the secretion within the end pieces cannot escape. The end pieces become closed epithelial sacs, known as *follicles* (Latin, *folliculus*, a leather bag, shell, or husk). In addition to the material enclosed within the follicles, the thyroid gland secretes substances which

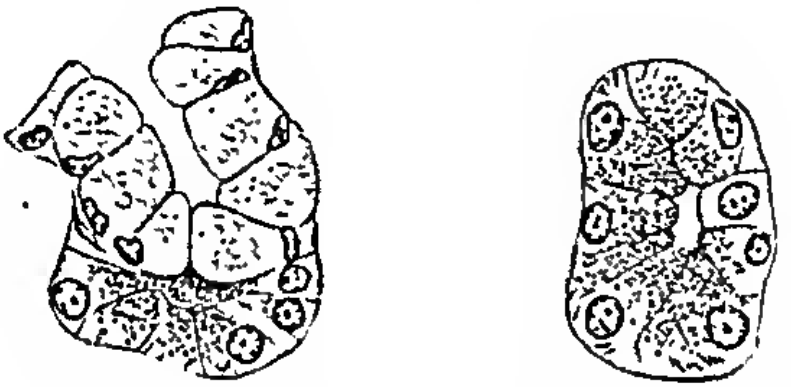


FIG. 57.—MUCOUS TUBULE WITH SEROUS HALF-MOON, AND A SEROUS END-PIECE. Human submaxillary gland. Sublimate fixation, Azan. (Heidenhain.)

are taken up by the surrounding blood vessels and lymphatic vessels. Secretions of this sort are called *internal secretions*.

The *epithelioid glands* are masses or cords of cells which produce internal secretions only. They are never provided with a duct or lumen, although in some cases their cells arise from the wall of an epithelial tube. They are closely related to the glands with obliterated ducts.

Finally there are glands which produce cells and are therefore called *cytogenic glands*. These include the ovary and testis, which are epithelial structures consisting of follicles and tubules respectively. They produce the ova and spermatozoa. The other cytogenic glands are non-epithelial bodies which produce various forms of blood corpuscles. They will be considered in a later section.

The classification of glands, as presented in the preceding paragraphs, is summarized in the following table:

I. *Epithelial glands, with persistent ducts, producing external secretions.*
   1. Unicellular glands.
   2. Simple glands.
      a. Ectodermal, *e.g.*, sweat glands.
      b. Mesodermal, *e.g.*, uterine glands.
      c. Entodermal, *e.g.*, gastric and intestinal glands.

3. Compound glands.
   a. Ectodermal, *e.g.*, mammary and lacrimal glands.
   b. Mesodermal, *e.g.*, epididymis and kidney.
   c. Entodermal, *e.g.*, pancreas and liver.

II. *Epithelial glands, with obliterated ducts, producing internal secretions.*
   a. Ectodermal, anterior lobe of the hypophysis (the duct of the posterior lobe is partially obliterated).
   b. Entodermal, thyroid gland.

III. *Epithelioid glands, never having duct or lumen, producing internal secretions.*
   a. Ectodermal (through their relation to the sympathetic nerves), chromaffin bodies; and medulla of the suprarenal gland.
   b. Mesodermal, cortex of suprarenal gland; interstitial cells of the testis; corpus luteum.
   c. Entodermal, islands of the pancreas; epithelioid bodies in relation with the thyroid gland; thymus (?)

IV. *Cytogenic glands, producing cells.*
   a. Mesodermal, *epithelial*—ovary and testis.
   b. Mesodermal, *mesenchymal*—the lymph glands, hæmal glands, spleen, red bone marrow, and many smaller lymphoid structures

PROCESSES OF SECRETION IN EPITHELIAL CELLS

The process of elaboration and discharge of the secretion or excretion may often be recognized by changes in the form and contents of the cell. A gland cell which is full of secretion, or discharging it, is called 'active,' and one in which the secretion is not apparent, though it may be in process of formation, is called 'resting.' The process involves a certain 'polarity' of the cell, the basal pole resting on the basement membrane and receiving the supply of nutritive material, which is elaborated within the cell and passed out as secretion from the opposite pole. For this reason gland cells are best studied when they are cut from base to free surface, not obliquely. The process of secretion is usually first recognizable by the presence of granules within the protoplasm. These have been considered as derived from mitochondria or from particles passing from the nucleus to the cytoplasm as the chromidial substance or elaborated in some way by the Golgi apparatus. According to Ludford[1] both mitochondria and Golgi apparatus may play a part in the process. "At the mitochondrial-cytoplasmic surface syntheses by enzymes occur. The resulting products continually diffuse into the cytoplasm, preventing an accumulation at the surface of the mito-

[1] Ludford, 1928.

chondria, which would inhibit further syntheses. At the surface of the Golgi apparatus the elaborated products are concentrated into droplets preliminary to their elimination."

Changes in the mitochondria and nucleus certainly accompany secretion, and the Golgi apparatus leaves its perinuclear position and moves toward the free pole of the cell. The process of secretion can be studied to a certain extent by the vital dye methods, for the reactions of the cells to these dyes is similar in many ways to secretion. As yet, however, we have no sure knowledge of the origin of the secretory droplets or granules.

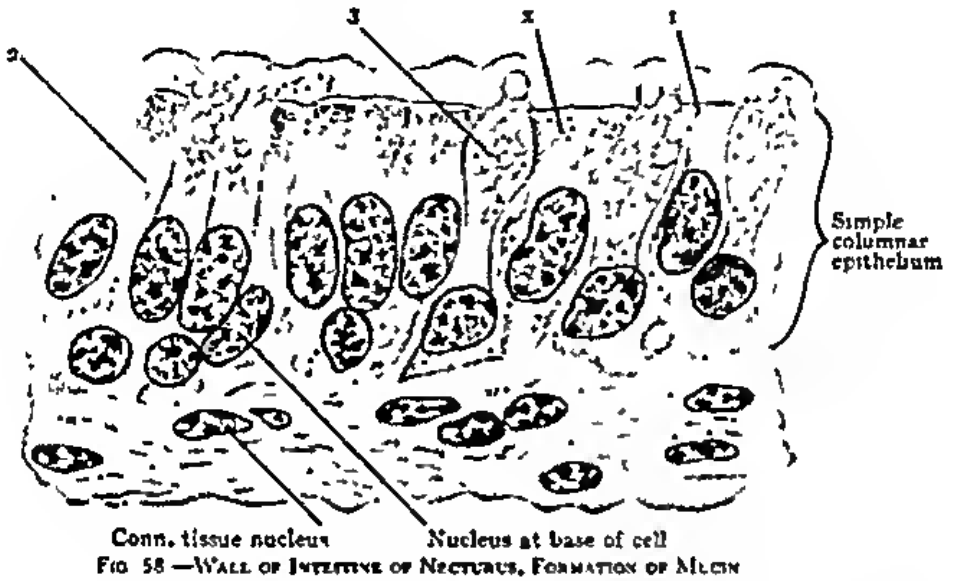


FIG. 58.—WALL OF INTESTINE OF NECTURUS, FORMATION OF MUCIN

1, 2, 3, cells in progressive stages from mucigen granules to mucin, "x," upper part of cell cut in thin section.

In the empty cell the nucleus is vesicular, with distinct chromatin granules, a fine reticulum, and often a clearly marked nucleolus. In cells full of secretion the nucleus shrinks, becomes darker by an increase or rearrangement of its chromatin, and the nucleolus may appear larger or disappear. In extreme cases the nucleus may become pycnotic. The appearances during secretion differ in the two main types of gland cells, —the *mucous*, which form thick, glairy secretions, like those of the nose and throat; and the *serous*, which produce watery secretions. The latter are also called *zymogenic*, because they elaborate specific enzymes, or chemical ferments. These will be considered in turn.

Mucous cells, when resting, are cuboidal or columnar, usually occurring in a simple epithelium and having top-plates and oval, centrally placed nuclei. The first evidence of activity is the appearance of minute granules in the cytoplasm beneath the top-plate. The granules stain differentially with iron hæmatoxylin and certain other stains. They increase in number in the upper part of the endoplasm, and then are changed into or replaced by clear droplets of mucus; hence they are

called mucigen or mucinogen granules. According to Duthie[1] the transition occurs in the Golgi network, the granule being actually surrounded by the osmiophilic material. The droplets form a discoid mass, which increases in bulk as the cytoplasm becomes increasingly transformed into secretion, and the remaining cytoplasm and nucleus are forced to the base of the cell. Sometimes the secretion causes the sides and top-plate to bulge; from their shape, like cups filled with mucus, such cells are called 'goblet cells.' Finally the top-plate either ruptures or becomes pervious to the enclosed mucus, which thus escapes to the surface, forced by the pressure of the surrounding epithelial cells. In preserved specimens the top-plate is frequently seen broken, but this may be an artifact. In the mucous cells of the intestine, secretion is formed below and discharged from the free surface at the same time. The cells, as seen in Fig. 59, arise near the bottom of tubular depressions lined with simple columnar epithelium. By the formation of new cells below them they are pushed toward the outlet of the tube. Thus the youngest cells are at the bottom of the pit and the oldest are at the top. For a time the secretion develops faster than it is discharged, and the cells enlarge as seen in the middle part of the gland; later, as elimination exceeds production, they become narrow, and their final stages, as compressed cells with a remnant of secretion, are found near the orifice of the gland. In other mucous glands the secreting cells apparently remain filled with mucin continually. The cells are arranged in a single layer around a small central cavity, the lumen, into which they pour their secretion. The cells are therefore wedge-shaped in one plane, the nuclei frequently crowded to one basal

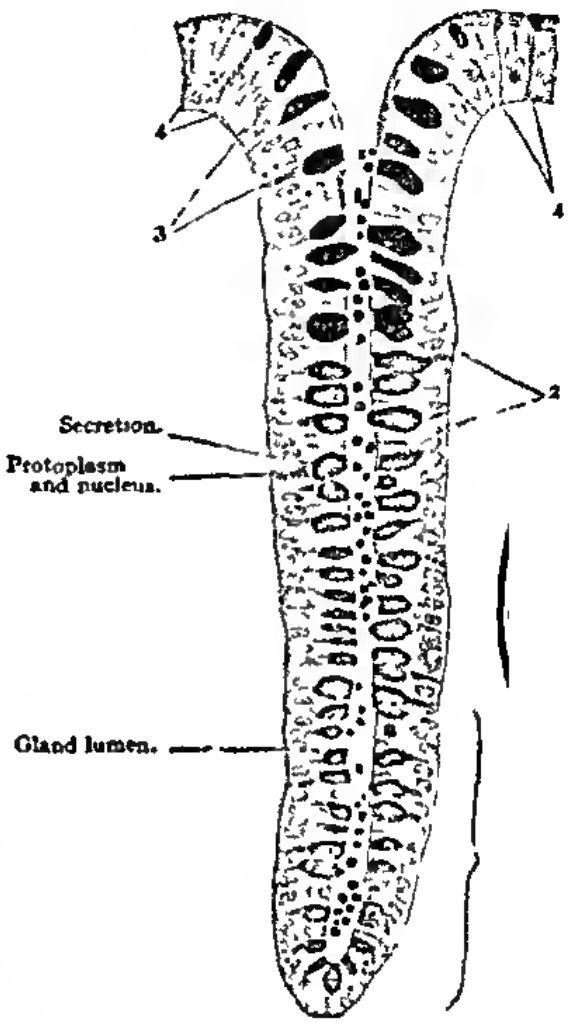


FIG. 59.—INTESTINAL GLAND FROM A SECTION OF THE HUMAN LARGE INTESTINE. X 165

The secretion formed in the goblet-cells is here colored blue. In zone 1 the goblet-cells show the beginning of secretion, that expulsion has begun is evident from the presence of drops of secretion in the lumen of the gland. 2, Goblet-cells with much secretion. 3, Goblet-cells containing less secretion. 4, Dying goblet-cells, some of which still contain remnants of secretion.

[1] DUTHIE, 1934.

corner. Mucus is presumably formed as rapidly as discharged; the gland looks pale and clear in ordinary sections, except when they have been treated with some special stain like muchematein, which has an affinity for mucin.

In certain stratified or pseudostratified epithelia, the formation of mucus has been seen to take place in some of the deeper cells, but the discharge of the secretion can occur only when these cells have reached the free surface.

Serous cells when empty are small with darkly staining protoplasm. As the formation of secretion begins, the cells, if prepared by appropriate

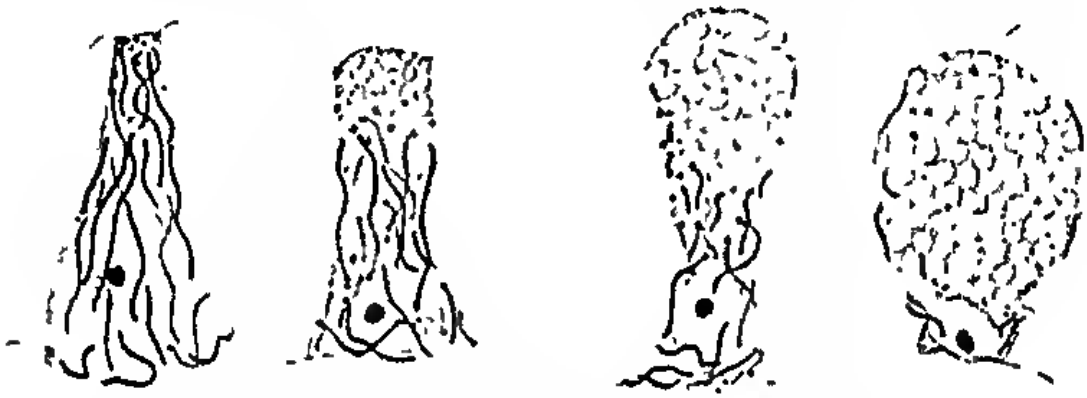


FIG. 60.—MUCOUS CELLS IN DIFFERENT PHASES OF SECRETION. SUBLINGUAL GLAND OF DOG. Potassium dichromate and formaldehyde fixation, acid fuchsin (Hoven.)

methods, exhibit specific granules which stain intensely. In fresh tissues these granules are highly refractive and conspicuous. They are not confined to an area under the top-plate, but occur usually above the nucleus. They enlarge and increase in number, even forcing the nucleus to the base of the cell. The cytoplasm becomes reduced to a network between them. They then lose their staining capacity and become transformed into droplets of fluid, giving the cytoplasm a light reticular appearance, which, however, is readily distinguished from that of mucous cells. Frequently the remains of the fading granules can be seen in the clear droplets. New granules may be detected in the strands of cytoplasm between the droplets, a source of new secretion. The secretion is discharged from the free surface of the cell, either directly into the gland lumen or into one of the intercellular canals. The actual method of discharge has been studied most intensively in the pancreas (*Fig. 61*). There is no evidence that the 'granules' are solid bodies; they may be droplets of stainable fluid. Covell[1] has shown that in the process of extrusion they bulge out, either as granules or vacuoles, and carry with them a portion of the cell wall which then closes behind them, leaving no trace. The granule or vacuole thus does not actually pass

[1] COVELL, 1928.

through the cell membrane. In the lumen the granules and their coating membranes soon dissolve, forming the fluid enzyme which is not detectable by the microscope.

Bowen[1] believes that the secretory granules develop only in contact with the Golgi apparatus and has noticed the difference in the activity of the latter in the two types of secreting cells. 'There is a marked tendency in cells of the serous type for the Golgi apparatus to be extended throughout the mass of developing granules, while in mucous

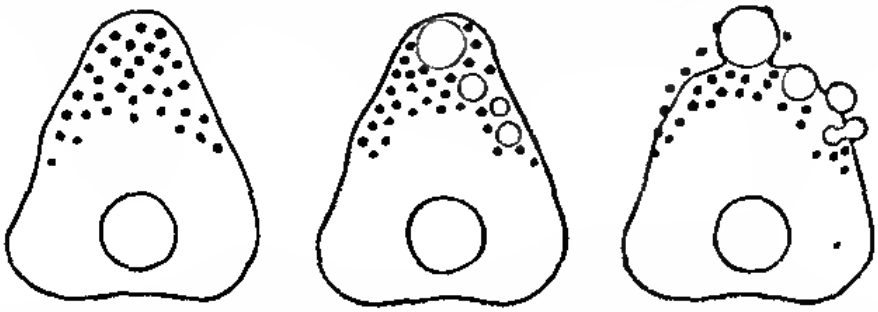


FIG 61.—SEROUS SECRETION

Formation of vacuoles and extrusion of granules and vacuoles, shown in successive stages. Diagram based on the paper by Covell.

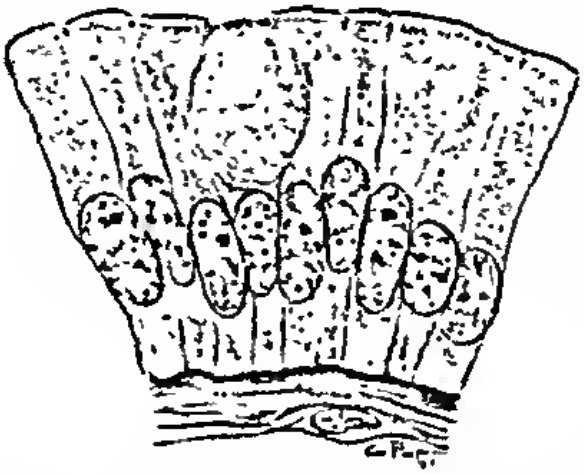
cells the apparatus tends to maintain a more compact and peripheral location. This is apparently correlated with the fact that in mucous cells the secretory granules are completed very soon after their formation, while in serous cells the whole content of granules seems to progress gradually toward a simultaneous completion.' Duthie[2] finds the granules in serous cells within the vacuoles, where they decrease in size with maturation and no longer show any staining ability. Their full maturation occurs in the meshes of the Golgi network. This has been denied by a number of recent workers whose findings have been reviewed by Dawson.[3]

Another type of serous cell is found in some of the ductless glands where the cells are arranged in cords without lumens. These cells contain secretory granules which, however, leave the cell by what would ordinarily be called its base, and pass into the surrounding tissue spaces, and thence to the lymph or blood.

Glands may consist entirely of serous or of mucous cells, but frequently they include cells of both sorts and are called *mixed glands*. The mixed glands contain some purely serous tubules or acini; the rest consist of both mucous and serous cells, so arranged that the latter appear more or less crowded away from the lumen. Often they form a layer outside of the mucous cells, partly encircling the tubule or alveolus and constituting a *crescent* (demilune), connected with the lumen by means of secretory capillaries. Sometimes the cells of the crescent are directly in contact with the lumen. Since the serous crescents are always associated intimately and somewhat irregularly with mucous cells, they were naturally interpreted as a functional phase of the latter. It is probably true that some crescents represent empty mucous cells which have been

[1] BOWEN, 1926. [2] DUTHIE, 1934. [3] DAWSON, 1942a.

crowded from the lumen by those full of secretion. No secretory capillaries lead to such mucous crescents, which moreover are not abundant. Another sort of crescentic figure is made by the basal protoplasm in mucous cells otherwise full of secretion. Finally, in oblique sections,



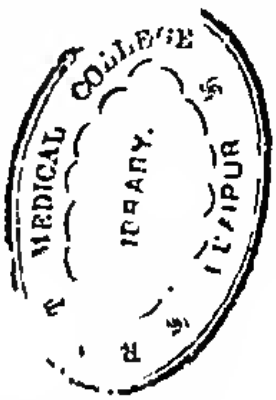


Fig. 62.—A Mucous Goblet and the Staining of Mucin (Light Blue).
Epithelium from a larger branch of the intrahepatic bile duct of a rhesus monkey. Nissl fixation. Arzt.

stellate cells associated with the basement membrane may resemble true crescents. Demilunes are further discussed on p. 324.

Though mucous and serous cells are usually considered as entirely distinct types, some authors recognize that the granules of certain apparently serous cells color to a slight extent with stains specific for mucin, and describe 'amphitrope' or seromucous cells. This suggests the possibility of a change of cell type during growth, and might explain the presence of the demilunes in mucous glands as younger forms, the serous cells changing to mucous cells.

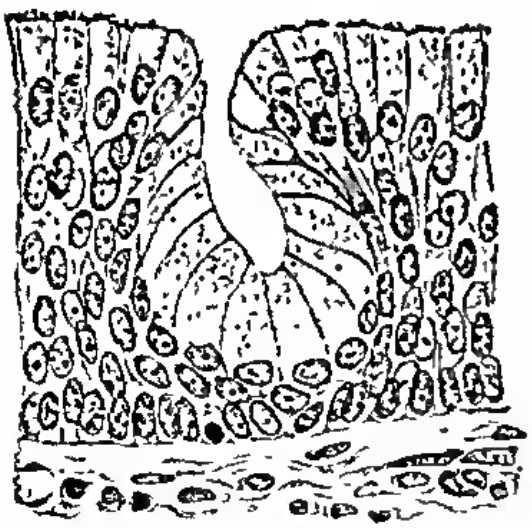


Fig. 63.—Intraepithelial Gland in the Pseudostratified Ciliated Epithelium from the Laryngeal Surface of the Epiglottis of a 72 Yr. Old Woman. × 534. (Patzelt.)

It is interesting to note the difference in size of the lumens of the two types of glands. Mucus is a slimy fluid and apparently needs a larger channel than the watery serous secretion; hence the lumen of mucous glands is much the wider. In fact the serous lumen is so small that the part of each cell forming its immediate wall is apparently too restricted for proper function. To overcome this difficulty secretory capillaries, either intercellular or intracellular, are frequently provided. These may be temporary, present only during gland activity. They serve to increase the area of free surface for the cells between which or within which they occur. The activity of cells in elaborating and giving off some specific substance is by no means limited to epithelial cells. All cells 'excrete' the waste products of their metabolism, and many 'secrete' some material useful to the body. The

evidences of secretory activity in connective tissue cells, for instance, are not readily recognizable, but the possibility of such activity should always be borne in mind.

In the sebaceous glands, secretion consists of the deposit within the upper cells of a stratified epithelium of fatty material and of the casting off of the entire fatty cell into the lumen, where it breaks down and allows the fat to escape along the hair follicle. In the mammary gland the fat accumulates in the upper part of the cell and is released with some of the cell protoplasm These different modes of secretion have been designated 'holocrine' (when the whole cell is discarded), 'apocrine' (when a portion of the cell is lost), and 'merocrine' (when the act of secretion leaves the cell intact).

THE MESENCHYMAL TISSUES

Mesenchyma (*μεσος* middle, *ἔγχυμα*, an infusion) is a term introduced by O. Hertwig, in 1883, for the tissue produced by cells which have wandered out from the epithelial germ layers into the spaces between them. It is found only in young embryos. In the adult it is represented by a large group of derivatives, including connective tissue, fasciæ and tendons, adipose tissue, cartilage, bone, smooth muscle fibers, and various special forms of cells. It also probably gives rise to the endothelium of the vessels, and to the blood corpuscles, either directly or indirectly. Mesenchyma arises chiefly from different parts of the mesoderm, as already described (p. 60), but in the head of the chick embryo a portion of it comes from the ectoderm, and in the wall of the intestinal tube, according to Hertwig, the entoderm contributes to its formation. Together with the blood islands it constitutes the entire non-epithelial tissue of the embryo in early stages.

MESENCHYMA

Mesenchyma (see Figs. 43 and 44) consists of a network of branching cells, in the meshes of which there is a homogeneous, fluid, intercellular substance, or matrix. It is usually considered as a syncytium, in that no visible cell walls divide the protoplasm surrounding one nucleus from that about the others. W. H. Lewis,[1] however, has raised the question whether it is not composed of discrete cells, with many branching and tapering processes, which merely adhere to those of their neighbors. The death of a single one of these units has been watched in tissue culture, confirming its individuality. In either case, it is convenient to speak of the protoplasm surrounding each nucleus as a mesenchymal cell. Such cells are embryonic in type and merit special study because of the variety of cytomorphic changes they may undergo in forming various tissues. The nucleus is large, oval or round in section, with very little chromatin, mostly in one or two masses. The nuclear wall is delicate, and usually indented slightly at one point to lodge the centrosome of the cell. The

[1] Lewis, W. H., 1922*a*.

protoplasm consists of the processes and of a compact portion, the body, surrounding the nucleus, but the body is often so small that the nucleus appears to be uncovered on one or more sides. Probably a thin film of protoplasm always covers the nuclei of all living cells, though they may often appear naked. The protoplasm throughout is finely granular and with no definite cell wall. The processes, extending from the body of the cell in all directions, and therefore frequently leaving the plane of any section, are longer or shorter depending on the density of the tissue. They may be rounded and rod-like, usually tapering, or flattened and sheet-like. They are apparently continuous with other processes, with the base of an epithelial layer, or with blood vessels. The intercellular substance is homogeneous and fluid; in it may be found occasional blood cells, outside of the vessels.

Fig. 64.—Diagram of Wide-meshed Cellular Connections in Mesenchyma (von Möllendorff.)

Although typical epithelium and mesenchyma are radically different, there are conditions in which they are comparable. Thus dense mesenchyma, in which the cells are closely packed and have very little intercellular substance, resembles epithelium, and it may give rise to groups or cords of epithelioid cells. Moreover epithelium may resemble mesenchyma by forming a *vacuolate syncytium*. F. T. Lewis[1] has pointed out how, by the development and enlargement of holes or vacuoles in the borders of contiguous cells in a solid epithelial tissue (as in Fig. 53), the tissue might become changed into apparently individual cells connected by processes traversing the intercellular fluid.

Fig. 65.—Mitosis in a Mesenchymal Cell. Head of 96 Hr. Chick Embryo. Bouin fixation; haematoxylin and eosin.

In addition to the true mesenchymal cells, which are more or less definitely fixed structures, other cells are infrequently found in the intercellular fluid, in which they may move or float about. These were called by Maximow the wandering cells. They apparently arise from the mesenchymal cells by simply withdrawing their processes from the syncytial net and becoming spherical. They are amœboid, and may put forth short pseudopodia. They may undergo changes similar to those seen in the developing blood cells within the blood vessels, but are themselves

[1] Lewis, F. T., 1933b.

found outside of the vessels (extra-vascular). In Fig. 44 they appear like the young intravascular blood cell.

Mesenchyma thus consists of fixed mesenchymal cells, intercellular substance, and wandering cells. It extends everywhere between the epithelial sheets or structures, and is in close relation with the blood vessels which traverse it. In the transformation from embryonic mesenchyma to its adult derivatives changes must be looked for in the nucleus and cytoplasm of both types of its inherent cells, and in the intercellular substance. Thus development may take place either intracellularly or intercellularly, or both.

THE CONNECTIVE TISSUES

The most common derivative of mesenchyma is connective tissue. In its development the cells becomes flattened or spindle-shaped, and may lose some or all of their cytoplasmic processes; the nuclei are altered in shape and the chromatin becomes more finely divided. The chief developmental change, however, is the appearance within the matrix of fibers, which may be few or many. Because of their differing morphology and chemical nature they are divided into several categories: reticular, white or collagenous, and elastic; according to the prevalence of the different kinds the tissue may be called reticular tissue, fibrous tissue, fibro-elastic tissue, and so forth. The fibers never entirely fill the intercellular spaces, but they and the cells are always bathed by the intercellular fluid. In some of the mesenchymal derivatives a non-fibrous material also appears in the fluid matrix, always as an addition to the fibers. If this is mucus, the tissue is called mucous (connective) tissue; in the case of cartilage or of bone, however, the tissue is called by its distinctive name.

Reticular Tissue. The tissue of the adult which most closely resembles mesenchyma is known as *reticular tissue*. Though it first arises rather late in embryonic life (*e.g.* in the œsophagus of embryos of 30 mm. and in the lymph glands which first appear in human embryos of 45 mm.) after true connective tissue has replaced the mesenchyma in most of the body, it is considered by some as a persistence of the primitive mesenchyma or a primitive form of connective tissue.

Reticular tissue forms the framework of lymph glands, the bone marrow and the spleen; it occurs as a layer immediately beneath the epithelium of the digestive tract, and has been reported in many other organs. It consists of a network of cells in relation with an abundant fluid intercellular substance. At the borders of the bodies and cytoplasmic processes of the primitive stellate cells there are stiff, slender fibers or narrow plates, (reticular fibers or lattice fibers), not especially notice-

able with the usual stains with which the cytoplasmic processes appear clear and homogeneous. Reticular fibers stand out prominently after impregnation with certain reduced silver methods and are often called *argyrophil*. The cells contain pale, flattened, oval nuclei, with few chromatin granules; the cytoplasm is clear and non-granular. The

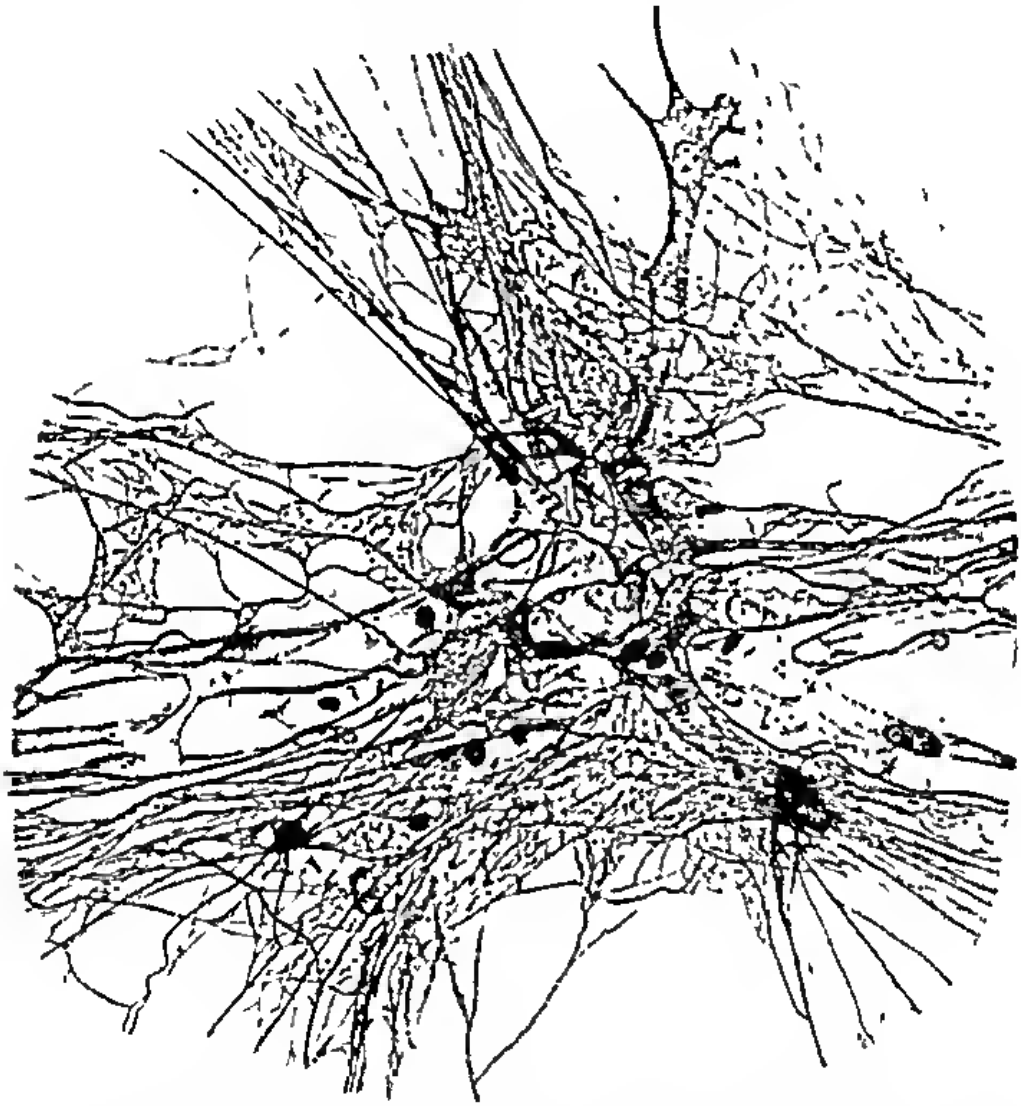


FIG. 66.—DEVELOPMENT OF RETICULAR FIBERS. TWENTY DAY TISSUE CULTURE.
Syncytial arrangement of fibroblasts in color. (Drawn from Maximow, Zeitsch. f. mikr.-anat. Forschg., 17, 1929.)

intricate relation of these cells to the blood vessels, and their probable function in producing wandering cells, are given elsewhere. In ordinary sections reticular tissue will be most readily recognized by the cells lodged in the fluid intercellular substance. These cells, which are chiefly lymphocytes (see p. 106), having round nuclei and a narrow rim of cytoplasm, are often so abundant that the tissue appears as a dense cellular mass in which the framework of reticular tissue is almost completely hidden. Upon careful examination, however, some of its nuclei and fibers can always be detected.

In order to study reticular tissue advantageously, the lymphocytes and other forms of free cells should be disengaged from its meshes. This may be accomplished by shaking or brushing the sections; or by artificially digesting the specimen (which if properly done will destroy the cells, including those of the reticular tissue, but will leave the network of fibers); or by the following ingenious method devised by Mall. A piece of fresh spleen is distended by injecting gelatin into its substance; it is then frozen and sectioned. The sections are put in warm water, which dissolves out the gelatin, carrying the loose cells with it, and leaves areas of clear reticular tissue. Mall has also shown how to wash out the pulpy contents of the entire spleen, so as to leave the framework of connective

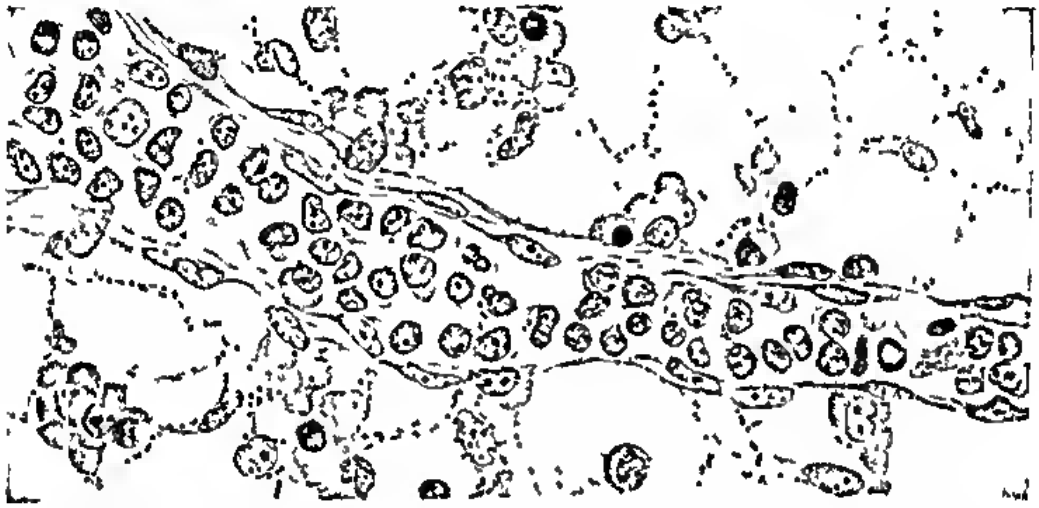


Fig. 67.—Lymph Gland of Dog, Injected with Dilute India Ink Solution, to Show Reticular Tissue Cells on Either Side of a Medullary Cord. ×850. (From Drinker, Wislocki and Field, 1933.)

and reticular tissue, which may be inflated and dried.[1] Such preparations give an idea of the intricacy of the reticular meshwork that can be obtained in no other way, and yet the finer ramifications have been destroyed by this process.

The fixed cells of the reticular tissue mesh, as opposed to those which may be floating in the intercellular fluid, are everywhere characterized by phagocytosis, the power to ingest particulate matter with which they may be brought in contact. This is well seen in the lymph sinuses of the lymph glands, where the intercellular spaces are relatively empty, especially in those of an animal previously injected with India ink[2] (Fig. 67) or lithium carmine. The ink or carmine granules are found within many of the reticular cells. Those that show this phagocytosis are sometimes called fixed macrophages; they may further become swollen, round up and leave the syncytium, being then free floating cells called true macrophages (see p. 102).

Reticulo-endothelial System. The intimate association of the vessels and the reticular tissue in certain localities has given rise to the term *reticulo-*

[1] Mall, 1900. [2] Drinker, Wislocki and Field, 1933.

endothelial system for those modified endothelial cells in many organs whose chief activity is phagocytosis.[1] In the sinusoids of the liver they seem to be interspersed among non-phagocytic cells to form a complete endothelium; in the lymph sinuses of the lymph gland the lining seems incomplete and the endothelial cells merge indistinguishably with the reticular tissue framework. The term is applied also to certain vascular cells of the bone marrow, spleen, suprarenal gland, hypophysis and other organs. Some authors include in the group the macrophages of the connective tissue and the monocytes of the blood, which they derive from the fixed cells of this system. The function of this system is protective, by removing injurious particulate matter from the blood, lymph and tissue fluids. It was first recognized, and has been intensively studied by the injection of vital dyes.

Mucous Tissue. The substance of the umbilical cord is composed of *mucous tissue*, which, while not strictly true connective tissue, has long been regarded as a particularly favorable material for the study of the white fibers. It differs from ordinary connective tissue by the deposit of a large amount of mucus in the intercellular spaces. At birth it is a peculiar gelatinous mass of pearly luster, known anatomically as Wharton's jelly.

Unlike the syncytium of mesenchyma, the cells of mucous tissue are difficult to recognize with the usual stains, because they are obscured by the material in the intercellular spaces. But when the specimen is stained with phosphotungstic acid hæmatoxylin they are marked clearly by dark blue fibrils which have developed along the cell borders. By their aid one can distinguish that the cells are stellate or spindle-shaped, with few processes, but still often forming a net, like the mesenchyma from which they were derived. Since the elongated cells are apt to lie parallel to one another, a favorable area in the section, where they are cut lengthwise (Fig. 68), should be chosen for study. The nuclei are elongated to conform to the shape of the cell. The chromatin is more finely divided than in the parent mesenchymal cell. The cytoplasm, especially in the processes, forms a thin non-granular layer, the limits of which could scarcely be determined were it not for the border fibrils.

The 'border fibrils' of connective tissue, first described by Mallory,[2] are similar to other fibrils found in the tissue which forms the supporting framework around the nerve cells and nerve fibers, thus binding them together. This tissue was called *neuroglia* (*νεῦρον*, nerve; *γλία*, glue), and the fibrils *neuroglia fibrils* (see p. 211). Other similar fibrils found at the periphery of young muscle cells are named *myoglia fibrils*. Mallory therefore named the fibrils in connective or fibrous tissue *fibroglia fibrils*. He described them as follows:

[1] METSCHNIKOFF, 1883. [2] MALLORY, 1905.

Neuroglia, myoglia and fibroglia fibrils morphologically and in certain staining reactions more or less closely resemble one another. They touch or form part of the periphery of the cell protoplasm, but continue away from the cell in two directions, *i.e.*, they do not begin or end in the cell which produces them. How far the fibroglia are accompanied by protoplasmic processes cannot be determined. The number of these fibrils to a cell is not constant, but it is usually in the neighborhood of a dozen.

The fibroglia fibrils are present at birth, and probably no tissue is more favorable for their study than the umbilical cord at term. Special

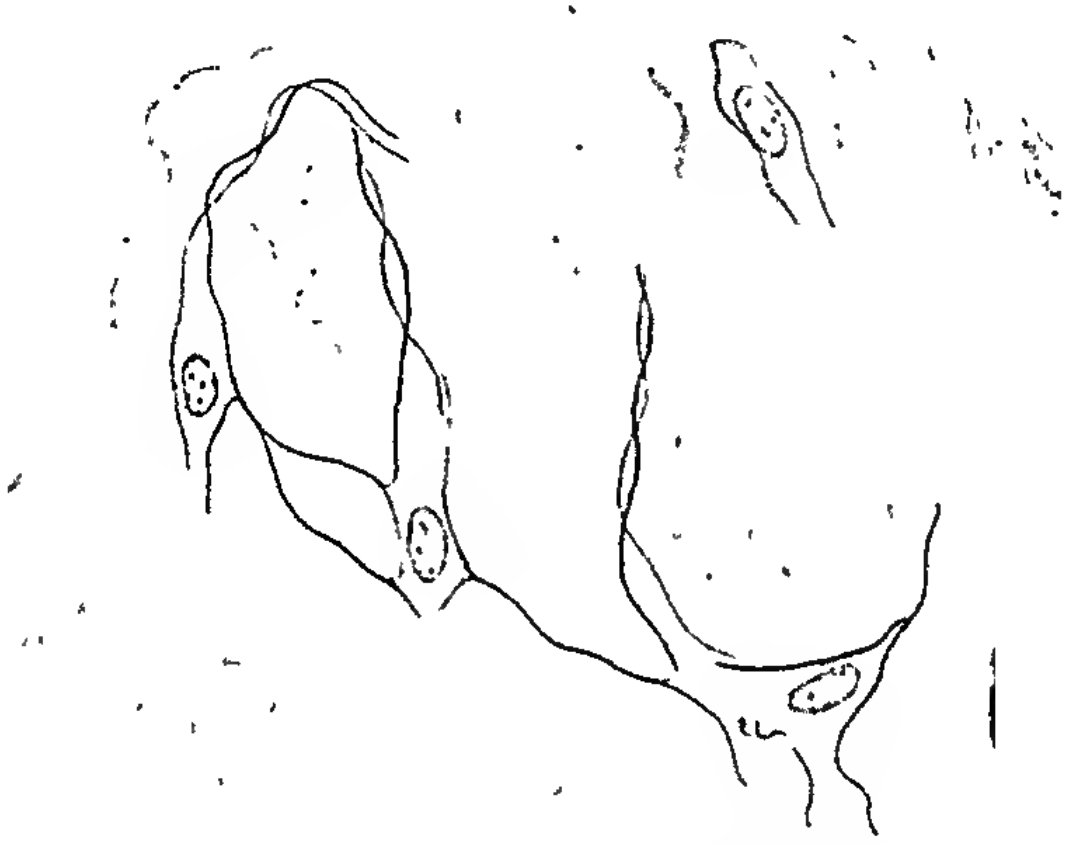


FIG. 68.—MUCOUS TISSUE, HUMAN UMBILICAL CORD. MALLORY'S PHOSPHOTUNGSTIC ACID HEMATOXYLIN.

stains are necessary to make them at all conspicuous, and except for their presence in new tissue (tumors) they seem to be relatively unimportant. The same may be said of the myoglia fibers; the neuroglia fibers, however, are an essential part of the adult tissue.

The intercellular spaces in mucous tissue, instead of containing only a homogeneous fluid or semifluid substance as in mesenchyma, are partially filled with mucus and white fibers. The mucus, like that produced in the goblet cells and that found in the cornea and vitreous body of the eye, is a translucent substance which contains *mucin*. In the mucus extremely delicate fibrils, the white or collagenous fibrils, are embedded. These are slender almost to the point of invisibility, but they are gathered into larger wavy bundles. They are in part in contact with the cell

borders, but may apparently leave the cells and lie free in the intercellular spaces. The individual fibrils are said not to branch, but the large bundles of them, or fibers, branch frequently. They are not elastic, and their presence helps to strengthen the tissue.

Chemically there are many varieties of mucins. They are compound protein bodies containing a carbohydrate complex in their molecules, and are therefore known as glycoproteins. True mucins are formed in abundance in goblet cells and in mucous tissue; to a less extent they occur in all embryonic connective tissue. Related substances, called mucoids, have been obtained from tendon, cartilage and bone. In the umbilical cord the mucus may be regarded as a secretion which is produced without the formation of special granules or vacuoles, and is discharged equally from all surfaces of the cells, making a homogeneous ground substance.

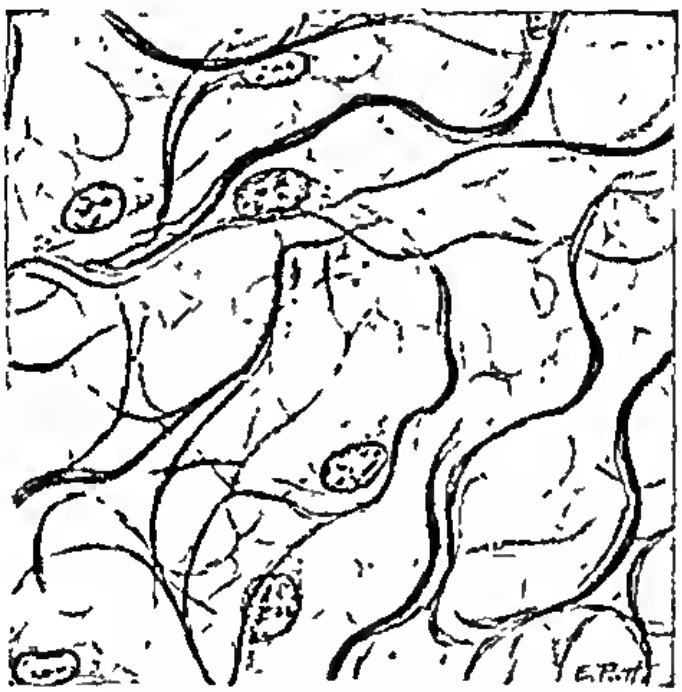


Fig. 69.—Embryonic Connective Tissue. Skin of Pig Embryo. Formaldehyde fixation, Azan.

The intercellular substance is not entirely filled by mucus and white fibers. There are spaces filled with a fluid through which the infrequent wandering cells may migrate. There are no capillaries, lymphatic vessels, or nerves within the mucous tissue of the human umbilical cord, and no elastic fibers. The three large blood vessels which pass through the cord, and the tissue in their walls, will be considered later.

Connective Tissue. Connective tissue proper occurs in various forms. As loose connective tissue or *areolar tissue* it is a spongy cobweb of delicate filaments, such as occurs between the muscles; as dense connective tissue it is a tough fibrous substance, such as that part of the skin from which leather is made; and as fascia or tendon it is specially modified to withstand various tensile strains. The difference depends largely

on the number and arrangement of the intercellular white fibers and elastic fibers, and the relative number and character of the essential cells and of the wandering cells.

As in mucous tissue, the cells of connective tissue are inconspicuous both in the fresh unstained specimen and with the usual histological stains. In this case, however, the use of phosphotungstic acid hæmatoxylin gives no help, since fibroglia fibrils, marking the cell borders, are not normally present in adult tissue. In areolar tissue, however, where the white fibers are few, the cells can be seen as faint, indistinctly outlined, and stellate or spindle-shaped. They are known as connective tissue cells, fibroblasts, or fibrocytes. (*βλαστὸς*, a bud, is used in many terms to indicate a formative cell, with a prefix which usually designates the structure produced. Since the fibers of connective tissue are probably not directly derived from the cells, the propriety of the term in this case may be doubted. 'Fibrocyte,' indicating a cell of fibrous tissue, is a better term but present usage favors 'fibroblast.') Their cytoplasm is a pale thin layer, almost homogeneous, ordinarily without granules, sometimes vacuolated. The nuclei are large (two or three times the diameter of a red blood corpuscle), oval and flattened. They have a delicate nuclear membrane and a small amount of finely divided chromatin. Seen on edge they are rod-shaped, and appear darker, since the light passes through a greater thickness of chromatin. Sometimes a small but distinct nucleolus is present. Occasionally a centrosome is seen, occupying an indentation in one side of the nucleus.

In dense connective tissue the cells exhibit broad *thin* cytoplasmic processes, which are bent to conform with the adjacent fibers, to which they are closely applied, and along which, in living tissue, they have been observed to migrate; but the flange-like processes are often so delicate that it is impossible to trace their extent. Often nuclei, seen in profile, stand out prominently along the edge of a fiber bundle with no visible cytoplasm surrounding them.

During mitosis the fibroblasts of both areolar and dense connective tissue retract their processes and become round or angular in form, apparently floating freely. After division both daughter cells resume their stellate form and become attached to their neighbors as fixed fibrocytes. The other cells present will be described later.

Between the fibroblasts, in the intercellular spaces, are found white fibers and elastic fibers, varying greatly in amount in the different types of connective tissue. These may be studied in the fresh state or in fixed and stained preparations. A small mass of fresh connective tissue, subcutaneous or intermuscular, may be spread out with needles upon a slide, thus forming a thin film. After adding a drop of water and apply-

ing a cover glass, it will present such an appearance as shown in Fig. 70. The bulk of the tissue is seen to consist of white or collagenous fibers felted together. They are the same in origin and structure as those already described in the mucous tissue of the umbilical cord, but in ordinary connective tissue their fibrils are gathered into denser bundles. Each bundle or *fiber* is composed of exceedingly minute *fibrils*, bound together by a small amount of 'cement substance.' The addition of picric acid causes the fibers to separate into their constituent elements. Often a bundle of fibrils turns aside from the main trunk, so that the fiber branches, but the fibrils themselves are unbranched.

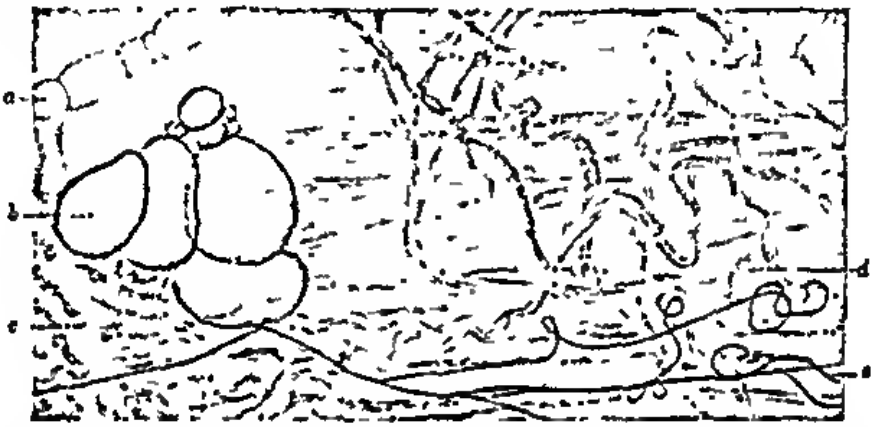


FIG. 70.—SUBCUTANEOUS TISSUE FROM A CAT

The fiber a has been treated with dilute acetic acid, the other fibers have been teased apart and examined, unstained, in water a, c, White fibers, b, fat cells, d, connective tissue cell, e, elastic fibers

Upon the addition of dilute acetic acid the white fibers swell and disintegrate, some of them passing through the condition shown in Fig. 70, a. Such fibers show a succession of constrictions at places where they are encircled by rings or spiral bands of a refractive substance not affected by the acid. These rings have been observed by Ranvier in living connective tissue fibers, and it is therefore improbable that they are remnants of a sheath which surrounded the entire fiber, as some have thought. They may be more resistant portions of a cement substance binding the individual fibrils together, of which the greater part is disintegrated by the acid. The relations of the white fibers to the intercellular fluid are not at all clear.

In addition to the white or collagenous fibers, connective tissue contains fibers of a second sort, known as *elastic fibers*. They are absent from corneal tissue, the mucous tissue of the umbilical cord and generally, though not always, from reticular tissue. They develop later than the

FIG. 71.—CONTROLLED FORMATION OF COLLAGEN AND RETICULUM GUINEA-PIGS RENDERED SCORBUTIC ON DIET DEFICIENT IN VITAMIN C AND RECOVERING ON GIVING ORANGE JUICE.

1. Collagen (in blue) deposited around an isolated cell within a blood clot 2 Collagen (in blue) around cells, penetrating fibrin strands (in red) 3 Argyrophil fibers formed with same relation to cells as collagen 4. A later stage, no argyrophil fibers. 5 Argyrophil fibers in endochondral bone formation 6. 'Endosteal' surface of rib in absolute scorbutus Fibroblasts and fibroglia are in red 7 'Endosteal' bone formation during a 40 hour recovery period Relation of collagen to cells active as osteoblasts Zenker fixation, Mallory's connective tissue stain (figs. 3 and 5 also impregnated with Foot's modification of Bielschowsky-Maresch silver method (Wolbach)

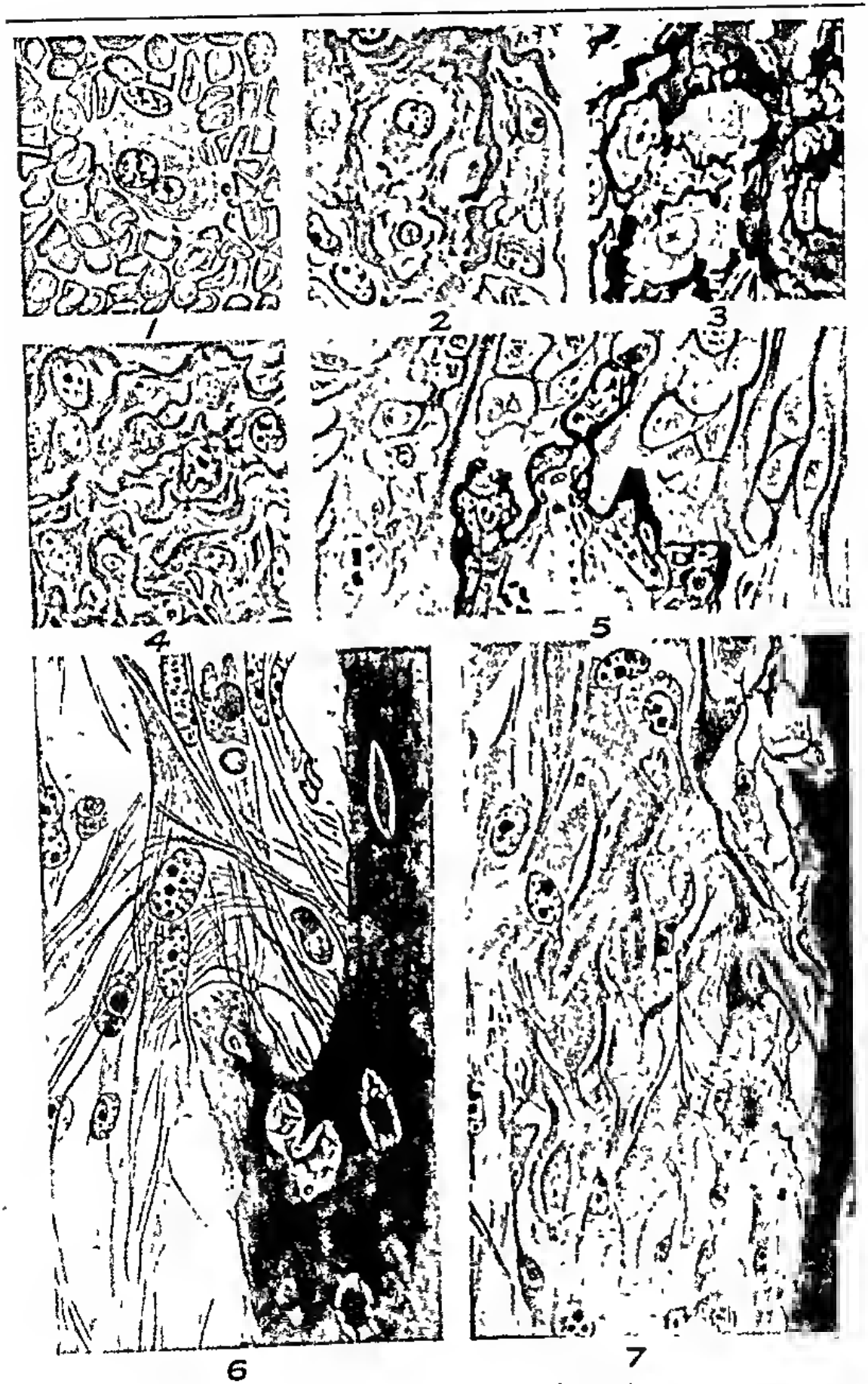


FIG. 71.—FOR DESCRIPTIVE LEGEND SEE OPPOSITE PAGE

white fibers, and are not found in the youngest connective tissue; but otherwise they are present, though varying greatly in abundance, in all forms of connective tissue. They are not destroyed by dilute acids or alkalies, and are described as composed of *elastin*, an albuminoid body which does not yield gelatin on boiling. Unlike the white fibers they are not composed of smaller elements or fibrils, but each fiber is a structureless homogeneous thread. In favorable cases, however, an enveloping sheath may be seen. In tissue which has not been torn apart the elastic fibers form a net. The fibers meet and fuse with one another; and across the angles thus formed, one or two delicate strands are commonly

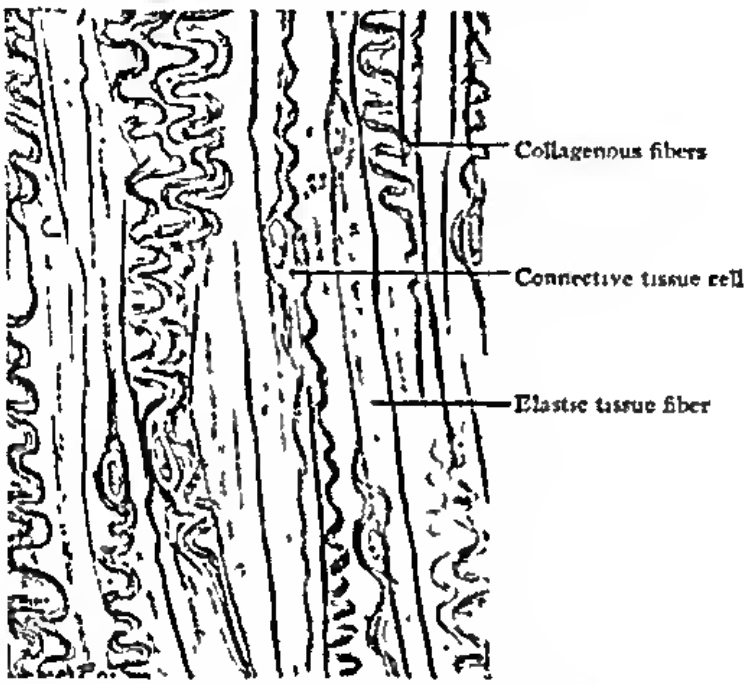


FIG 72.—ELASTIC TISSUE. LIGAMENTUM NUCHAE OF OX.
The elastic tissue fibers are broad and straight, the collagenous fibers are in wavy bundles. Note the scarcity of connective tissue cell nuclei. Formaldehyde fixation, Azan.

to be found. When the tissue is pulled apart so that the net is broken, the fibers kink and recoil like tense wires. In cross section they may be round or flattened.

Although elastic fibers are clearly seen in fresh connective tissue, they are often invisible in specimens stained with hæmatoxylin and eosin. In order to determine their presence, sections may be stained with resorcin-fuchsin, which leaves the white fibers nearly colorless, but makes the elastic fibers dark purple; or other special stains may be used. In some situations, however, the elastic tissue is highly developed and may be seen with any stain. This is true of the fenestrated membranes found in many blood vessels. A fenestrated membrane is a network of elastic fibers in which the fibers are so broad that they appear to form a perforated plate. The greatest development of elastic tissue probably occurs

in the ligament of the neck in grazing animals, which consists of very coarse elastic fibers with very little white fiber. It is therefore commonly used for the histological and chemical study of elastic tissue (Fig. 72). In man the stylohyoid ligament and the *ligamenta flava* are of this class, and they exhibit the yellowish color which is characteristic of elastic tissue. Elastic fibers are found also in the ground substance of certain cartilages, which will be described later.

The usual action of elastic fibers is to tend to return to their normal length after being stretched. The larger sheets of this tissue, however, seem to resist being crumpled by muscular action, so that their recoil would lengthen the tissue. These crumpled masses of elastic tissue are especially noticeable in contracted arteries.

Where connective tissue comes in contact with an epithelial layer it frequently, but not invariably, is modified to form a 'basement membrane' (see p. 78). This is usually a structureless sheet, sometimes collagenous, sometimes elastic. But in some regions of the body the basal membrane may be fibrous and may even contain flattened cells. The membrane may be of extreme thinness, as is the case around the secreting portions of most glands, or of notable width, as in the trachea (Fig. 49). Though the basal membranes are usually considered as modifications of the connective tissue, in a few localities, as in the cornea, the epithelium is said to take part in their formation by a sort of basal secretion.

There has been much discussion as to the chemical relationships of the white, elastic, and reticular fibers, and as to their origin. That any of the fibers are derived by differentiation of the exoplasm of the cells, either directly or through chemical alterations of the fibroglia fibers, is no longer credited. Henle (1841) considered that they arose in the intercellular substance, the cells taking no direct part in the process other than the production of the intercellular gel. Other comparable colloidal gels (such as solutions of egg albumen, gelatin, etc.) may precipitate a fiber network under the proper chemical conditions, much as fibrin is formed in blood plasma.[1] The mesenchymal cells may be the agents, by secretion, for supplying the proper conditions. Maximow[2] states: "Thus, abundant formation of true collagen can be obtained in cultures of adult mammalian connective tissue. The process follows the same paths as in the body. At first argyrophil fibrillar networks appear which in every respect are identical with the so-called reticulin or lattice fibers. Later, with continued increase in quantity, the fibers become arranged in parallel, wavy bundles, lose the argyrophilia and begin to stain in the fashion of mature collagen. Nothing could be observed which would substantiate the idea of the transformation of the cellular protoplasm or exoplasm into reticulum or collagen. The first fibrillæ appear in the medium surrounding the cells, as the result of precipitation or, perhaps, of transformation of some colloidal sol into a gel under the influence of an unknown factor, probably of chemical nature, which originates in the cell body of the fibroblasts and diffuses into the surrounding medium. Therefore the fibrillæ first arise in the immediate neighborhood, sometimes directly on the surface of the cytoplasm.

[1] Isaacs, 1919. [2] Maximow, 1928.

They, however, extend at once far into the medium, away from the cells. It is probable, that, as Heringa and Lohr[1] suggested, the particles of the colloids in question are rod shaped. This causes the gel to assume a fibrillar structure. The fibrin threads of the plasma clot seem to serve as pathways for the precipitating material. Whether the fibrin itself is transformed into reticulin and later into collagen (Baitsell[2]) is doubtful.' Reticular fibers differ from the elastic elements of connective tissue, since reticular fibers are dissolved by both acids and alkalis which leave the elastic fibers intact; and they are not destroyed by pancreatic digestion which causes the elastic fibers to disintegrate. Mallory and Parker[3] believe that reticulum and collagen are chemically the same. Collagen is produced by the fibroblasts and there are no reticular cells other than fibroblasts. Wolbach[4] finds that the alignment and distribution of collagen is determined by the shape of the cell and its processes and that the only difference between reticulum and collagen fibers is physical. A similar conclusion is arrived at by Mary L. Stearns[5] who studied the formation and development of connective tissue in the living animal. The morphological distinction then between the two tissues is that reticular fibers are separated while collagen fibers occur in bundles Therefore when treated by appropriate impregnation methods the reticular fibers are blackened, but the collagen fibers are not. The fine reticular fibers having a lesser surface would have a greater deposit of silver upon them than the coarser bundles of collagen fibers. Scott[6] has shown with the electron microscope that connective tissue fibers exist which cannot be seen with ordinary instruments.

The Free Cells of Connective Tissue. Within the tissue spaces, floating freely in the intercellular fluid which bathes the fibers and the fibroblasts, other cells are present in varying numbers. Many of the cells of the blood may occasionally be found, sometimes in disease in great numbers, for they may leave the blood stream and wander through the tissue spaces; but other cells remain so permanently in the connective tissue that they may be considered a normal part of it. The differentiation of these cells from fibroblasts and from the leucocytes of the blood, and their classification among themselves, are rendered difficult by the fact that the varieties are not always comparable in the different animals studied, and that hence the nomenclature has become greatly involved. The differentiation of the various classes depends not only on the form of the cytoplasm and nucleus, but also on the staining reactions, the activity of the cells in living conditions, and the avidity with which they ingest particulate matter.

The *histiocyte*, *macrophage*, or *wandering cell*, is apparently the most common type. These cells vary greatly in number, however, in different parts of the body, being infrequent in connective tissue where blood vessels are few. They are large cells of various shapes, flat, rounded or angular, often spindle-shaped in profile or showing blunt pseudopodia. They lie either singly or in small groups in the tissue spaces, sometimes

[1] Heringa and Lohr, 1926. [2] Baitsell, 1915. [3] Mallory and Parker, 1927.
[4] Wolbach, 1933. [5] Stearns, 1940. [6] Scott and Anderson, 1942.

stretched along the white fibers forming a net like mesenchyma, sometimes floating free in the tissue spaces. In the first case they are called 'fixed' or 'resting'; they become true wandering cells by withdrawing

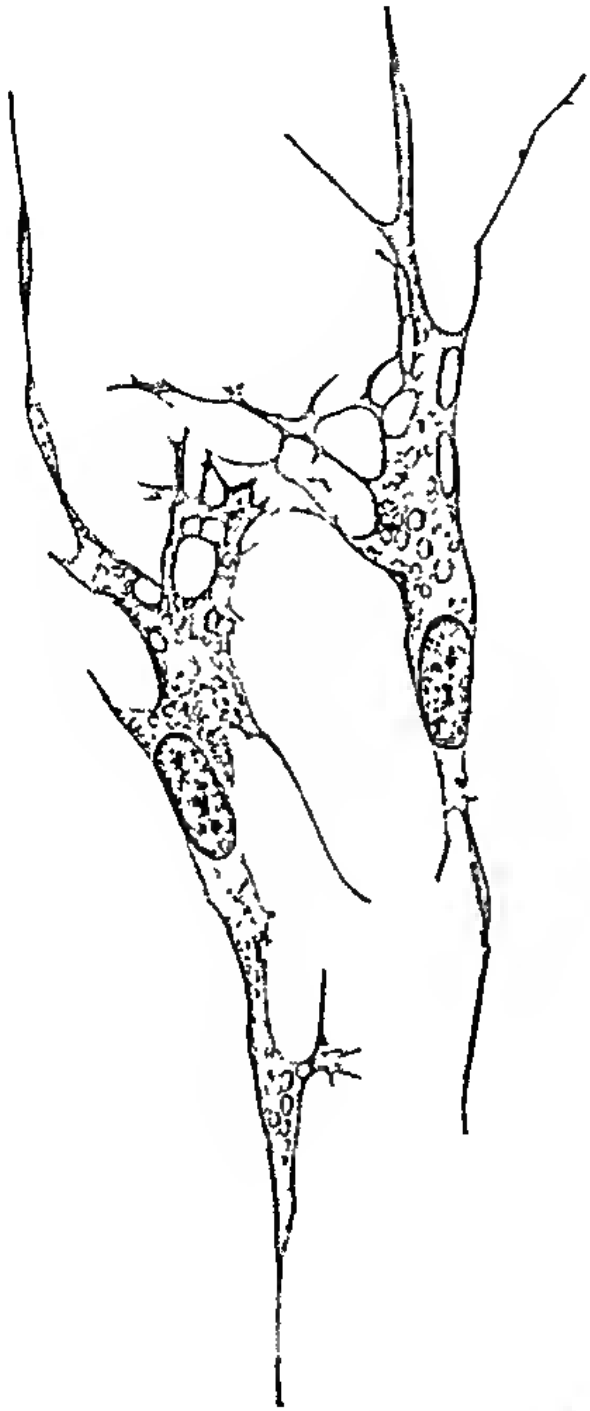


FIG. 73.—FIBROCYTES IN LOOSE SUBCUTANEOUS CONNECTIVE TISSUE OF MAN. × 800 (von Möllendorff.)

their processes and becoming spherical. Their nuclei are smaller than those of fibroblasts, round or oval, with coarser nuclear membrane and larger chromatin granules, so that in stained specimens they appear darker. Their cytoplasm is often darker, granular or vacuolated with sharp cell border, often irregular. The chief distinction between fibro-

blast and macrophage, however, is brought out by vital or supravital staining, that is, the injection of a particulate stain into the living animal or the use of such stains on fresh tissue. Under these conditions the fibroblasts are found to remain almost unstained, while the macrophages have ingested many of the dye granules (Figs. 75 and 76). Their phagocytic activity often leads to the formation of vacuoles of digestion within the cytoplasm. *Phagocytosis is their most pronounced characteristic*, and gives the name 'macrophage,' 'large eating cell,' by which they are most commonly known. They are also known as 'histiocytes,' or 'tissue cells,'

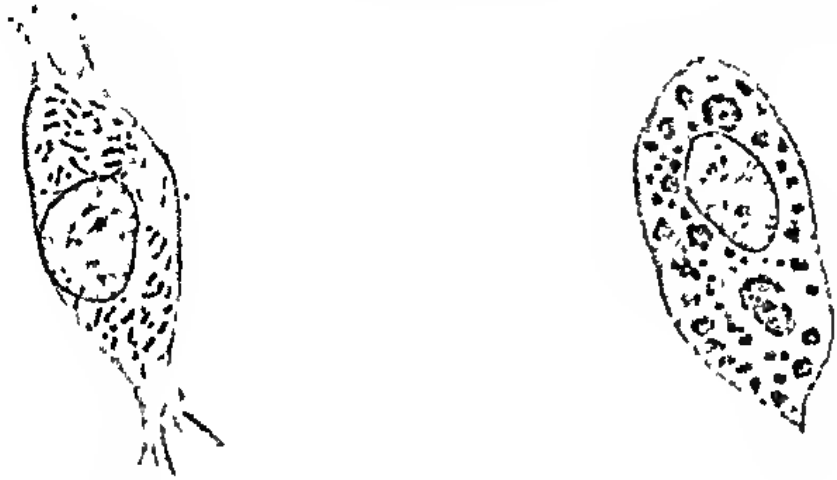
FIG. 74.—RESTING WANDERING CELLS (HISTIOCYTES) FROM THE SUBCUTANEOUS CONNECTIVE TISSUE OF MAN. × 800. (von Möllendorff.)

and as 'clasmatocytes,' or 'breaking cells,' a name suggested by Ranvier from the fact that after treatment with osmic acid small round particles of the exoplasm of the cells were seen to break off. They are probably mesenchymal cells differentiated, more than the fibroblasts, for phagocytosis, like those of the reticular tissue. Whether they are the same cell as the endothelial phagocyte of the blood is a matter of controversy.

Mast cells are large irregularly round or oval cells, often showing *short pseudopodia; or they may be flattened or spindle-shaped, stretch*ing along the fiber bundles. Their nuclei are small, round, or oval, and stain darkly. They may lie singly or in small groups, especially in vascular areolar tissue. Mast cells were so called by Ehrlich[1] because he believed that they arose from over-nourished connective tissue cells (Mast is the German term for the acorns on which animals are fattened) located near the blood vessels. Their most striking characteristic is the presence in the cytoplasm of large granules, sometimes filling the cell so that the nucleus is obscured, and staining intensely with basic dyes. The granules, however, are imperfectly preserved by certain common fixing reagents, especially those containing much water. With poly-

[1] EHRLICH, 1879.

chrome methylene blue the granules stain a deep purple. Such granules which assume a color different from that of the stain employed are called *metachromatic*. With neutral red the granules are red. Mast cells are not phagocytic and only feebly motile. Cells with similar granules are found normally in the blood, but are said not to be of exactly the same type, and are therefore called 'basophilic leucocytes' as distinguished from the 'mast cells.' Michels[1] in a comprehensive review of mast cells states



a b

FIG. 75

Fibrocyte (a) and histiocyte (b) from loose connective tissue around the kidney of an albino rat. The animal was given 14 subcutaneous injections of 1 cc. each of 0.5% aqueous solution of Trypan blue. Susa fixation, paracarmine.

that the chemistry of the granules is unknown and that any speculation as to the relation of them to mucin is based solely upon the similarity in staining. Their reactions suggest that they contain protein. Holmgren and Wilander[2] think that the metachromatic substance in the granules is identical with heparin,[3] a coagulating hindering substance formed in the liver.

Eosinophils, or tissue eosinophils, are cells with granules within the cytoplasm which stain intensely with the acid dye eosin. Otherwise they resemble the mast cells in size and nucleus. The ordinary fixing fluids do not affect these granules. They are especially prominent in the lactating breast. It has been suggested that the eosinophilic granules were derived from ingested particles of red blood corpuscles, but there is little to uphold this idea except the similar staining reaction of corpuscles and granules. Some claim that basophilic granules ripen into eosinophilic. Others regard the tissue eosinophils as cells which have passed from the blood stream into the tissues.

Plasma cells usually have very round nuclei with characteristic coarse masses of deeply staining chromatin. These masses may appear as

[1] MICHELS, 1938. [2] HOLMGREN AND WILANDER, 1937.
[3] HOWELL AND HOLT, 1918.

wedge-shaped bodies with their broad ends against the nuclear membrane so that they resemble the spokes of a wheel ('Radkern'); or the chromatin blocks may suggest the squares of a checkerboard. The nucleus occupies an eccentric position in the mass of dense and deeply staining protoplasm. Specific granulation, such as occurs in mast cells and eosinophils, is absent. In certain plasma cells, vacuoles are seen which contain a 'homogeneous semifluid, colloid-like substance which

FIG 76.—MESENTERY RAT VITAL STAINING WITH PYRROL-BLUE, COUNTERSTAIN, NEUTRAL RED (KIYONO)
B v, blood vessel, Fb., fibrocyte, Ly, lymphocyte, M 1, M 2, M 3, macrophages, Mst., mast cell

has a strong affinity for acid dyes.' If the affinity for such dyes has become well marked, these vacuoles form conspicuous structures, known as Russell's bodies. Usually they are regarded as degenerative products, but some investigators consider them as secretions. It is thought by some, because of the different staining reactions of plasma cells in different regions of the body, that more than one type of cell is included under this term.

Lymphocytes are often found associated with plasma cells. They differ from plasma cells in having only a small rim of pale cytoplasm about the nucleus, but the nuclei of these two sorts of cells are very similar. They also form a large percentage of the cells of the blood, and make the main part of 'lymphoid tissue,' where they are gathered in great numbers.

They are called large or small lymphocytes, according to the amount of their cytoplasm, and vary considerably in size. They are only infrequently phagocytic to vital dyes, and are apparently the same type as are found in the blood stream.

The origin of the various free cells of the connective tissue spaces has been the subject of considerable controversy. The lymphocyte is considered by some as the parent stem of most or all of the cells of the blood, and also as the forerunner of the plasma cell. Others derive the plasma cell directly from the fibroblast. A short discussion of this point is given by Kingsley.[1] Maximow[2] recognizes the presence in connective tissue, especially near blood vessels, of an embryonic or mesenchymal type of cell, which because of its lack of differentiation is able to produce all the cells of connective tissue, as well as the fibrocytes. The macrophage has been especially studied by Evans and Scott.[3] The original differentiation of cell types by the staining of their granules is given by Ehrlich;[4] their activity when submitted to vital dyes is described by Kiyono.[5]

Connective tissue contains two additional types of cells, which are so distinct that they may be regarded as separate tissues. These are the pigment cells and the fat cells; the latter will be described as adipose tissue.

Pigment Cells. The color of the various tissues is due to pigments, which may be in solution, like the hæmoglobin in red blood corpuscles and the lipochromes in fat; or they may occur as granules embedded in the cytoplasm. The granules, which are yellow, brown, or black, often retain their natural color in stained specimens. They are said to consist of 'melanin,' which represents an ill-defined group of substances, some of which are hæmoglobin derivatives. These substances, which are widely distributed in the body, are described by Jacobsen.[6] In the lung, inhaled soot is taken into the cytoplasm of certain cells which thus become pigmented with extraneous material. Pigment granules are widely distributed, and may be found in the liver, spleen, heart, brain, and other organs.

In certain situations, pigment is extensively developed in branched connective tissue cells such as are shown in Fig. 10. In man these are of limited occurrence, being found near the eye, and in the pia mater especially under the medulla oblongata and upper portion of the spinal cord. Weidenreich considers that this represents the remains of a general pigmented sheath for the entire nervous system. In lower vertebrates branching pigment cells are often abundant in the subcutaneous tissue, and changes in color, such as occur in frogs, are due to the extension or

[1] Kingsley, 1924. [2] Maximow, 1929. [3] Evans and Scott, 1921.
[4] Ehrlich, 1879. [5] Kiyono, 1914. [6] Jacobsen, 1934.

retraction of these processes. Such pigmented connective tissue cells are called chromatophores or *chromatocytes*. But in the human skin the pigment granules are in the epidermis, chiefly in the basal layers. In the stratified epithelium of the conjunctiva of the eye, toward the cornea, numerous pigment granules are found in the basal layers, and scattered groups occur also in the outer layers. Pigment in this situation occurs frequently in the Caucasian race, and regularly in the other human races. Simple epithelium may be densely pigmented, as in the external epithelium of the retina. Thus it is seen that pigment cells are by no means limited to connective tissue.

Tendon. Tendons consist essentially of very dense connective tissue. They are composed almost wholly of parallel white or collagenous fibrils, compactly bound together in bundles. The cementing matrix contains *tendomucoid*. Closely applied to the bundles are the tendon cells which produced them.

In ordinary longitudinal sections of tendon the nuclei appear in rows, but the cytoplasm of the cells is often indistinct. This is because the narrow slits in which the cells lie between the massive fiber bundles are seldom found parallel to the line of vision, and the dense color given by the fibres obscures the more delicate color of the cell cytoplasm. When a clear view is obtained the cells are seen connected end to end, as in Fig. 77. In special preparations, particularly in those of the delicate tendons found in the tail of a rat or mouse (Fig. 78), it is seen that the cytoplasm of tendon cells forms a plate-like layer which is folded about the fiber bundles, tending to encircle them. Moreover the cells are provided with lamellar or wing-like projections, which extend out between adjacent fiber bundles, and may anastomose with those of adjacent cells. Thus, as in connective tissue, the original syncytial arrangement of the mesenchyma is partially preserved.

The primary tendon bundles, which consist chiefly of white fibers and tendon cells, contain also a small amount of elastic tissue in the form of fine, wide-meshed networks. The elastic fibers are said to occur especially near the cells and their processes. The primary bundles are generally grouped in secondary bundles or fasciculi, which are bounded by partitions or septa of looser connective tissue. Within the septa there are nerves and blood vessels in relatively small number. Lymphatic vessels are said to be confined to the sheath of connective tissue which surrounds the entire tendon, with which the septa are continuous.

The fibrous sheath or *vagina fibrosa*, which surrounds the tendon, may contain a cavity filled with fluid. It is then called a mucous sheath or *vagina mucosa*. The cavity arises as a cleft in the embryonic connective tissue and its walls are formed of mesenchymal (or false) epithelium. The

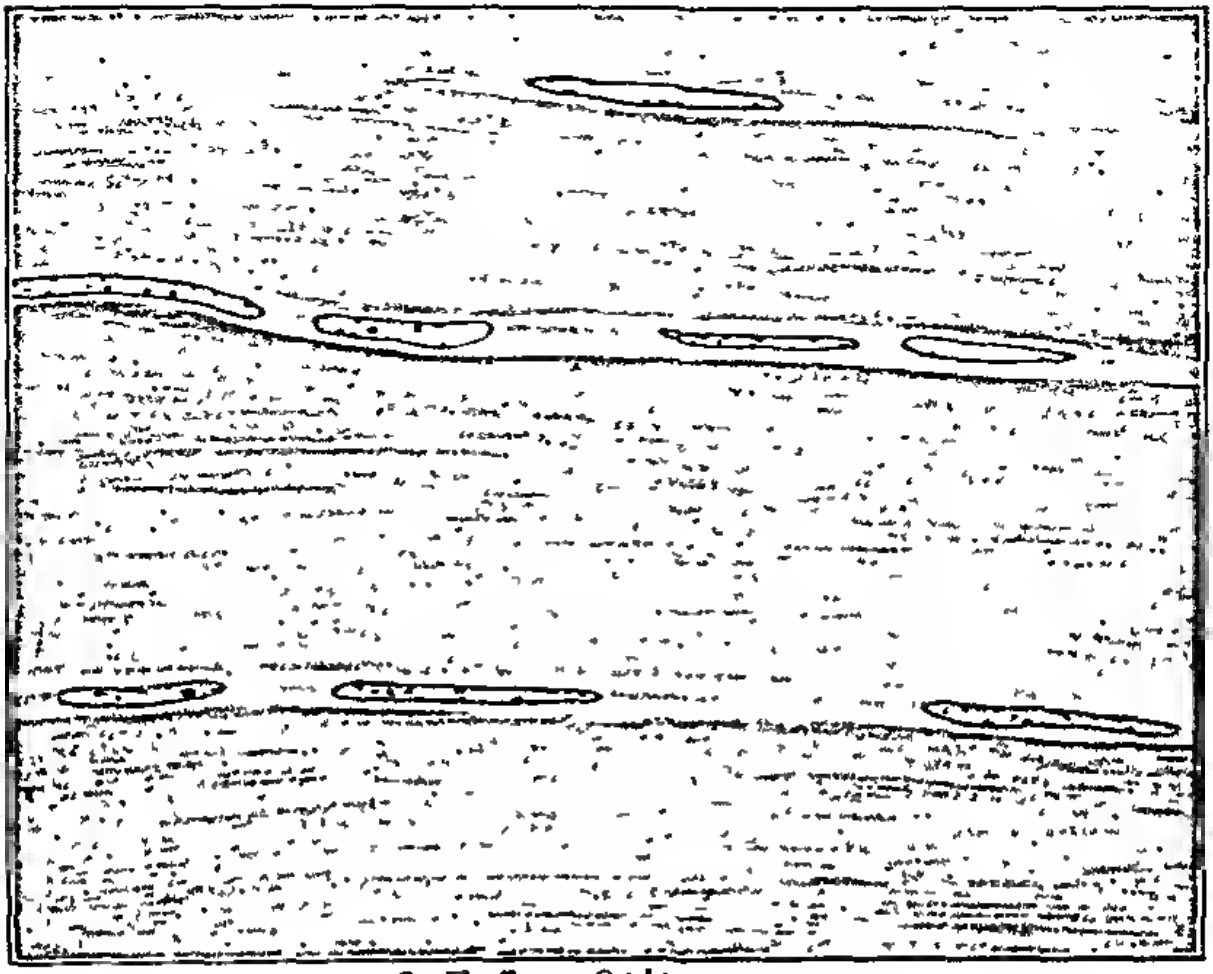


FIG. 77.—TENDON CUT LONGITUDINALLY
Chains of tendon cells between tendon fibers.

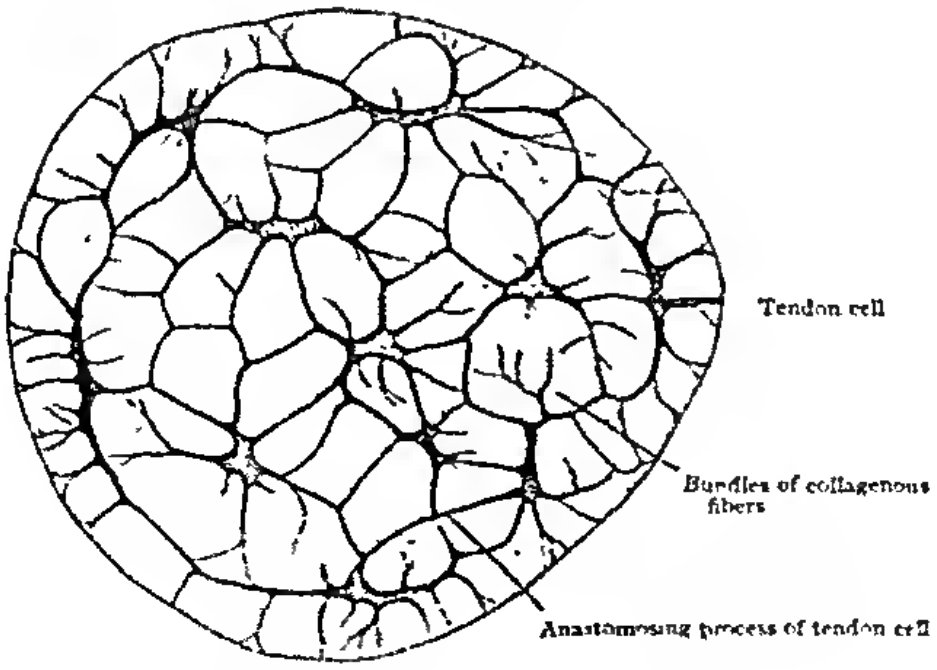


FIG. 78.—CROSS SECTION OF A THIN TENDON IN A MOUSE TAIL. GOLD CHLORIDE IMPREGNATION. (Schaffer)

cells have become flattened and the fibers felted together to bound the space. It contains a fluid like that of the joint cavities, being chiefly water and a mucoid substance which renders it viscid, together with protein material and salts. The function of the mucous sheath is to facilitate the movements of the tendon. By its formation the tendon is freed from the local connection with surrounding tissue, and the sheath generally occurs where such connection would especially interfere with motion. The *mucous bursæ* are similar structures in relation with muscles or bones. The joint cavities, to be described later, belong in the same class, having a similar origin and function.

Aponeuroses, fasciæ and ligaments are connective tissue formations, resembling tendon in possessing a more or less regular arrangement of cells and fibers. Elastic elements may be abundant.

ADIPOSE TISSUE

If in a freshly killed animal a loop of intestine is drawn out of the abdominal cavity, the blood vessels ramifying in its mesentery will be seen to be embedded in a band of fat, which branches when the vessels branch, and diminishes in width toward the intestine as the vessels become small. The close relation between the distribution of fat and the course of the vessels is notable also in sections. Fat cells occur in groups or lobules around the vessels, and are found, with few exceptions, wherever there is loose connective tissue. They may also occur singly, as in some parts of the denser connective tissue of the breast. The distribution of fat is not confined to any region or organ and there are only a few places in the body where it is not stored. In general the greatest amount is found in the subcutaneous tissues, in the deeper connective tissue around the kidneys, in the mesenteries and frequently, especially in some older persons, around the heart. Fat may be associated with other supporting tissue as in the epiglottis, the lyssa of the tongue and in the cavernous tissue of the penis and clitoris in some animals.

When examined fresh, each fat cell appears as a large, round oil-drop, which is more or less compressed into a polyhedral shape by the surrounding cells. It is highly refractive, having a border which becomes alternately bright and dark on changing the focus. The liquid fat or oil which fills the cell, leaving only an imperceptible film of cytoplasm around it, may escape by the rupture of the membrane, thus forming smaller drops. In the specimen shown in Fig. 70 the fat was seen coming out from the upper surface of one of the cells, and the droplets thus emerging ran together forming larger ones. As fat cells develop, a coalescence of small drops occurs in the cytoplasm. After prolonged injections of dyes, like trypan blue into a living animal, particles of

the dye may be seen in the cytoplasm of adipose cells. The storage of the particles is more like that seen in fibrocytes than in histiocytes (macrophages).

The earliest formation of adipose tissue is said to occur in human embryos of the fourth month. It may be studied advantageously in the subcutaneous tissue of embryos of the fifth month (Fig. 79). In such specimens there are areas of loose and very vascular mesenchyma, found at the level of the roots of the hairs, in which certain cells exhibit

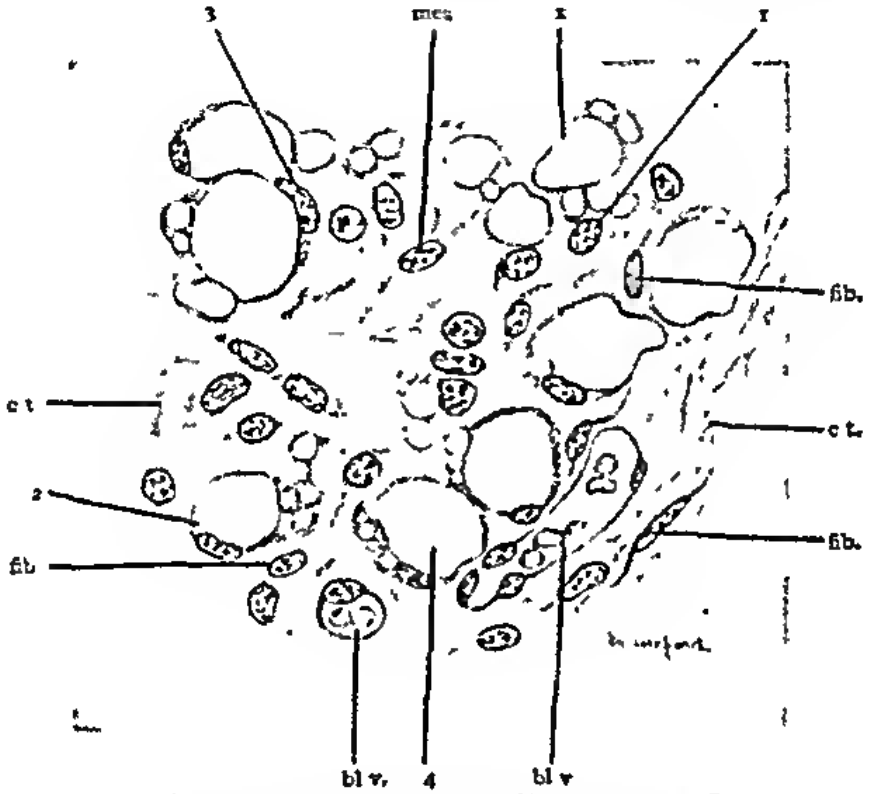


FIG. 79.—FAT ISLAND SUBCUTANEOUS TISSUE OF A HUMAN EMBRYO OF FOUR MONTHS
mes., mesenchymal cell, fib., young fibrocyte 1, 2, 3, stages in the development of fat cells; 2, young fat cell cut without showing nucleus, c.t., white fibers

vacuoles. These cells are at first quite like the surrounding fibroblasts, being fusiform or stellate. Their cytoplasm contains several small vacuoles, some of which unite to form one large drop, and the nucleus, together with the greater part of the cytoplasm, is pushed to one side. Sections of such cells have the form of 'signet rings.' Frequently small vacuoles are seen in the accumulation of cytoplasm beside the nucleus. With further development the fat droplet becomes so large that the cytoplasmic rim appears as a mere line or membrane, just within which is the greatly flattened nucleus. During the formation of the fat cells, the branching processes become very short, but it is doubtful whether they are altogether *lost*.

For some years after birth fat cells containing several vacuoles are found in certain situations, as around the kidney and in the outer layer

of the œsophagus. Usually these are regarded as immature forms, and the groups have been compared to the fat organs and hibernating glands of lower animals. The fat cells in these groups may contain pigment in diffuse form or as granules in the cytoplasm and are especially well provided with blood vessels; hence they are known as 'brown fat.'

Adipose tissue of the adult, when well preserved, presents cells of rounded form as shown in Fig. 80; often, however, their thin walls are bent or collapsed. If the sections are thick, a network of a different pattern, representing another layer of cells, will come into view on chang-

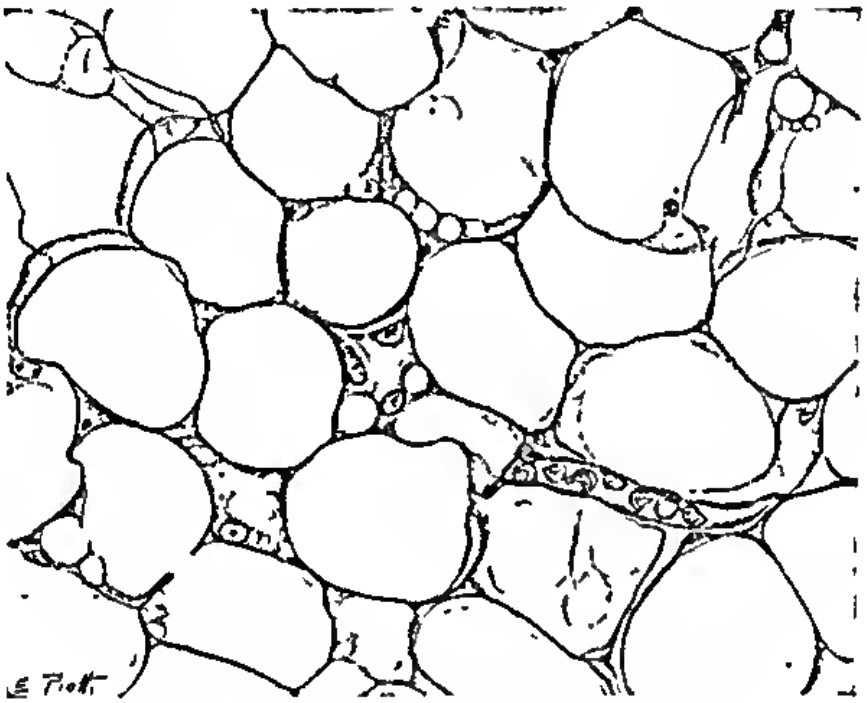


Fig. 80.—Adipose Tissue from the Anterior Mediastinum of a Monkey. Formaldehyde fixation, hæmatoxylin and eosin.

ing the focus. The nuclei of the fat cells are pale, oval bodies, with finely granular chromatin, often containing one or two small vacuoles. The *cytoplasm around the nucleus forms such a thin layer that it is scarcely* appreciable on surface view. Both nucleus and cytoplasm are much darker when seen on edge, since a thicker layer of substance is thus presented. When sectioned in this position the nuclei within the cells must be carefully distinguished from those of the connective tissue just outside. Many of the fat cells will show no nuclei, since the entire cell is usually not included within the limits of one section.

Adipose tissue is never composed entirely of fat cells. Some of the *original mesenchymal cells of the area of development become fibroblasts* instead of fat cells, and the two tissues are always intimately mingled. Sometimes single fat cells may occur in connective tissue. A careful study of adipose tissue stained with Mallory's connective tissue stain reveals

the collagen fibrils in larger or smaller bundles forming a network between the fat cells binding them together and affording passage for numerous blood vessels.

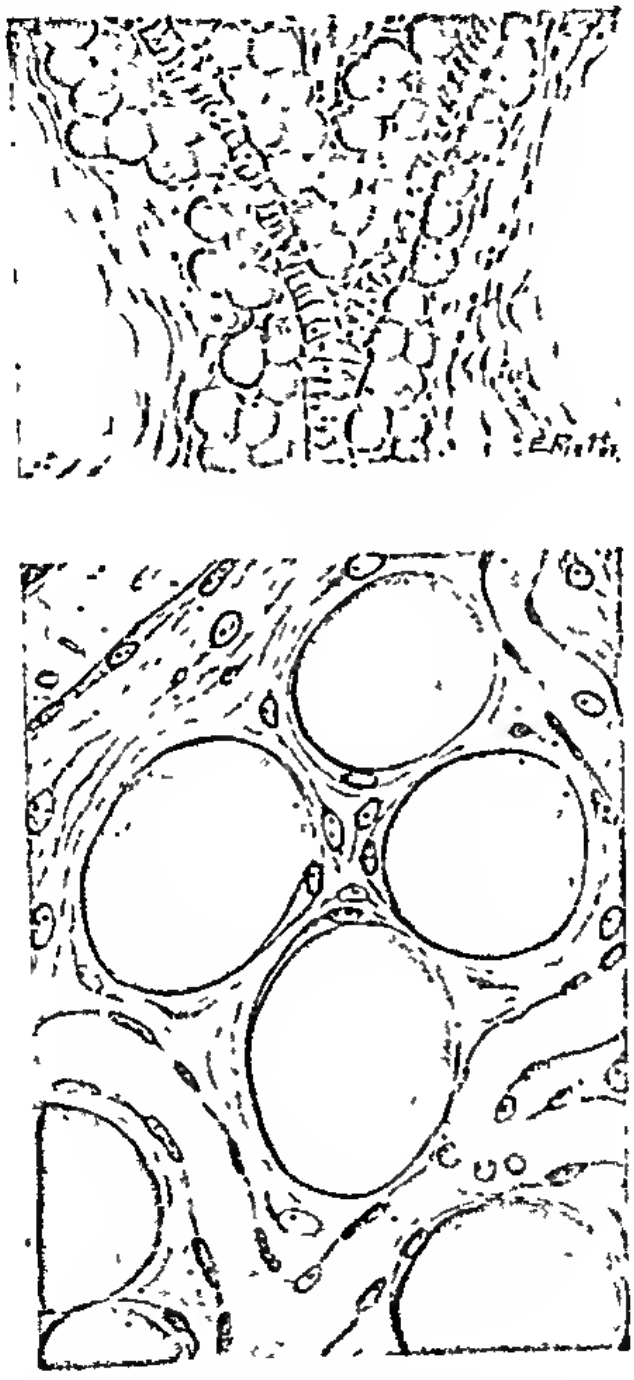


FIG. 81.—FAT CELLS IN THE MESENTERY OF A CAT.

Above, under low power note the relationship of the cells to a small blood vessel. Below, under higher magnification, see that a single globule of fat fills nearly the whole of the cytoplasm. Formaldehyde fixation; Sudan III and hæmatoxylin.

Flemming[1] regarded fat as being laid down in ordinary fixed connective tissue cells or fibroblasts, while Hammar[2] and others thought it took place in partially differentiated mesenchymal cells. Another view of the formation of fat is brought forward by Wassermann,[3] who considers that in a reticulo-endothelial network fat droplets develop within

[1] FLEMMING, 1871. [2] HAMMAR, 1895. [3] WASSERMANN, 1926.

the endoplasm regardless of cell areas, and by enlargement force the exoplasm to form the boundaries of large compartments. The exoplasm becomes changed chemically into a stiff outer layer, a kind of cuticle, staining blue with Mallory's connective tissue stain. He also finds that the reticulo-endothelial tissue may at the same time form blood corpuscles, and compares the fat islands, called by him the primitive organs, to the bone marrow and spleen. Dees-Mattingly[1] finds special thickenings or fibers in these sheets of cuticle, making a framework between the 'fat corpuscles.' She also describes a protoplasmic (endoplasmic) network within the fat drop. These views exclude the presence of connective tissue among the fat cells. These several views were arrived at from the

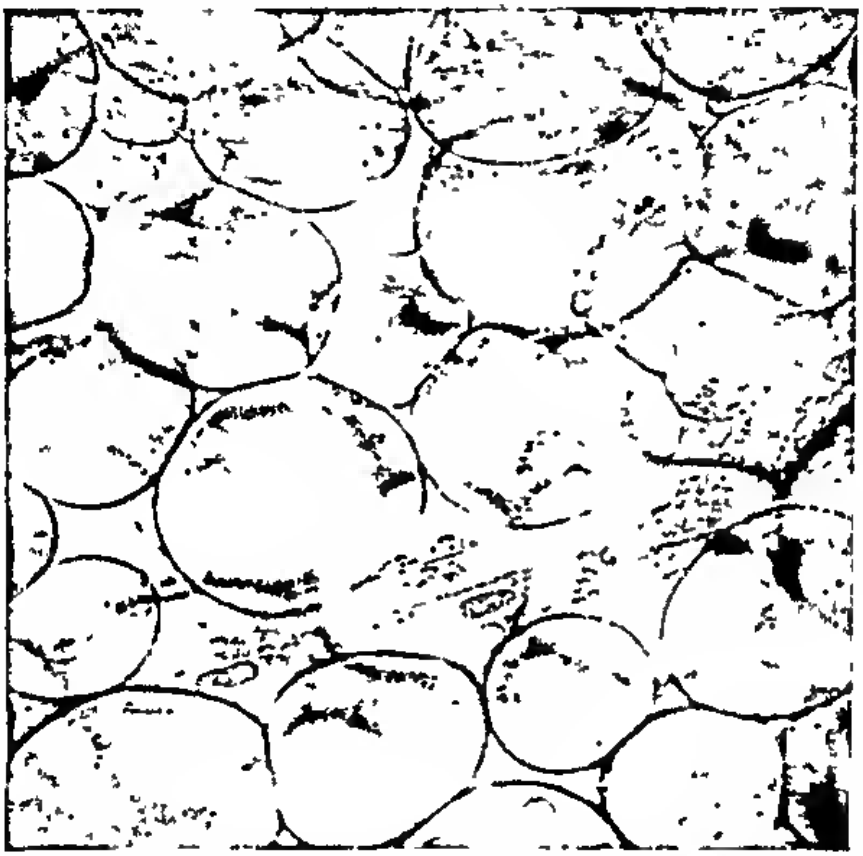


FIG. 82.—FRESH FAT FROM THE MESENTARY OF AN ALBINO RAT STAINED WITH 0.1% METHYLENE BLUE IN GLYCEROL.

study of sections of embryos and adult tissues. Clark and Clark[2] were able to see the formation of fat cells in the living animal. They found refractile droplets in cells which they were unable to distinguish from fibroblasts. These were seen to acquire, lose, and regain fat which apparently entered in soluble form and not by phagocytosis of visible globules

In extreme emaciation, the fat cells become small and the cytoplasmic rim thickens, so that the cells again assume the signet-ring form. A delicate reticulum appears between the shrunken cells. Some of the fibers proceed directly from the fat cells, indicating that the processes have never wholly disappeared. Others come from the fibrocytes which from the first are scattered among the fat cells.

The great difference between the appearance of fresh fat cells and those seen in sections is due to the fact that fat is dissolved by the reagents

[1] DEES-MATTINGLY, 1927 [2] CLARK AND CLARK, 1940a.

ordinarily used in preserving the tissue. Thus the sections usually show empty vacuoles and no fat whatever. Occasionally, as a result of cooling, the fat has formed insoluble crystals in the shape of radiating needles, and these, or an amorphous precipitate which takes a bluish stain with hæmatoxylin, may be seen within the cells. Although fat is the commonest substance to be found within the vacuoles in human tissues, it is not the only material which may have filled them, and therefore to *demonstrate the presence of fat, special methods must be employed.* Fresh tissue may be preserved in osmic acid, which blackens not only fat

FIG 83.—BROWN FAT FROM A NEW-BORN CHILD. MALLORY'S STAIN (WEIGERT)

but some related substances; or frozen sections of tissue may be stained with certain dyes soluble in fat, such as Sudan III, Scharlach R or Sudan black B, demonstrating in even minute droplets tones of orange, red or blue-black. These stains may also be used after preservation of the tissue in formaldehyde. It may be noted that Sudan III has been fed to animals, thus imparting a pink color to the living adipose tissue. If the animal is lactating, the fat globules in the milk also become pink.

Fat vacuoles occur in many sorts of cells which do not belong to adipose tissue, such as the cells of the liver, cartilage, and striated muscle. These cells are not called fat cells, even if their cytoplasm contains many vacuoles, and they do not resemble the cells of adipose tissue.

CARTILAGE

Cartilage (χόνδρος, chondros) is a solid, tough, resistant substance, not rigid and frangible like bone, yet strong enough to form the skeleton in some of the adult fishes and in the fetal forms of other animals. Many, but not all, of the bones of the adult skeleton are represented in the fetus by cartilages of similar shapes. The cartilage material consists of a

matrix in which are scattered *cartilage cells*, and each piece of cartilage is enclosed by a sheet of *perichondrium* which merges with the surrounding connective tissue.

Cartilage develops from mesenchyma in the *centers of chondrification*. We do not know the causes which initiate this development in certain positions, but certain of the vitamins are necessary for their proper regulation. The mesenchymal cells multiply and become crowded, forming a dense nodule. The nuclei lie close together, as the cytoplasm is much reduced. Then the central cells increase in size, the nuclei are more separated, and in this form the tissue is known as *precartilage*. Between the cells a thin network of matrix appears, first along the angles of the cells, which encloses the individual cells in separate spaces or compartments, called *lacunæ;* they are then called *cartilage cells*. At first the matrix stains lightly with eosin, but as it becomes denser it takes on the characteristic blue stain with hæmatoxylin.

The matrix is a mixture of collagen, chondromucoid, chondriotin-sulphuric acid in combination, and albuminoid substances. It is probably the result of some secretion of the precartilage cells poured out from the whole surface of the cells and acting on the intercellular fluid. It can increase between cells already buried in it, and the bulk of the cartilage also increases by the peripheral extension of the process. The precartilage and cartilage cells contain mitochondria, Golgi apparatus, and segregation vacuoles (neutral red vacuoles) which increase as the matrix is being actively formed, and decrease in older cartilage cells.

In becoming precartilage the mesenchymal cells are usually supposed to lose their branching processes and become discrete individuals. Renaut and Dubreuil,[1] however, state that the syncytial character of the tissue is maintained, though the processes are reduced to short bridges, and that the matrix forms around them by the transformation of the intercellular substance, through which at first free cells can wander. As the substance becomes denser the free cells are excluded, and as it increases in amount the intercellular processes become drawn out very thin, fragment, and are absorbed. In the skeletal cartilages of some of the lower forms minute canaliculi exist between the lacunæ, which may represent the channels formed as the matrix solidified around the persistent syncytial processes (cf. bone canaliculi). In mammalian cartilage *no* such channels exist, and the cells in the lacunæ are entirely isolated from the nutritive tissue fluids, receiving only substances which can seep through the matrix.

Once the precartilage has formed its matrix it becomes surrounded by connective tissue in which the fibers are in general parallel to the

[1] Renaut et Dubreuil, 1910.

surface. This layer is called the perichondrium, and begins adding to the cartilage already present, which thus enlarges by 'appositional' growth. The collagen fibers of the perichondrium are incorporated in the matrix. They are usually unnoticeable because they are of nearly the same index of refraction as the matrix; but they can be brought out by special treatment, as by artificial digestion or polarized light. The matrix forms around the perichondrial cells nearest the cartilage

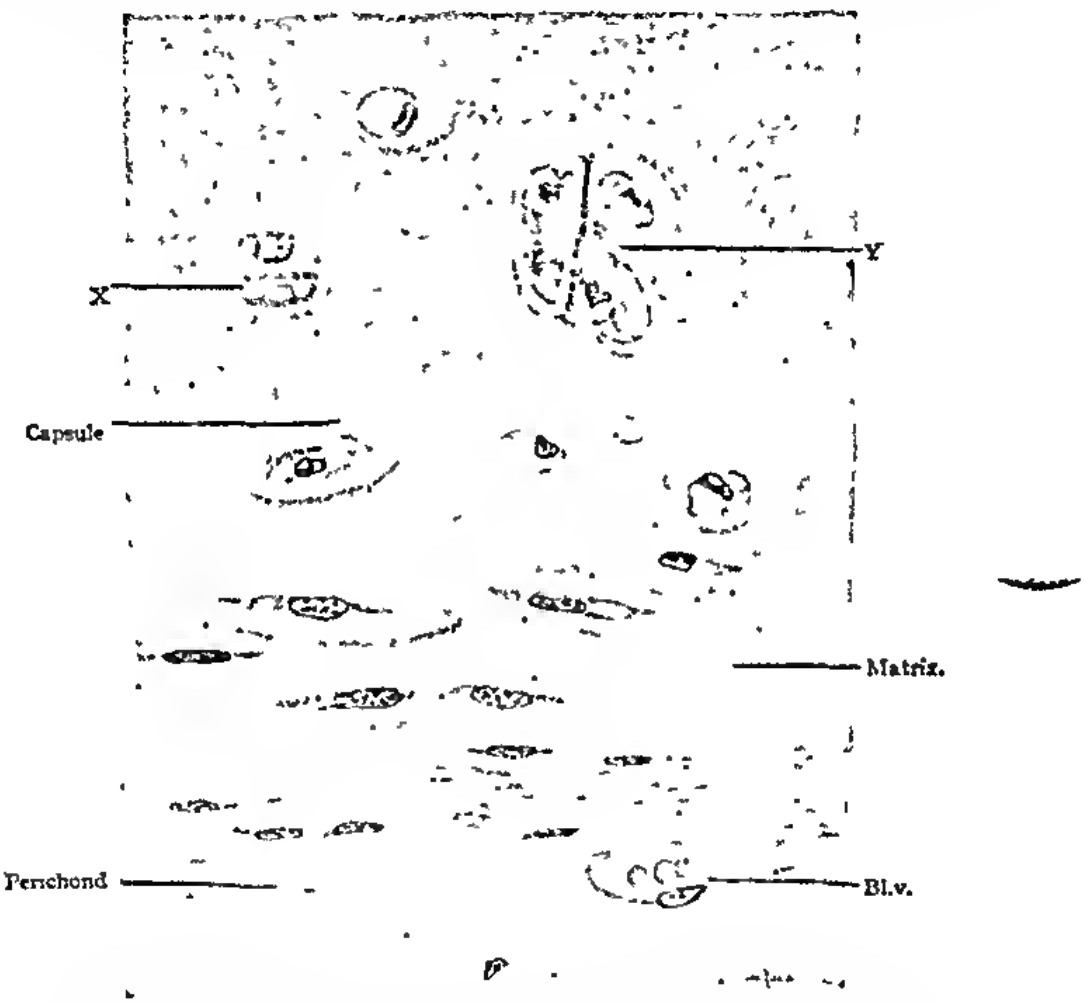


Fig. 84.—Hyaline Cartilage, Human Trachea, Including the Perichondrium 'x,' periphery of cells cut showing no nucleus, 'y,' new matrix between daughter cells

(chondroblasts), and they thus become cartilage cells living in lacunæ. In life the cells probably fill the lacunæ but in fixed material they may shrink, leaving a certain amount of space.

Immediately around the lacunæ the cartilage matrix may stain much more deeply with hæmatoxylin. This appearance varies in different specimens, in some being inconspicuous or absent (as in Fig. 84), in others giving the picture of rather broad bands, which may be concentrically striated, indicating that the matrix was deposited in successive layers. Such dense rims around the lacunæ are called *capsules*. The deep color is probably due to *chondromucoid*. Peripherally the color blends with

that of the intervening matrix, which takes a pale blue stain. Within their lacunæ the cells may divide, and after division two of them are found in a single capsule. They then move apart, and a partition of

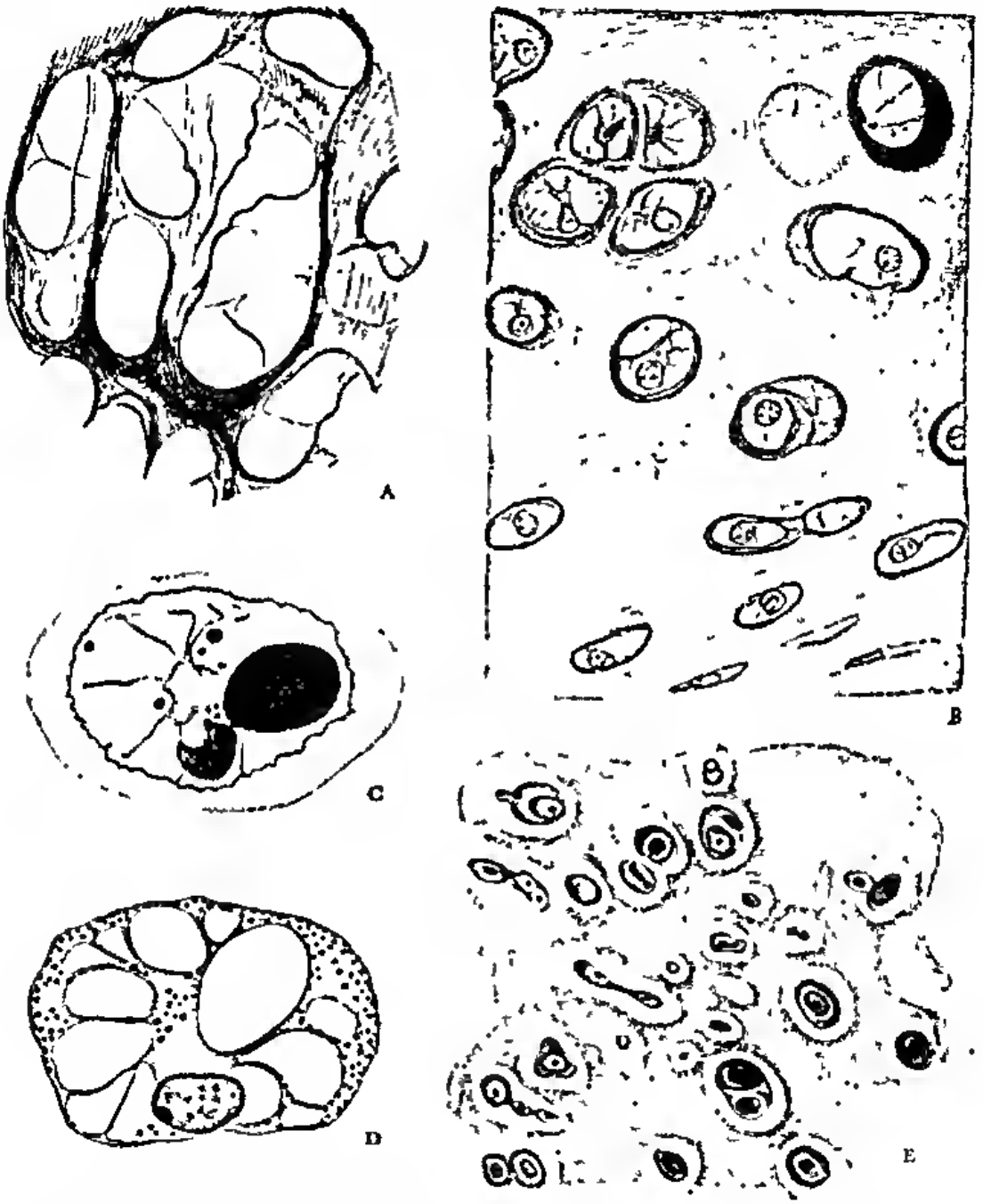


Fig. 85.—Hyaline Cartilage.

A, fibrous structure left after tryptic digestion Mallory stain (Rupprecht), B, trachea of a child Hæmatoxylin, carmine and orange G C, fresh cartilage cell Fat stains with Sudan III, nucleus with hæmatoxylin, D, Glycogen, alcohol fixation, Best's carmine and hæmatoxylin (Orujima), E, protein in the ground substance shown with Millon's reagent. (Morawitz)

matrix, at first very slender, is formed between them. They may remain grouped as a pair, forming a bisected elliptical figure, or they may divide again producing either a cluster of three or four (Fig. 84), or a row of cells (Fig. 98). Since the cells change their positions in the dense matrix

only with difficulty, by what is called *interstitial growth*, they are regularly found in very characteristic groups.

In becoming cartilage cells, the connective tissue cells of the perichondrium (chondroblasts) undergo an interesting cytomorphosis. In Fig. 84, a section of cartilage is shown extending from the perichondrium below to the middle of the cartilage above. From below upward the cells are seen to enlarge, and to become rounder, losing the processes which may have connected them. As they are buried deeper in the matrix they form small fat droplets within their cytoplasm. This is probably a sign of degeneration, and is often found pathologically in other tissues (*e.g.*, fatty degeneration of the liver) as a result of poor nutrition. The fat droplets increase in number, and may coalesce, but not usually to a single drop. In ordinary specimens, since fat is dissolved in alcohol or ether, the cells appear riddled with holes, the cytoplasm reduced to strands and a thin surface layer. The nucleus, which at first enlarges, may also contain fat, and finally becomes shrunken and pycnotic. Besides the fat, cartilage cells also contain glycogen.

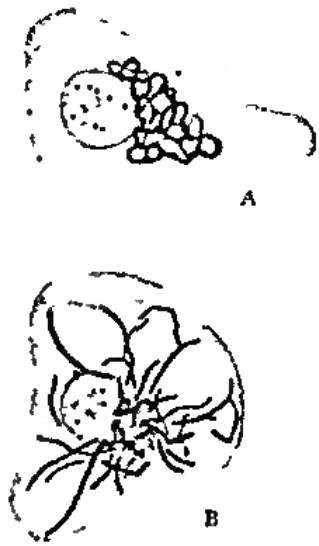


FIG. 86.—CARTILAGE CELLS
A, Golgi apparatus, costal cartilage of cat. B, mitochondria thyroid cartilage of Guinea-pig (Penna.)

Glycogen is a carbohydrate which resembles starch and is therefore sometimes called '*animal starch.*' *It is soluble in water, and soon after death it becomes converted* into glucose. For both of these reasons it disappears from ordinary sections. Fresh tissues, preserved in strong alcohol and stained with tincture of iodine, exhibit glycogen as brownish-red granules which may be aggregated in masses of considerable size. Glycogen is found not only in cartilage cells but also in striated muscle and in the cells of the liver. In the embryo it has a wider distribution. At certain stages of development, according to Gage, it occurs in the cells of the nervous system and is abundant in the epidermis, the digestive tube, and the cœlomic epithelium. *Its production, like that of fat,* varies with nutritive conditions, and it accumulates in well-nourished individuals.

The division of cartilage cells may also be considered the result of their insulation. J. Loeb[1] points out that certain ova divide parthenogenically after treatment with CO_2 or some other waste products of cell metabolism, which in the case of cartilage cells might be retained within the lacunæ. Amitotic division has also been considered as often accompanying the injurious treatment of cells. In old cartilage certain cells are said to undergo a mucoid degeneration, and become lost in the matrix, leaving only dark spots, staining intensely with hæmatoxylin. Such spots should be carefully differentiated from tangential sections of deeply staining capsules.

[1] LOEB, 1916.

It will be seen that the perichondrium is the formative layer of cartilage, from the cells of which the cartilage cells have been derived at successive ages. Cartilage grows very slowly, and the cells may remain in their various stages of cytomorphosis for long periods. After injury or surgical operations, a more or less perfect regeneration of cartilage may occur if the perichondrium is left in place, but not otherwise.

The perichondrium contains vessels and nerves, none of which ordinarily penetrate the matrix of the cartilage. In some cases[1] however, vascular connective tissue occupies an excavation in its peripheral

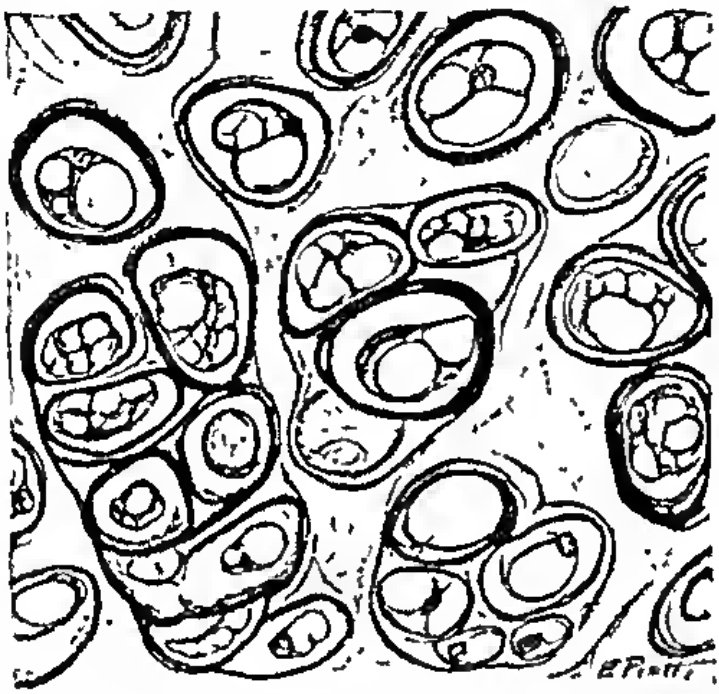


FIG. 87.—CARTILAGE CELLS FROM THE EXTERNAL EAR OF A RABBIT
Note the scarcity of matrix. Formaldehyde fixation, hæmatoxylin and eosin.

portion, and in the cartilage models for some of the larger short bones of the fetus (like the astragulus or patella, which become quite thick before calcification occurs) channels may be hollowed out through the matrix, from the periphery inwards, to carry blood vessels toward the center of the supply of the deeply buried cartilage cells. The blood vessels take part in the later formation of bone in the center of ossification. Similar vascular canals are also found in some of the epiphyseal cartilages of the long bones; they are destroyed with the completion of ossification. Most mammalian cartilages are in the form of thin strips, even the bulkier costal cartilages being composed of trabeculæ with spaces between, like the bone of the ribs. In old age, perhaps because the diffusion through the matrix is lessened, or because the cartilage sheet has become too thick, a deposit of calcareous granules occurs in the matrix. The degenerated cartilage matrix may thus become calcified, but does

[1] HURRELL, 1934.

not become true bone, lacking the characteristic pattern of the bone matrix. In degenerating portions of the laryngeal and costal cartilages, fibers having a luster like asbestos (or the mineral *amianthus*) are sometimes seen; according to Prenant these 'amianthoid' fibers are neither white nor elastic.

Fig. 88.—Elastic Cartilage, Human Larynx, Resorcin-fuchsin and Hæmatoxylin and Eosin.

The three principal forms of cartilage—hyaline, elastic, and fibrocartilage—and the exceptional 'vesicular supporting tissue' may be further described as follows:

Hyaline cartilage, the commonest type, is characterized by its clear, pale bluish or pearly translucent matrix, which is ordinarily free from distinct fibrils. The nasal cartilages, most of the laryngeal cartilages, and the tracheal and bronchial rings are of this variety, together with the xiphoid

and costal cartilages, and the articular cartilages which cover the joint surfaces of the bones. In embryos the greater portion of the skeleton is at first formed of hyaline cartilage. It is in this apparently homogeneous type of matrix that fibers can be discovered by suitable methods, as explained above.

Elastic cartilage contains, in its matrix, in addition to the usually invisible collagenous fibrils, fibers or networks of elastic substance (Fig. 88); consequently its color is yellowish. It is found in the external ear, the auditory (Eustachian) tube, the epiglottis, and in certain small cartilages of the larynx, namely the corniculate and cuneiform cartilages

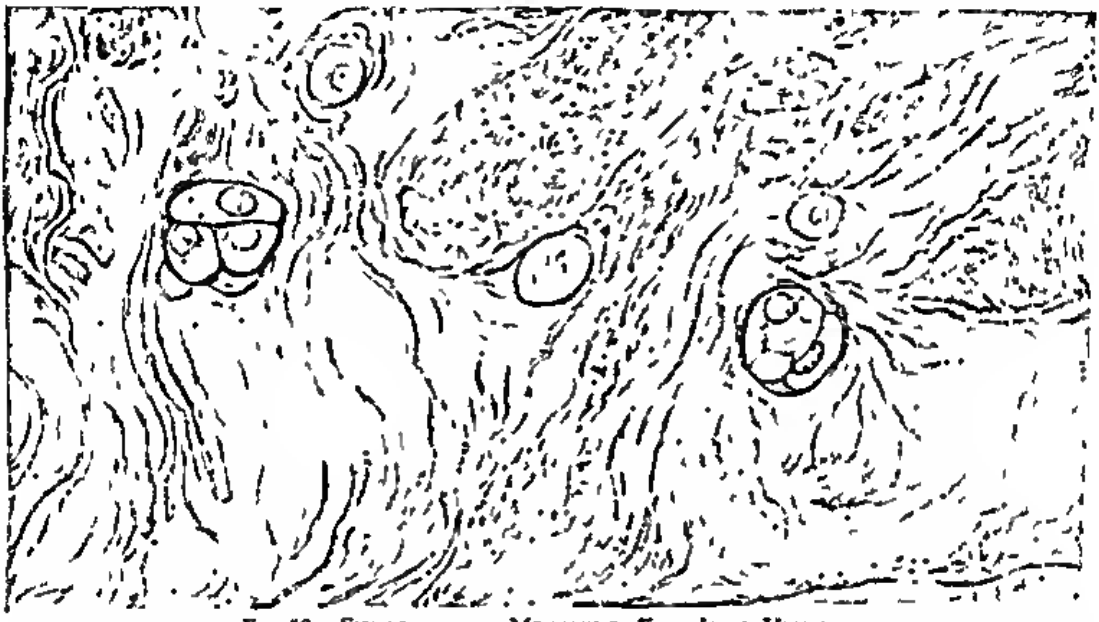


FIG. 89.—FIBROCARTILAGE, MENISCUS OF KNEE JOINT, HUMAN

and the vocal processes of the arytenoid cartilages. It develops from hyaline cartilage, which it closely resembles. The elastic fibers apparently originated from granules deposited within the matrix; authors differ as to whether or not the cells take any part in their formation. Later, with appositional growth, the elastic fibers present in the perichondrium are successively incorporated in the cartilage matrix. In Fig. 88 such fibers can be traced from the perichondrium into the matrix. The same cartilage may be hyaline in one region and elastic in another. The cells of elastic cartilage show less of the accumulation of fat and glycogen in the cytoplasm and fewer cases of cell division, as though they were receiving better nutrition. Perhaps the elastic fibers offer pathways for tissue fluid through the matrix.

The elastic nature of fibers within the cartilage matrix can be demonstrated by special stains, such as resorcin-fuchsin; they stain like the elastic fibers of connective tissue.

Fibro-cartilage cannot be regarded, like elastic cartilage, as a late modification of hyaline cartilage. In its early development, as seen in the

intervertebral disc of an embryo, its matrix is primarily fibrous. It is composed of dense connective tissue, the fibers of which here and there blend with a hyaline cartilaginous matrix containing round or oval lacunæ with enclosed cells. The proportion of collagen fibers and cartilage matrix varies in different positions of the body, so that it becomes at times a matter of opinion whether the tissue is fibro-cartilage or dense connective tissue. It is found typically developed in the inter-

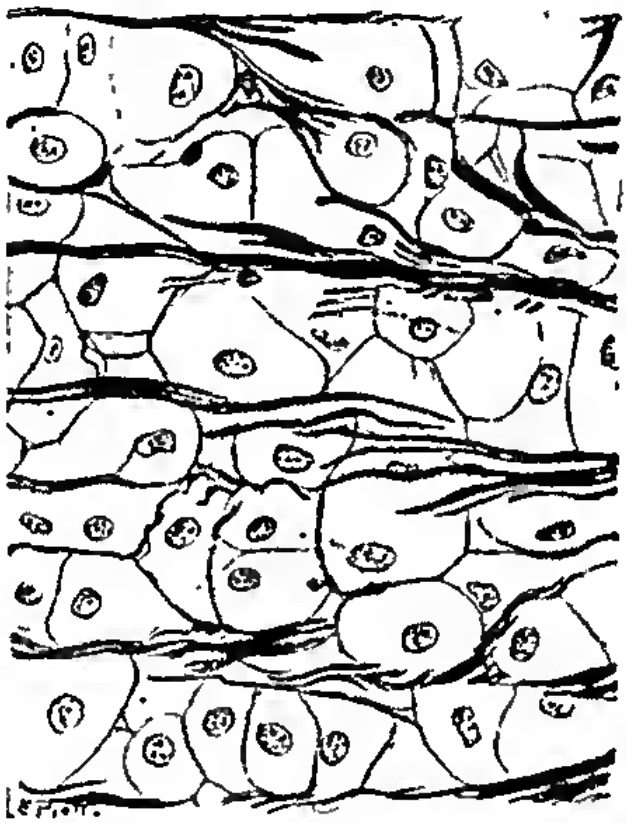


Fig. 90.—Chondroid Tissue or Pre-cartilage from a Spindle Nodule in the Tendo Achillis of a Frog. Formaldehyde fixation. Azan. The dark fibers are collagenous.

vertebral and interpubic fibro-cartilages. According to Stöhr it forms the articular cartilage lining the sterno-clavicular, acromio-clavicular, and mandibular joints, together with the joints of the costal cartilages, and it covers the head of the ulna. Usually it is said to form the rims deepening the sockets of the shoulder and hip joints, together with the inter-articular discs of the mandibular, sterno-clavicular and knee-joints, and it lines certain bony grooves through which tendons run. When typically developed, fibro-cartilage consists chiefly of interwoven bundles of white fibers. With hæmatoxylin and eosin this ground substance is diffusely stained, since the fibers, colored by the eosin, are embedded in a chondro-mucoid matrix which stains with hæmatoxylin. The cells are not flattened as in connective tissue. They are lodged in well-rounded lacunæ bounded by capsules and zones of blue-staining matrix; and they are frequently arranged in pairs or small groups such as occur in other forms

of cartilage. Their cytoplasm is extensively vacuolated and is sometimes shrunken.

'Vesicular supporting tissue' is a form of precartilage which consists of large vesicular cells in close contact, bound together by firm walls; it is a 'cartilage without a matrix.' In many invertebrates it is an important tissue, but in adult mammals it is of limited occurrence. In man such a tissue is said to be present on the inner surface of the tendon of

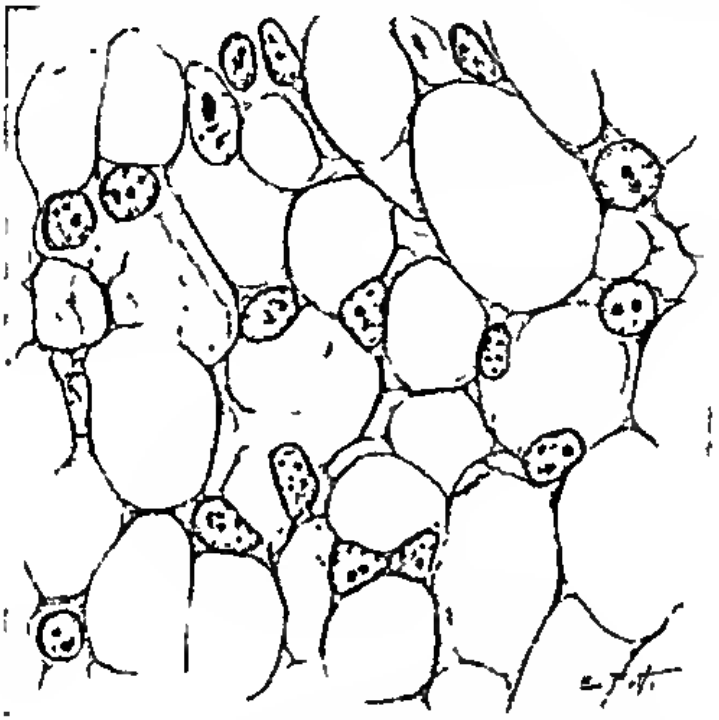


Fig. 91.—Notochordal Tissue from a 96 Hr. Chick Embryo. Bouin fixation, haematoxylin and eosin.

insertion of the M. quadriceps femoris, and in the sesamoid cartilage in the tendon of the M. peroneus longus. This form of cartilage resembles the notochordal tissue at a certain stage of development, and it is called 'chordoid tissue' by Schaffer.

Notochordal Tissue. The notochord gives rise to a tissue which has often been called cartilage. Notochordal tissue differs, however, from any of the types thus far considered. The principal stages in its development in the pig have been described by Williams[1] whose account may be summarized as follows:

In an embryo measuring 5.5 mm. the notochord is a rod of cells surrounded by a thin notochordal sheath. A cross section contains about eight wedge-shaped cells. In an embryo measuring 9 mm it is larger, and a cross section shows about fifteen cells at the periphery, and three or four at the center. In an embryo of 11 mm. the cells have lost all definite arrangement and are more or less vacuolated. The vacuoles increase in size and number, and are found to contain mucin or a gelatinous mucin-like substance. In

[1] Williams, L. W., *1908.*

an embryo measuring 17 mm. the cell walls, which up to this time have remained intact, are breaking down (or being absorbed) and the mucin escapes from the vacuoles. The cells are united by strands of cytoplasm and the notochordal tissue now resembles mesenchyma. The syncytial network continues to enlarge, both by growth, and by the formation of a greater number of vacuoles. In a much older embryo (250 mm.) the formerly continuous peripheral sheet of syncytial tissue is broken in many places by large masses of mucin. In the center of this accumulation, the slender syncytial network seems suspended (cf. Fig 91). In the adult the syncytium has become divided into groups of vacuolated cells embedded in a gelatinous matrix Thus it acquires a resemblance to cartilage in several particulars, but it should be regarded as a distinct tissue.

The human notochord undergoes a development similar to that of the pig. After it has ceased to be an epithelioid rod of cells, its most

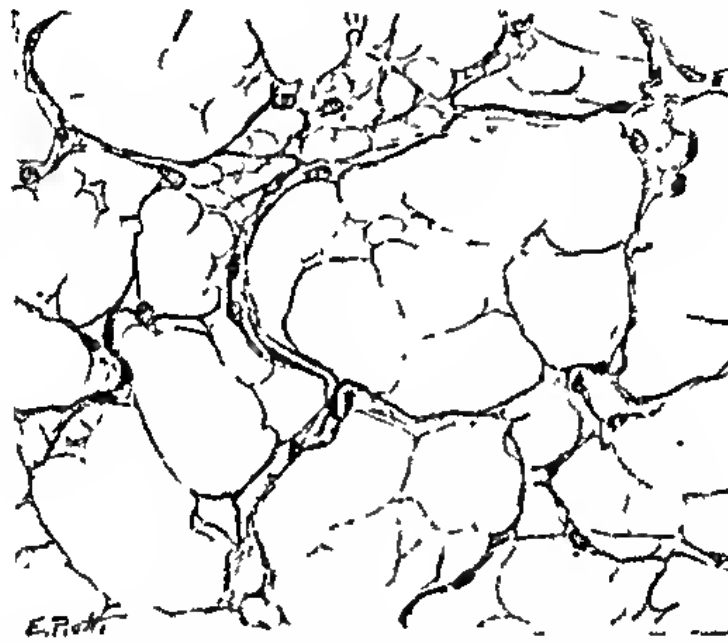


Fig 92.—A Portion of a Nucleus Pulposus from an Intervertebral Disc of a 2½ Yr Old Child. Notochordal syncytium is seen in the center of a mucoid matrix. Formaldehyde fixation, Azan.

characteristic condition is that shown in Fig. 92, which includes a portion of the nucleus pulposus from a child of two and one-half years old. The notochordal tissue forms a vacuolated syncytium suspended in the gelatinous matrix, which, at the periphery of the nucleus pulposus, is bounded by a structureless membrane. Very rarely the notochord is the source of tumors which are composed of tissue similar to that normally found within the nucleus pulposus.

BONE

Bone develops relatively late in embryonic life, after the muscles, nerves, vessels, and many of the organs have been formed. The skeleton at that time consists of hyaline cartilages, which are later replaced by the corresponding bones of the adult. According to Kölliker, Robert

Nesbitt was the first to point out that the bones are not indurated or transmuted cartilages, but are new formations, produced around the cartilages which are later destroyed. Moreover, in his 'Human Osteogeny Explained in Two Lectures' (London, 1736), Nesbitt showed that certain bones develop directly from connective tissue without having been preformed in cartilage. These are now called *membrane bones* in distinction from *cartilage bones*. The membrane bones are the bones of the face and the flat bones of the skull. They include the interparietal or upper part of the occipital, the squamous and tympanic parts of the temporal, the medial pterygoid plate of the sphenoid, the parietal, frontal, nasal, lacrimal, zygomatic (malar) and palate bones, together with the vomer, maxilla and almost the entire mandible. Nesbitt correctly concluded that there is but one method of bone formation, whether or not it takes place in relation with cartilage, but he was unaware of the existence of cells, and believed that bones were produced from an ossifying juice derived from the blood.

Development of Membrane Bone. Bone formation begins by the alteration in small areas of the intercellular substance of young connective tissue to a more gelatinous consistency. What part the tissue cells or fibrils play in this process is not clear. The collagenous fibrils are incorporated in this substance, and seem to be more conspicuous just on its border, but once engulfed they lose their identity, perhaps because they and the substance have the same index of refraction; the colloidal substance, or bone matrix, is thus apparently homogeneous. Soon after its formation the matrix attracts certain chemical salts, and calcareous granules are deposited in it. It stains characteristically red with eosin, after decalcification.

Around these isolated masses or spicules of bone matrix the mesenchymal or young connective tissue cells multiply and enlarge, and apply themselves in an epithelioid layer to the surfaces of the matrix. Such cells are called *osteoblasts*, and are generally supposed to add new bone matrix, as their name implies. Another interpretation of their presence will be given later. The new matrix forms a narrow bordering layer, at first uncalcified and staining less intensely.

Two characteristic differences in the behavior of the original mesenchymal cells lead to the formation of cartilage on the one hand and of bone on the other. In cartilage the cells lose their original cytoplasmic processes, and matrix is formed on all sides of them. In bone the mesenchymal network is retained, and the cells deposit the matrix only from one surface, that which rests on an already formed spicule. The young osteoblasts are thus still connected by fine cytoplasmic processes both to each other and to the neighboring unaltered fibrocytes. A variable

number of white fibers, already present in the intercellular spaces, become incorporated in the bone matrix.

Before sections of bone can be cut, the tissue must be decalcified to remove the inorganic constituents of the matrix This is usually accomplished by the use of dilute acids and may cause some shrinkage of the cellular bodies and rupture of the cytoplasmic processes. With Mallory's connective tissue stain a specimen of bone formation like that

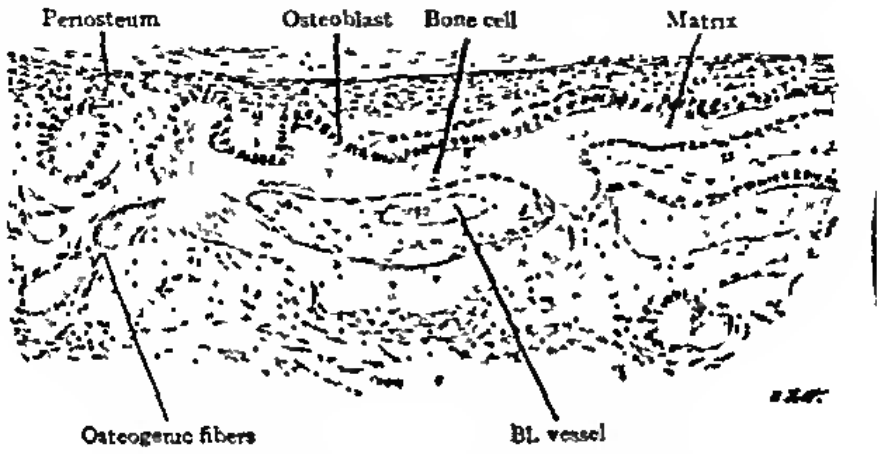


Fig 93 —Bone Formation, Jaw of Pig Embryo, Low Power.

in Fig. 94, shows young white fibrils differenually stained blue and the cytoplasmic processes of the osteoblasts, red. Eosin stains both the fibrils and processes red, and they are usually indistinguishable.

There is great variation in the shape of osteoblasts. Often they are pyramidal, but they may rest upon the bone either by a broad base or a pointed extremity. Their round nuclei may be in the part of the cytoplasm next to the bone, or away from it as far as possible. Active osteoblasts tend to be cuboidal or columnar, but as bone production ceases they may become quite flat. Their nuclei show changes similar to those seen in gland cells, changing from clear and vesicular when the cells are most active to dark and cloudy when activity has passed its peak, and pycnotic when the cells become inactive and flat. The cells form bone only along that surface which is applied to the matrix, and only in the intercellular spaces. New matrix is thus formed around any cell or cytoplasmic process which lies at the edge of the matrix, and osteoblasts which have ceased to produce matrix are buried by their neighbors. Since the inactive, flat osteoblasts are those most often thus buried, the lacunæ formed are often flattened and parallel to the surface; and since the cytoplasmic processes still connect the buried cells with the active ones, the lacunæ are also connected to the surface or to each other by *canaliculi*, the little canals formed by the deposition of matrix around the processes. Buried osteoblasts are called *bone cells* or *osteocytes*. They differ from cartilage cells in having access through the canaliculi to the tissue fluids. Therefore they do not develop fat

droplets and rarely divide or die, like cartilage cells. If they continue to produce matrix, thus becoming more widely separated, it is only to a slight extent and in young bones; they are therefore quite inactive. The matrix around the lacunæ resists strong hydrochloric acid which destroys the ordinary matrix, and so may be isolated in the form of 'bone corpuscles.' The 'corpuscles' correspond with the capsules of cartilage, which may be isolated in the same way. The bone cells nearly fill the lacunæ and send out very slender processes into the canaliculi. These may be continuous with processes of neighboring cells, as can be seen in the

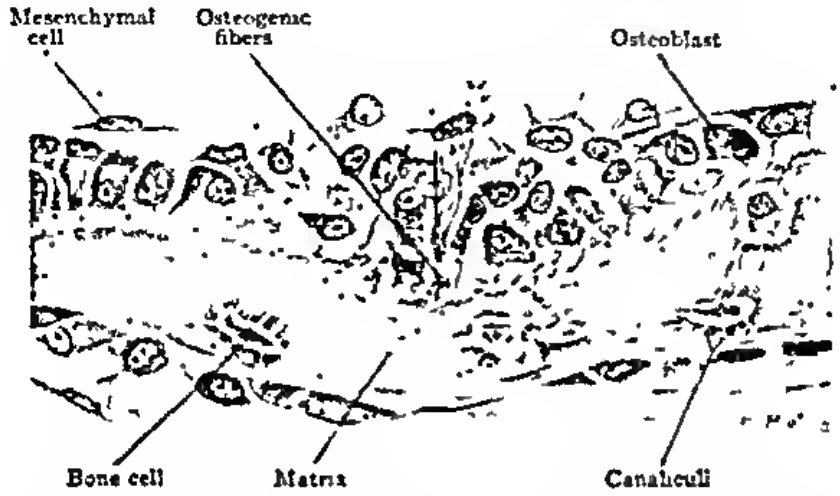


Fig. 94.—Bone Formation. Detail of Osteoblasts and Bone Cells.

embryo, but it is doubtful if this condition is retained in the adult. The processes, moreover, are so fine that ordinarily they are invisible. In ordinary specimens the canaliculi also are invisible, except where they enter the lacunæ, which are thus made to appear stellate. They can be clearly shown, however, in very thin sheets of bone, such as some of the nasal bones of the mouse (Fig. 97), and in thin layers of dried bone, ground until thin enough to be translucent. The lacunæ, with the canaliculi projecting from them, are then empty, except for air and particles of bone dust. The specimens are mounted in thick balsam, which spreads over the bone without filling the lacunæ and canaliculi. When seen under the microscope these structures appear black (Fig. 108), the air within them being highly refractive. In such preparations the way in which the canaliculi pass from one lacuna to another, and their manner of branching, may be readily observed.

Leriche and Policard,[1] and many other authors, oppose the view that the osteoblasts are active agents in the deposition of new bone matrix. They consider that the osteoblasts represent 'reactionary forms of the connective tissue cells in the presence of the modifications of the connective tissue medium in which they live. The transformation of the connective tissue cells into osteoblasts would be the result of a change of medium.' In many cases pathologically the wandering cells of the connective tissue or blood cluster

[1] Leriche and Policard, 1928.

around an injurious agent. The osteoblasts, however, do not seem to have the usual characteristics of free wandering cells, since they retain their cytoplasmic connections with cells of the surrounding connective tissue and of the bone, essential to the formation of the canaliculi. Their active participation in the formation of new matrix is by no means certain, but young osteoblasts are said[1] to show a great increase in the number of mitochondria and secretory granules, usual signs of secretory activity, which are again much reduced in the flattened forms of osteoblasts and in bone cells. It has been shown[2] also that osteoblasts, bone cells and old cartilage cells produce an enzyme (phosphatase) which acts in freeing phosphate ions that may unite with the calcium in the tissue fluids of the immediate neighborhood, thus fixing it in a collagenous matrix already prepared.[3] F. T. Lewis[4] has pointed out that the spacing of the cell bodies and nuclei is similar within the bone spicules and in the connective tissue outside, but interrupted in the denser layer of osteoblasts It may be that this condensation, with its consequent grouping of cellular processes, provides for the dense arrangement of the canaliculi in bone and the consequent better nourishment of the bone cells Lewis also calls attention to the intricate readjustment of processes entailed as certain cells become buried while others retreat. It seems certain that every bone cell must appropriate canaliculi made by others

The spicules of bone, containing bone cells and beset with osteoblasts, increase in size and unite with one another, so as to form a spongy network enclosing areas of vascular connective tissue. These areas are not entirely surrounded by bone, but retain connections with the exterior, through which the vessels may enter and leave. It is evident that if the spicules continued to thicken, while new ones were added at the periphery, the bone would soon become quite solid and heavy. This is prevented by the destruction or resorption of certain spicules, which begins at a very early stage. It may be studied advantageously in the developing mandible of a pig embryo 60 mm. in length. At this stage the teeth are growing rapidly, and around each tooth the spicules of bone are being destroyed so as to produce a larger socket; at the same time the jaw is increasing in thickness by the formation of new bone over its outer surface. Toward the area of resorption the osteoblasts become flatter and less numerous, finally disappearing.

In sections of bone, the places where resorption is going on may be recognized by the presence of large multinucleate cells, which Kölliker in 1873 named 'bone destroyers' or ostoclasts (preferably spelled *osteoclasts*). They are shapeless masses of protoplasm without any limiting membrane, containing usually from two to twenty nuclei and therefore also called *polykaryocytes*. In the largest of them Kölliker counted from fifty to sixty nuclei. They have nothing in common, except their large size, with the giant cells of the bone marrow (megakaryocytes) which may appear in the same sections. Osteoclasts are found along the

[1] Dubreuil, Charbonnel et Massé, 1933.
[2] Robison, Macleod and Rosenheim, 1930.
[3] Ham, 1934. [4] Lewis, F. T., 1935.

surface of the bone, sometimes forming rounded elevations or caps at the extremities of spicules, and sometimes embedded in shallow

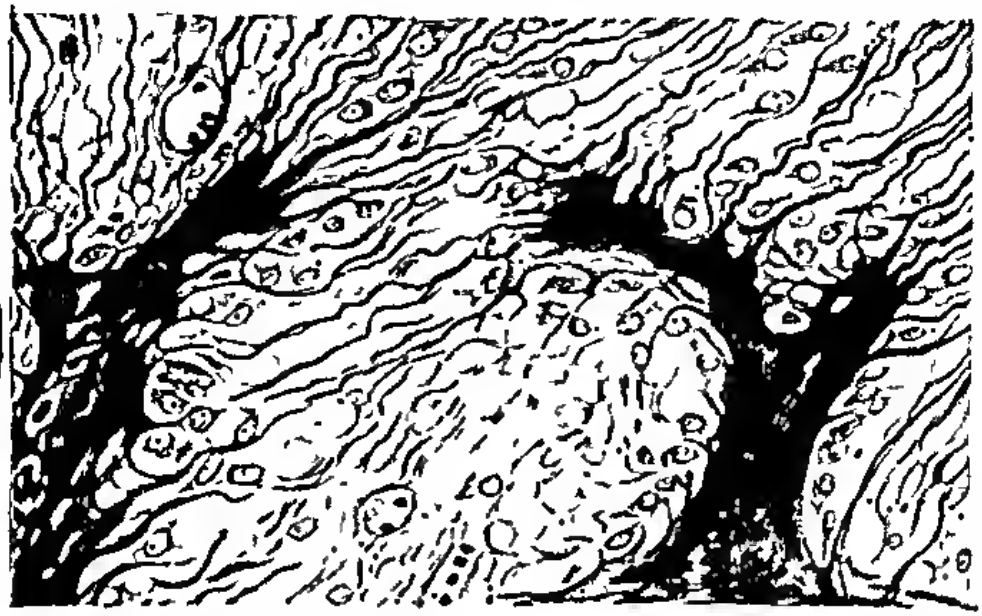


Fig. 95.—Intramembranous Ossification. The Formation of a Fibrous Matrix. From the maxilla of 210 mm. pig embryo. Formaldehyde fixation, Azan.

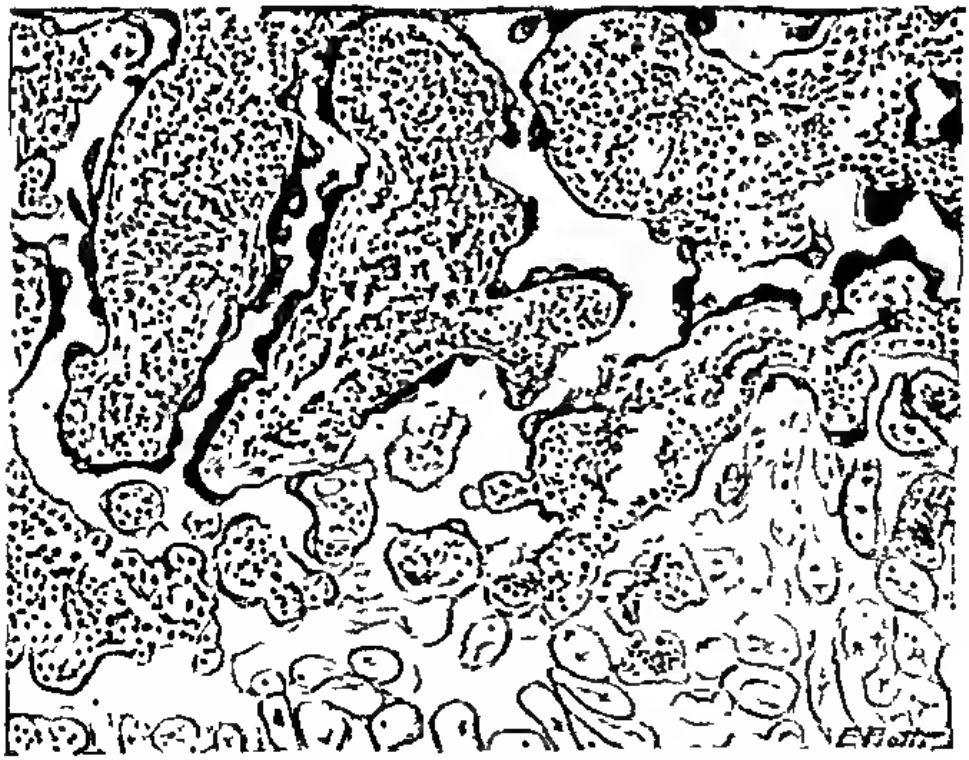


Fig. 96.—Endochondrial Ossification. From the Body of a Vertebra of a 2½ Yr. Child. The bone is a purplish-red, the cartilage a pale blue. Formaldehyde fixation, Azan.

avations known as *Howship's lacunæ*. Their cytoplasm is slightly dophilic, finely granular, and may contain many vacuoles. Against

the matrix it may show fine vertical striations, almost like cilia, but these may be artifacts due to shrinkage. Their origin is uncertain. They have been considered as fusions of osteoblasts (the nuclei seem to be similar); as fusions of bone cells freed by the dissolution of the matrix (on the other hand they are often seen to contain ingested bone cells); or as new products from the connective-tissue cell strain. The osteoclasts may be degenerating cells. There is no proof that they are the active cause of bone destruction. They are not phagocytic to injected trypan blue, but they may secrete some chemical solvent for the bone matrix. For a discussion of this subject see Arey[1] or Weidenreich.[2]

The processes of bone formation and resorption just described take place both in membrane and in cartilage bones. As the membrane bones enlarge, the central portion, through resorption, becomes loose, spongy bone (*substantia spongiosa*), which is enclosed on all sides by an outer layer of compact bone (*substantia compacta*). In the flat bones of the skull the compact substance forms the outer and inner 'tables,' which have the spongy 'diploë' between them. The cartilage bones likewise consist of spongy and compact portions.

Replacement of Skeletal Cartilage. 'Bone tissue always arises from connective tissue, even though the bone has a cartilaginous model as an antecedent.' The changes within the skeletal cartilages during the formation of bone may be studied advantageously in longitudinal sections of any developing 'long bone,' or in transverse sections of the vertebræ from pig embryos measuring about 10 cm. The vertebræ exhibit several processes which will be cut lengthwise in transverse sections. Figure 98 represents longitudinal sections of two phalanges around which ossification has begun. On either side of the shaft of hyaline cartilage, the matrix of which stains blue with hæmatoxylin, there is a strip of bone, the matrix of which is stained red with eosin. These strips are sections of a band of bone which completely encircles the middle part of the cartilage. It has been formed by osteoblasts which developed in the perichondrium. The portion of the cartilage which is surrounded by bone has begun to degenerate. Its capsules have been resorbed, and the enlarged lacunæ are beginning to coalesce. The matrix of the cartilage in this region takes a deep stain, and calcareous granules are being deposited within it.

What initiates the degeneration of the cartilage matrix in a definite area is not known. It is not the thickness of the cartilage, for the center of ossification is seldom in the thickest part of the future bone. The formation of the band of perichondrial bone, which might conceivably

[1] Arey, 1920. [2] Weidenreich, 1930.

deprive the cartilage cells of their meager nutriment, does not precede the change but rather accompanies it.

On the left of Fig. 98, a bud of perichondrial tissue is seen entering the shaft of the cartilage, and similar buds may invade it from other sides. One of these openings through the perichondrial bone is the future nutrient foramen. Within the cartilage the ingrowing perichondrial tissue forms the *primary marrow*, which is very vascular connective tissue. As the walls of adjacent lacunæ are resorbed, thus setting free the cartilage cells, the primary marrow spreads into the vacant spaces (Fig. 99).

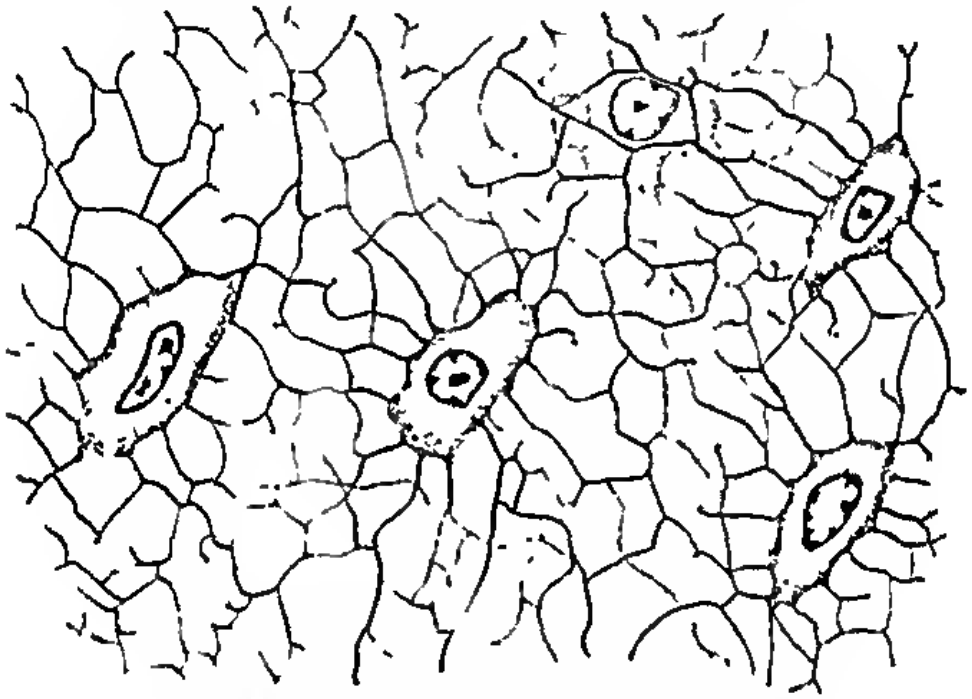


FIG. 97.—BONE CELLS, THIN NASAL BONE OF MOUSE, METHYLENE BLUE. The whole thickness of the bone is shown.

Meanwhile the cartilage continues to grow, especially in length. This is brought about by successive transverse divisions of the cells of the shaft, so that they become arranged in more or less definite longitudinal rows. The thin transverse walls of the lacunæ in these rows are dissolved more readily than the thicker longitudinal walls, and the ragged spicules of calcified matrix which are thus produced, stained deep blue by hæmatoxylin, are therefore generally elongated. Osteoblasts, derived from the primary marrow, arrange themselves on these spicules, and form bone in the same manner as elsewhere. Thus the spicules of calcified matrix, staining blue, become encased in the matrix of bone which stains red with eosin. Formerly it was thought that the freed cartilage cells turned into osteoblasts, but now it is generally recognized that they die without further function. Reconstructions have shown that no osteoblasts are present in closed lacunæ which the primary marrow has not reached.

From what has been said, it is clear that bone is formed both around the cartilage (perichondrial bone) and within the cartilage (endochondrial bone). In long bones and flat bones, ossification is at first perichondrial and later endochondrial; in short bones it is endochondrial until the cartilage has been entirely replaced. Thus the part taken by endochondrial and perichondrial ossification varies greatly in different bones. As the bone grows, the older parts which have formed in relation with

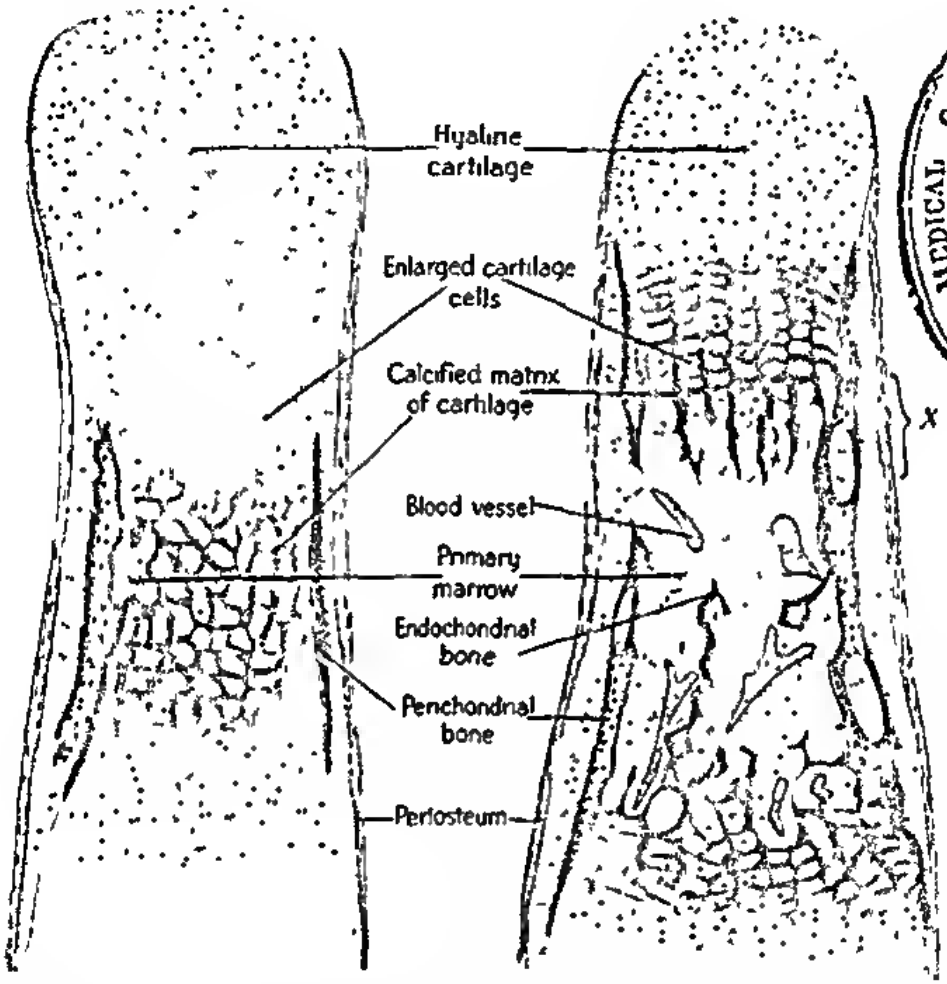


Fig. 98.—Longitudinal Section of Human Phalanges, Two Stages of Ossification. Hematoxylin and Eosin.

the cartilage are resorbed. In the shaft of the humerus from a human embryo of the fourth month (Fig. 100), only a thin and interrupted layer of calcified cartilage remains to mark the boundary between perichondrial and endochondrial bone, and in the adult all traces of this layer have disappeared.

The final stages in the replacement of the cartilages by bone take place long after birth, when the bones have increased greatly in diameter and length. The growth in diameter is accomplished by the deposition of new layers externally, and the enlargement of the marrow cavity,

through resorption, internally. The diameter of the adult marrow cavity in a long bone is greater than that of the embryonic cartilage which it replaced. Thus all of the original cartilage of the shaft and of the bone that was formed immediately upon it has been secondarily resorbed. This explains why a band of metal placed around the bone of a young animal is later found within the marrow, and why none of the original cartilage matrix remains in most of the adult bones. In the auditory ossicles and the otic capsule, *i.e.*, bones without marrow cavity and of very slight growth, the calcified matrix remains throughout life. The internal

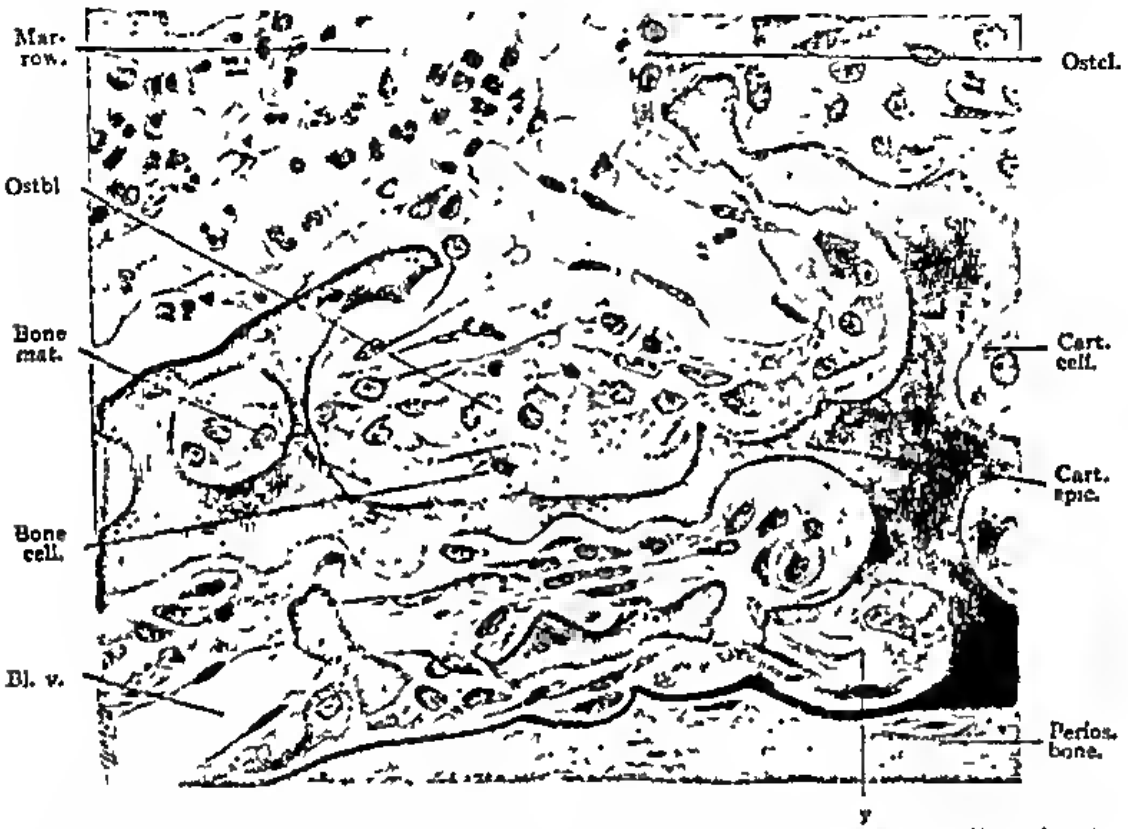


FIG. 99.—DETAIL OF AREA LIKE THAT AT 'x' FIG. 98. INVASION OF CARTILAGE LACUNÆ BY OSTEOGENIC TISSUE (AT 'y').

resorption takes place in such a way that a meshwork of spicules and plates, denser toward the periphery, remains within the shaft, and the marrow occupies its interstices. To a limited extent new bone is formed in the interior of the shaft by osteoblasts in its lining membrane, called the *endosteum*. The deposition of new layers externally is produced by osteoblasts in the *periosteum*, which is a specialized connective tissue layer surrounding the bone. It replaces, and apparently is derived from, the perichondrium of the original cartilage. The extent to which new bone is formed, and its distribution, may be determined by feeding madder to growing animals. This dye, as has long been known, imparts a red color to the matrix of bone deposited while it forms a part of the diet. By this means Kölliker determined that the deposition of periosteal bone is not

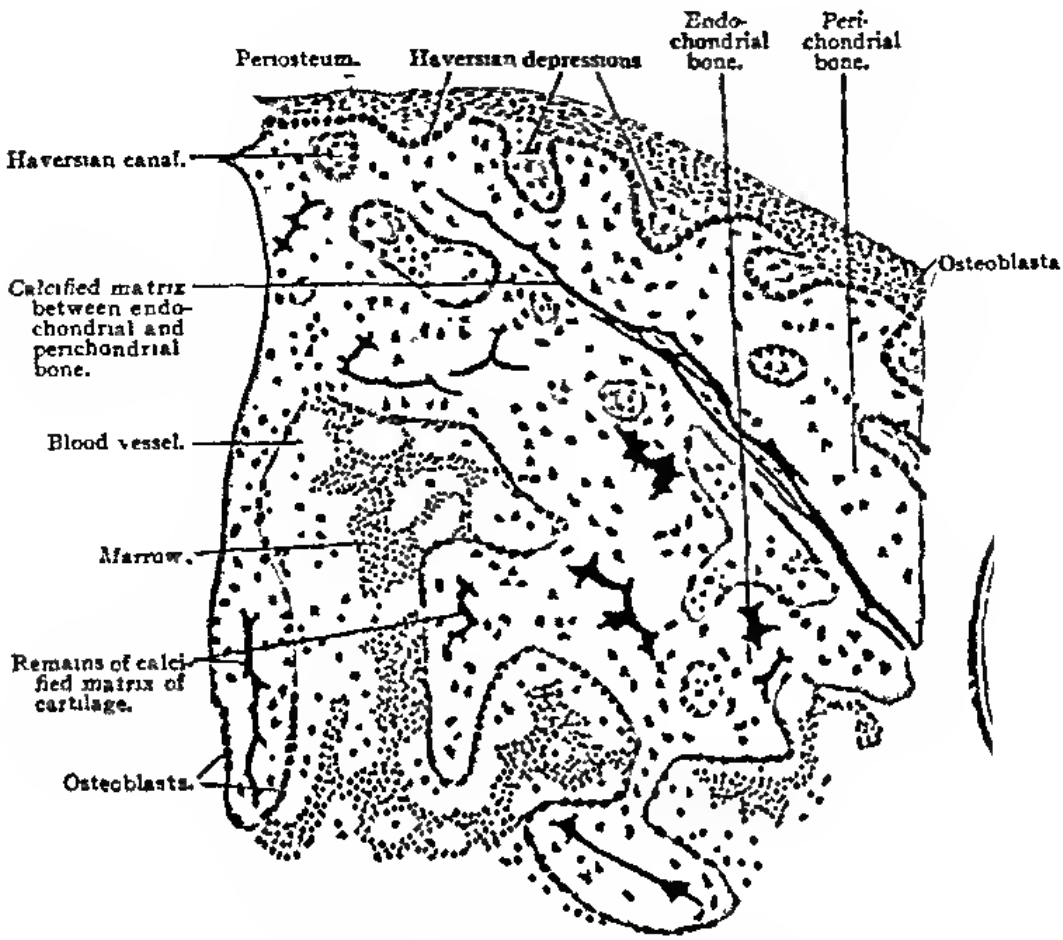


Fig 100.—From a Cross Section of the Shaft of the Humerus, from a Human Embryo of the Fourth Month. × 80

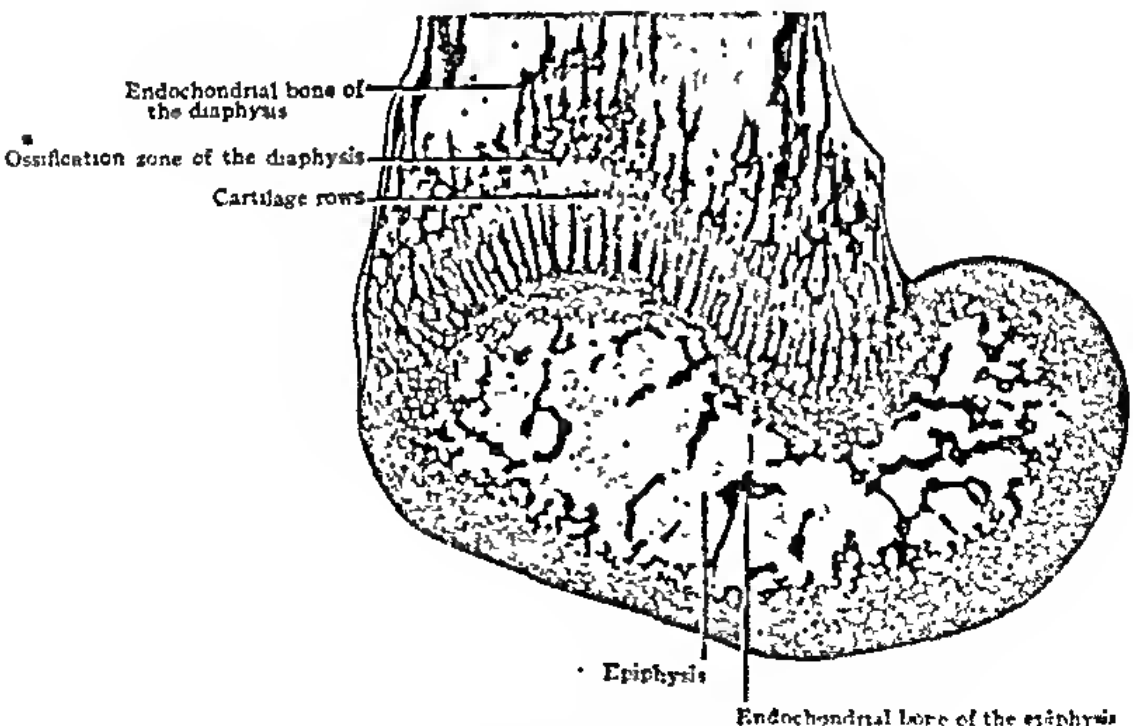


Fig. 101.—Frontal Section through the Femoral Condyle of a 4 Wks. Old Mouse. Zenker fixation, Mallory's acid fuchsin. (Schaffer.)

uniform. In a given bone there will be unstained areas, where no new bone is being formed, or where an external resorption is taking place. In this way the bones acquire their characteristic modelling.

Growth in length occurs chiefly through the activity of the uncalcified cartilage. In a long bone ossification first produces a band of bone

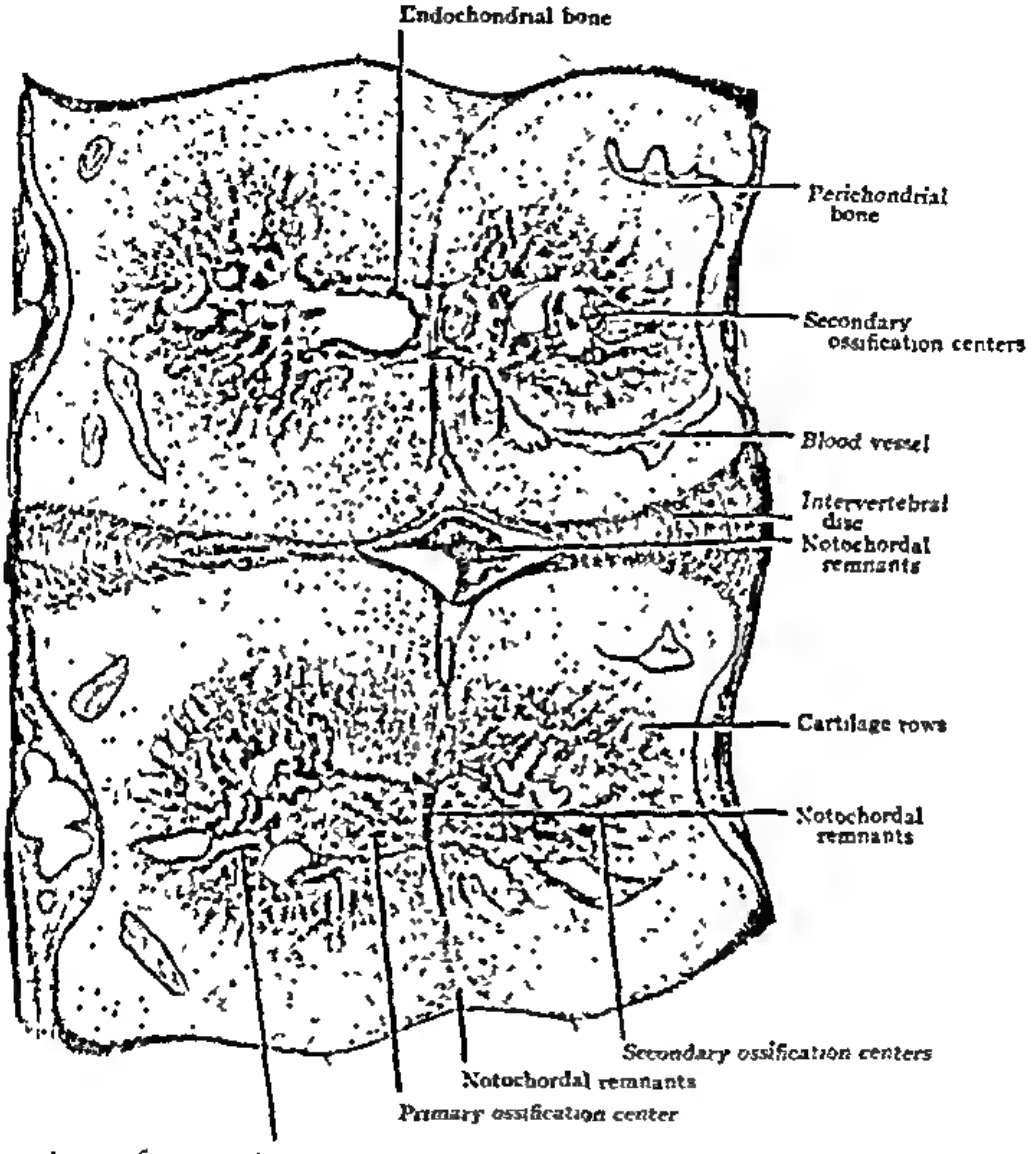


FIG. 102.—A MEDIAN SECTION THROUGH TWO THORACIC VERTEBRAE OF A 17 CM. LONG HUMAN EMBRYO. Müller—formaldehyde fixation (Schaffer).

encircling the cartilage, and then a hollow shaft of bone with a rounded mass of cartilage at either end. The cells in these masses continue to divide, prolonging the longitudinal rows of cells. As ossification takes place at one end of these rows, new cells are formed at the other, and thus the length of the shaft or *diaphysis* increases. Certain bones have been found to grow more at one end than at the other. After a time osteogenic tissue invades the cartilages at the extremities of the bone, extending into them from the marrow cavity of the shaft. It forms a small bone within

each, and these are known as *epiphyses* (Figs. 101, 104 and 105). Between the epiphysis and the diaphysis there remains a layer of cartilage, called the *epiphyseal synchondrosis*, which allows further growth in length. The cells which it produces are added chiefly to the shaft.

The relation of the epiphyses to the growth of bone was demonstrated by early experiments, in which metal pegs were placed in the bones of young animals. Pegs in the shaft scarcely separate from one another during growth, but a peg in the epiphysis moves away from one in the diaphysis. The epiphyses are formed at various times after birth, or, in the tibia, shortly before birth; they unite with the diaphyses usually between the eighteenth and twenty-second years, when the bones have acquired their full length. The rate of division of the cartilage cells is apparently governed by the secretion of the pituitary gland, an excessive activity of this organ in youth resulting in long-boned 'giants.' After the union of the diaphyses and epiphyses, a similar excessive activity leads to the thickening of the bones, since the possibility of lengthening has been lost with the loss of the epiphyseal cartilage. At that time nothing is left of the original cartilage except the layer of *articular cartilage* which covers the joint surfaces. Details in regard to the time when ossification begins in the various bones, the number of centers involved (for many bones have more than the three which have here been described), and the time when these join the main bone, will be found in textbooks of anatomy, and in

FIG. 103.—THE FOREARM AND HAND OF A HUMAN FETUS OF ABOUT SIX MONTHS.

The bone is stained with alizarine and the preparations cleared by the Spalteholz method. (Preparation by Dr. Don Fawcett.)

FIG. 104.—HUMERUS OF A THREE YEAR OLD CHILD. THE EPIPHYSES ARE UNUNITED. (Hasselwander.)

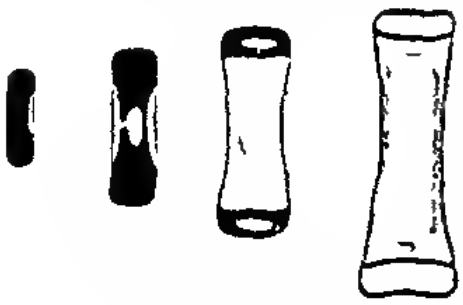


FIG. 105.—DIAGRAM OF GROWTH OF LONG BONE.

Cartilage, black; bone, gray; marrow cavities white. The epiphyses are shown

articles by Stevenson[1] for man and by Dawson[2] for the rat. The differences in ossification in the two sexes have been shown by Pryor,[3] and may serve to indicate the sex of late fetuses.

Structure of Bone in the Adult. Adult bones are composed of inner cancellous or spongy portions and outer cortical or compact portions. Compact bone is formed from the irregular bone spicules of the embryo in the following manner. On every surface of the spicules osteoblasts add

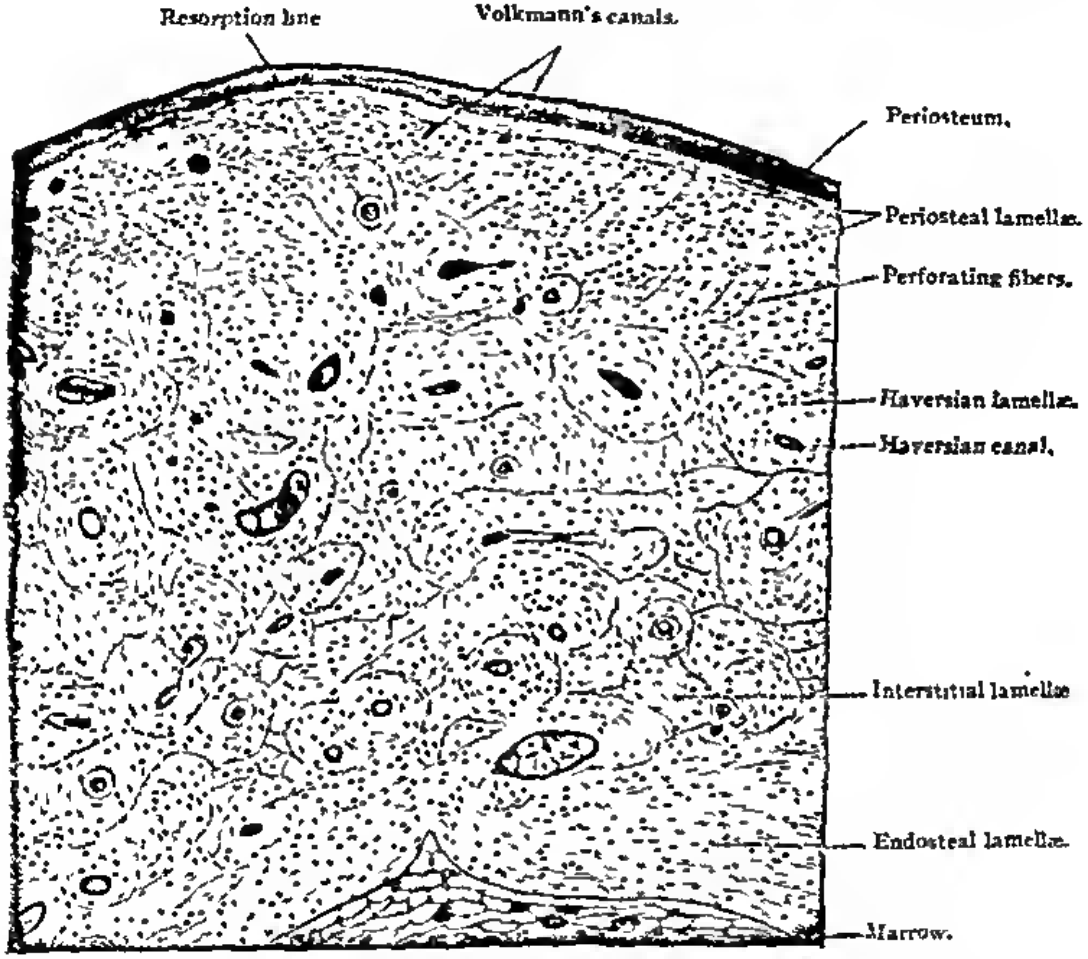


FIG. 106.—PART OF A CROSS SECTION OF A DECALCIFIED PHALANX FROM AN ADULT

more matrix, so that the many spicules by growing larger soon meet to form an irregular trabecular network, enclosing large marrow spaces. Additional layers of bone are added, the corners of the network are filled in, until rounded tunnels are formed, enclosing marrow and blood vessels. The bone cells lie parallel to the inner surface of matrix, and thus have a concentric arrangement. The collagen fibers of the marrow are successively arranged in different orientation as they are buried in the matrix, resulting in optically different, concentric lamellæ. These 'decussating fibers,' occurring in parallel sets which tend to cross each other at right angles, thus producing a lattice work, are seen only in

[1] STEVENSON, 1924 [2] DAWSON, 1925. [3] PRYOR, 1923.

special preparations in which successive lamellæ have been peeled off, so that they can be examined in surface view. Such a concentric arrangement of bone cells and lamellæ is called an *Haversian system*, and the enclosed vessels occupy an *Haversian canal* (named for the English anatomist, Clopton Havers). An Haversian canal often contains two vessels, an artery and a vein, together with a small amount of connective tissue and occasional fat cells; flattened osteoblasts may rest against the surrounding bone and send processes into it. The deposition of bone

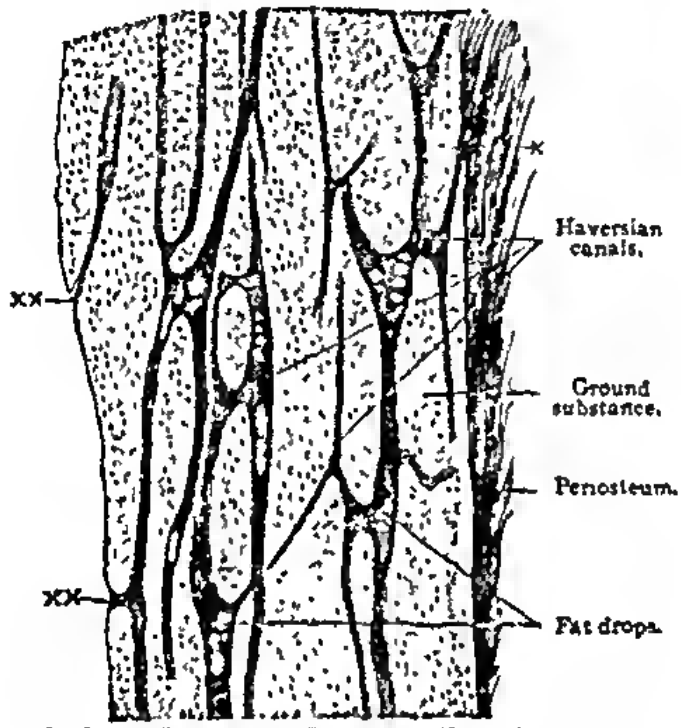


Fig. 107.—From a Longitudinal Section of a Human Metacarpal. × 30.
Fat drops are seen in the Haversian canals. At x and xx vessels reach the outer or inner surface of the bone through Volkmann's canals.

ceases when the blood vessels within the canals are crowded. All the bone cells of an Haversian system receive nutriment through the canaliculi opening into the canal. The vessels are a part of the vascular network of the original marrow. Meanwhile the bone is growing by addition of new layers from the periosteum, in which again the lacunæ are parallel to the surface. In young bone this surface is irregular, formed of many separate spicules, so that Haversian systems are also present in this region. The vessels are derived from the periosteum but connect with those of the marrow. In older bones the periosteum becomes a smooth layer, and the bone formed from it is in parallel lamellæ. These surround and embed the already existing fibers (perforating fibers) and the vessels, which thus come to lie in canals without concentric lamellæ (Volkmann's canals).

Volkmann's[1] canals have often been wrongly described as communications between Haversian systems, but they are secondary vascular channels penetrating already formed bone. They may connect with the vessels in the systems or pass irregularly through the entire width of the bony cortex. When first formed the Volkmann's canals have irregular or serrated borders which become smooth through resorption[2] (Fig. 110).

If this solid appositional growth continued unaltered the whole bone would be made of lamellæ parallel to the surface, and the more centrally placed bone cells would derive their nutriment through many layers of lacunæ. But local resorption, accompanied by osteoclasts, and starting from the marrow cavity or from some Haversian canal, forms a wide tunnel toward the poorly-nourished area. Into this grow new vessel sprouts and osteogenic marrow cells. The fresh cells become osteoblasts and build against the circular walls of the tunnel, thus forming a new Haversian system, carrying nutriment to the distressed cells. The new canaliculi connect with the old lacunæ. The process is continually repeated. The direction taken by the lamellæ in a section of bone will reveal its history. The newest Haversian systems are completely circular, the outer edge perhaps cuts into the side of an older system, which itself may have invaded an area of layers parallel to the surface, originally laid down by the periosteum. In the finished bone no cell is separated by more than a few layers from some source of nutriment. The junction of the older matrix with that more recently added is often marked by an optical difference in the matrix, called the 'cement line,' best seen in sections of decalcified bone. The lines thus mark the extent of each Haversian system.

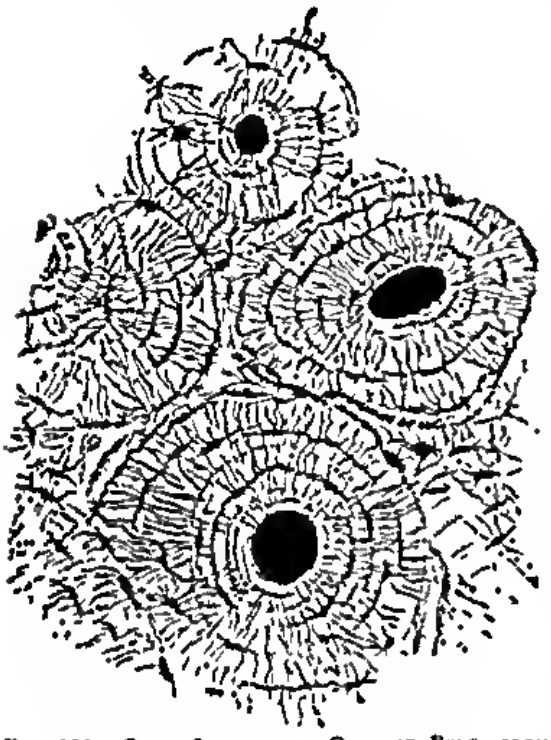


Fig. 108.—Cross Section of Compact Bone, from the Shaft of the Humerus showing Three Haversian Systems and Part of a Fourth (Sharpey.) Dried and Ground

Cross sections of long bones show 'periosteal lamellæ,' parallel to the bone surface, just beneath the periosteum; 'endosteal lamellæ,' parallel to the border of the marrow cavity; and between these two, recent and old Haversian systems with 'concentric lamellæ' and 'interstitial lamellæ,' which latter may be the remnants of former periosteal layers or of former Haversian systems.

[1] Volkmann, 1863 [2] Jaffé, 1929.

Longitudinal sections of bone show the way in which the Haversian canals connect with one another (Fig. 107). The lamellæ are not so strikingly subdivided into the groups seen in cross sections, since both the concentric lamellæ and the ground lamellæ are longitudinal layers.

The matrix of bone consists of organic and inorganic constituents intimately blended, and perhaps chemically combined. Of the inorganic

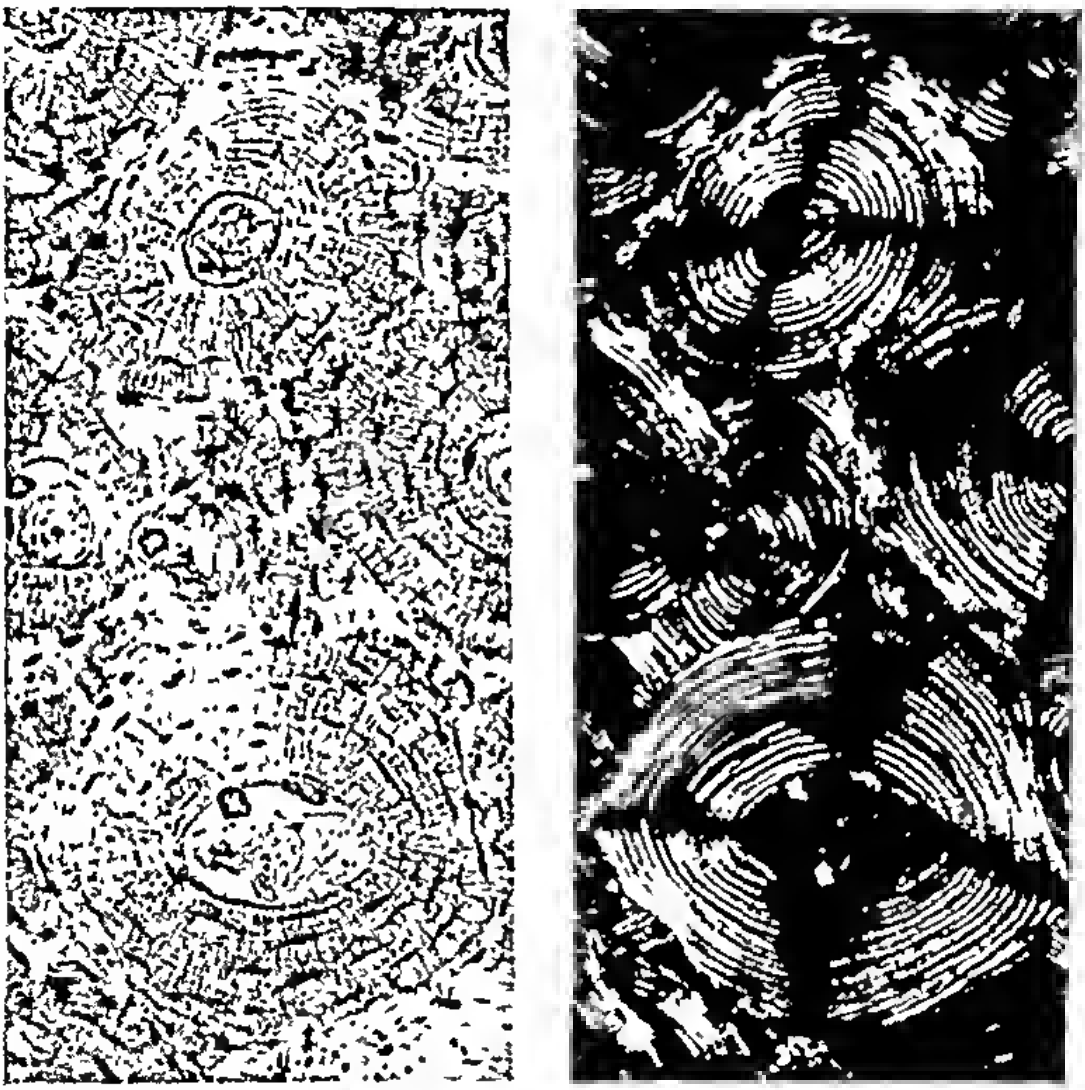


Fig. 107.—Photographs of a Ground Section of Bone from the Adult Human Femur. At the left as seen in transmitted light, at the right in polarized light.

matter, over 80% is calcium phosphate, $Ca_3(PO_4)_2$; the remainder includes chlorides, carbonates, fluorides and sulphates of calcium, sodium, potassium and magnesium. The calcium seems to be in an unstable state, since it can be drawn on for the body metabolism; the bones are considered a store house for calcium. In order to cut sections of bone, this inorganic matter must be removed, and decalcification is usually accomplished by placing the specimen, after it has been preserved, in dilute acid for several days or weeks. The matrix then has

the consistency of cartilage. Its organic portion, which remains, is composed chiefly of collagen, together with osseo-mucoid. The collagen occurs in the fine white fibrils which are gathered in bundles, arranged in the *lamellæ*. The calcareous matter is said to be deposited in the cement substance between the fibers, and not within them. Coarser uncalcified fibers are found in embryonic bone and in certain situations in adult bone—for example, at the sutures and the places where tendons are inserted. Their presence is due to the continued deposition of matrix around the tendon fibers of the embryo, or to the incorporation of fibers as the periosteal bone is laid down. They are called 'perforating fibers'

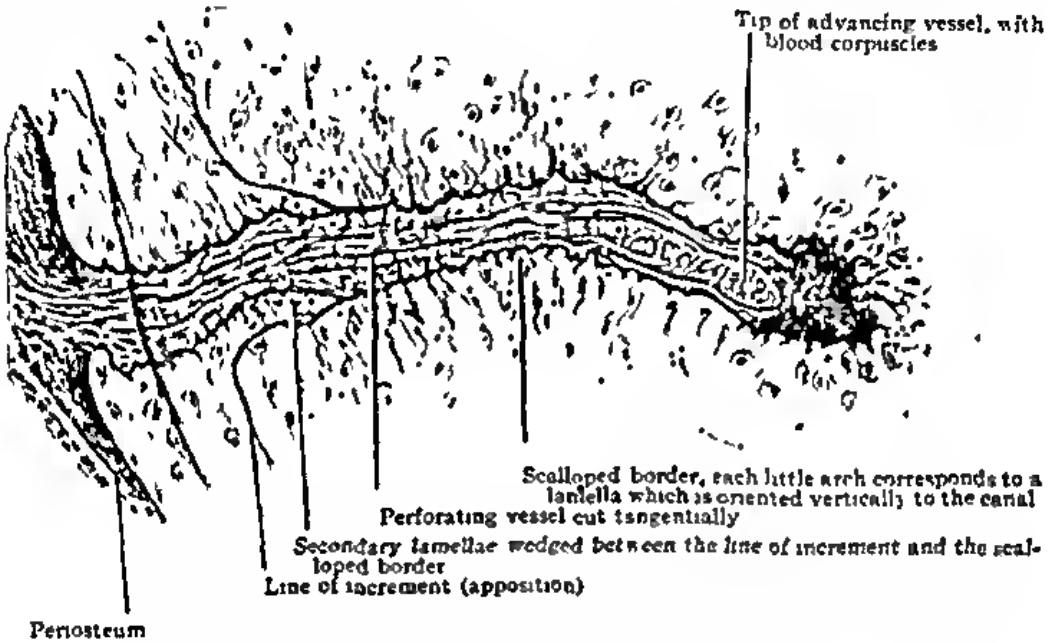


FIG 110.—A PERFORATING OR VOLKMANN'S CANAL FROM THE BONY ALVEOLUS OF A MANDIBULAR MOLAR TOOTH OF A 25 YR OLD WOMAN X 170

Formaldehyde fixation, Delafield's hæmatoxylin and eosin (Schaffer)

(Sharpey's fibers); in the bone of the adult skull they are entirely collagenous, but in other bones elastic fibers may accompany the perforating fibers.

The *periosteum* consists of two ill-defined layers. The outer is of dense connective tissue, rich in blood vessels and containing also lymphatic vessels and nerves, which blends with the surrounding looser tissue or with fasciæ and tendons. The inner layer has fewer vessels, but contains an abundance of elastic fibers. They are chiefly parallel with the long axis of the bone, but in the periosteum of the bones of the skull they form an interlacing network. The cells of the inner layer of the adult periosteum are spindle-shaped or flattened, but in the young there are in addition numerous rounded or cuboidal osteoblasts, which may be so numerous as to form a third layer of the periosteum where appositional bone growth is active. Presumably the cells of the inner layer are all able to be converted to osteoblasts, though ordinarily dormant in the adult,

for the adult periosteum is apparently able to form new bone after injury. The periosteum, even in bodies which have been kept a week at 15° C., is said to be capable of producing bone when transplanted to another body; and after operations in which a shaft of bone has been shelled out from its periosteum, a new shaft may be formed.

Leriche and Policard[1] deny that the periosteum as such can produce new bone, and cite numerous instances of failure. According to them if the periosteum is dissected so as to include some living bone chips regeneration will ensue; if these are absent

Fig. 111

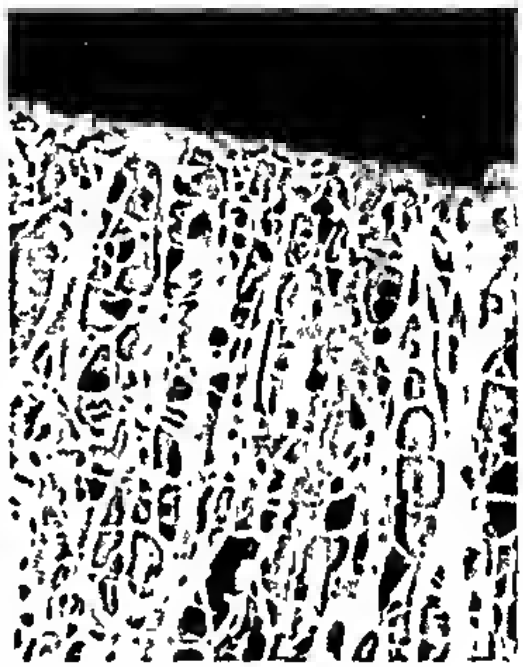


Fig. 112

Fig. 111.—Normal Spongiosa, the First Lumbar Vertebra. Human. × 5. (Schmorl.)
Fig. 112.—Senile Osteoporosis, the First Lumbar Vertebra. Human. × 5. (Schmorl.)
The bony trabeculæ and plates are much thinner and the spaces wider than in the normal

failure will result. Dubreuil[2] maintains that the inner layer of the periosteum is the essential element for success, since it contains the cells capable of becoming osteoblasts as the result of stimulation, though dormant and inconspicuous in adult life. The outer layer alone, which can readily be stripped separately, would not give the desired result.

Vessels and Nerves in Bone. The blood vessels of the marrow, bone and periosteum freely connect with one another. Small branches from the arteries and veins of the periosteum enter the bone everywhere, through the Volkmann's and Haversian canals, and anastomose with the vessels in the marrow. The marrow receives its blood from the *nutrient artery*, which gives off branches on its way through the compact bone and forms a rich vascular network in the marrow. Of the larger veins which drain this network, one passes out beside the nutrient artery and others connect freely with the vessels in the compact bone. Lymphatic vessels are found only in the outer layer of the periosteum. Numerous medul-

[1] Leriche and Policard, 1928. [2] Dubreuil, Chabbonnel, and Massé, 1933.

lated and non-medullated nerves are present in the periosteum, where some of them end in lamellar corpuscles. Others enter the Haversian canals and marrow, chiefly to innervate the vessels.

The Joints. Bones may be joined in two ways, either by a *synarthrosis* which allows little or no motion between them, or by a *diarthrosis* which permits them to move freely upon one another.

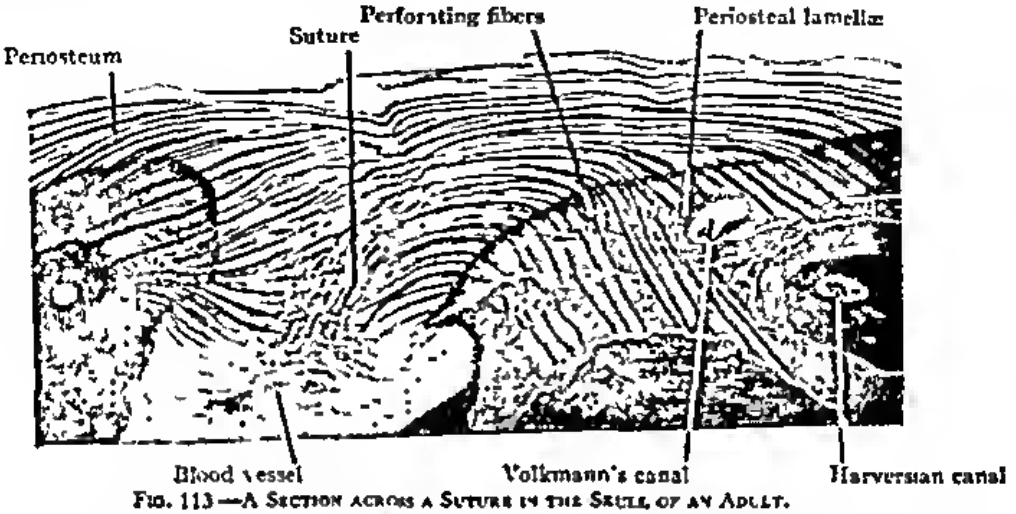


FIG. 113.—A SECTION ACROSS A SUTURE IN THE SKULL OF AN ADULT. Prepared by Bielschowsky's method × 80.

In a synarthrosis the mesenchymal tissue between the adjacent bones may form dense connective tissue, such as passes from one bone to another across the sutures of the skull (Fig. 113); or it may form cartilage, in which case the joint is known as a *synchondrosis*. The cartilage may be hyaline, as in the epiphyseal synchondroses, but often it is fibrous, as in the intervertebral synchondroses.

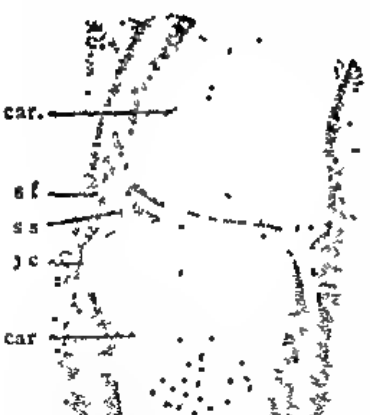


FIG. 114.—PHALANGEAL JOINT FROM A HUMAN EMBRYO OF THE FOURTH MONTH

car., Cartilage, j c, joint cavity, s. f, stratum fibrosum, s. s, stratum synoviale

In a diarthrosis the connective tissue between the bones remains comparatively loose in texture, and a cleft forms within it, containing tissue fluid. This is the *joint cavity* (Fig. 114). It is bounded by the opposing articular cartilages at the ends of the two bones, and by the joint capsule, a continuous curtain connecting their sides. The *articular cartilages* are hyaline, except in certain instances noted on p. 123. They vary in thickness from 0.2 mm. to 5.0 mm., and conform to the shape of the joint. At the sides a perichondrium is present and continuous with the periosteum of the bone, but this gradually becomes thinner and disappears over the portion of the cartilage which actually rubs on its neighbor. Where they come in contact with motion the two articular cartilages are thus naked. The cells near the free surface are flattened.

In the middle strata they are rounded and are often arranged in groups; in the deepest layers they tend to be in rows perpendicular to the surface. The matrix becomes calcified as the cartilage connects with the bone, and a line of demarcation separates the calcified from the uncalcified portion. In the uncalcified cartilage, the normal slow attrition at the joint surface is compensated by amitotic division of the flattened cells.[1]

FIG. 115.—PHOTOGRAPH OF THE ANGLE OF A JOINT CAVITY, SHOWING BONE, JOINT CARTILAGE, AND SYNOVIAL VILLUS WITH INCOMPLETE COVERING. ×120

The *joint capsule* consists of an outer layer of dense connective tissue, the *stratum fibrosum;* and an inner loose layer, the *stratum synoviale* (Fig. 114). The fibrous layer is continuous with the perichondrium of the articular cartilage or with the periosteum, and is especially thickened in various places to form the ligaments of the joint. In some joints, as in the shoulder and hip, it forms a rim of dense fibrous tissue, called the *labrum glenoidale*, which deepens the joint socket. Large folds or plates of dense fibrous tissue, covered by the synovial layer, may project into the joint, thus forming the *menisci* of the knee joint and the various *articular discs*. Frequently these folds are transformed into fibro-cartilage (see p.

[1] ELLIOTT, 1936.

123); in these cases the synovial membrane may be absent over the portions which are rubbed during joint movement. Nerves and vessels are absent from the articular cartilages of the adult, and also from the labra and articular discs. Whatever meager nutriment they receive must come from the synovial fluid.

The synovial layer consists of loose connective tissue, generally with abundant elastic fibers. In many places it contains fat cells, either singly or in great numbers; and macroscopic accumulations of fat cells, similar

FIG. 116.—A SECTION OF SYNOVIAL MEMBRANE
The superficial cells are arranged in a row as in endothelium. Formaldehyde-alcohol fixation, hæmatoxylin and eosin (Hammar).

to the appendices epiploicæ of the colon, may bulge into the joint cavity. The inner surface of this layer has been described as having a continuous epithelium comparable to the mesothelium of the body cavities, and stomata have been noted and denied. But according to most authors this is not a true picture. The surface bordering the joint cavity is merely covered by flattened or even spindle-shaped fibroblasts, with somewhat shrunken nuclei,[1] between which the connective tissue fibers are often exposed. The cells are sometimes infrequent, as in places of direct pressure. Elsewhere they may be spread in a single thin layer, or heaped together in three or four strata. Macrophages are present near the surface, as is proved by the ingestion of particulate matter injected into the cavity.[2] The synovial membrane may be thrown into coarse folds (plicæ) or into minute club-shaped or tapering projections (villi). It merges with the perichondrium of the cartilaginous ends of the bones, but is not continuous over the apposed cartilaginous surfaces. Thus the cavity is bounded variously by cell groups and flattened cells, which might be construed respectively as stratified and simple mesodermal epithelium, and by naked connective tissue and naked cartilage (Fig. 115).

The synovial layer has nerves which may terminate in lamellar corpuscles and in the portions not exposed to pressure numerous blood vessels and lymphatics, which form rich superficial and deep plexuses. The *synovia* (synovial fluid) consists chiefly of water (94%), the

[1] SIGURDSON, 1930 [2] KUHNS AND WEATHERFORD, 1936.

remainder including salts, albumin, mucoid substances, fat droplets, blood cells,[1] fragments of cells shed from the membrane, and often bits of cartilage. How the fluid is maintained is not known. The synovial villi, though vascular, are not especially equipped to act as glands; production and absorption probably are functions of the whole membrane, but

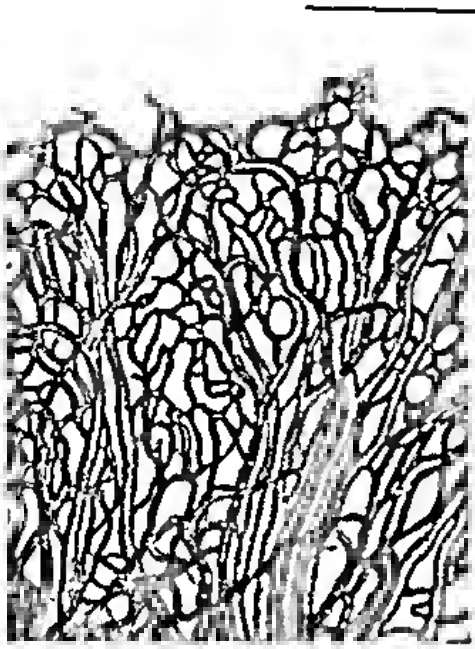


FIG. 117.—A SECTION OF SYNOVIAL MEMBRANE FROM THE KNEE JOINT OF A NEWBORN CHILD WITH INJECTED BLOOD VESSELS. ×60. (von Mollendorff.)

mucin granules have been found in the cells of well-defined areas,[2] perhaps showing that the fluid is a true secretion, not an exudate.[3]

MUSCULAR TISSUE

Contractility is a fundamental property of protoplasm which in muscle cells attains its highest development. Muscle cells are elongated structures, known as *muscle fibers*, which contain numerous longitudinal fibrils, the *myofibrils*, within their protoplasm. By the shortening of these fibrillated cells, muscular action results. The muscle fibrils may be free from transverse markings, as in *smooth muscle;* or they may exhibit a succession of dark and light transverse bands, as in *striated muscle.* In young smooth muscle cells an additional type of fibrils, the border fibril, may also be present, but apparently takes no part in contraction. Smooth muscle fibers enter into the formation of the viscera, and their action, almost without exception, is *involuntary.* Striated muscle, in so far as it constitutes the entire system of skeletal muscles, is *voluntary,* or under the control of the will, but the striated fibers of the diaphragm and upper

[1] KEY, 1928. [2] KLING, 1931. [3] BAUER, ROPES AND WAINE, 1940.

part of the œsophagus are apparently involuntary. The special form of striated muscle, known as *cardiac muscle*, which makes the bulk of the heart and extends some distance in the wall of the pulmonary veins, is involuntary. The three principal forms of muscle,—smooth, skeletal, and cardiac—are mesodermal in origin; the developmental forms of the cells are called *myoblasts*. Within the basement membrane of the sweat glands and mammary glands there are elongated ectodermal cells which have been described as smooth muscle fibers, but their contractile nature is still questioned. It is well established, however, that the muscles of the iris, which control the size of the pupil, are derived from ectodermal cells which bud off from those forming the optic cup. Ectodermal muscles in man are limited to these examples.

SMOOTH MUSCLE

The mesodermal smooth muscle cells are derived from a mesenchymal syncytium or from young connective tissue cells. They may lie singly or in small groups, as in the lamina propria mucosa of the intestinal tract and in the capsules and trabeculæ of certain glands, or in more closely packed bundles. Usually they are produced in layers which surround some tubular organ, such as a blood vessel, a duct, or a part of the digestive tube. The fibers in these layers are generally parallel and are disposed either circular or longitudinal in relation to the organ which they envelop. Occasionally they are oblique, or irregularly interwoven as in the gall bladder and uterus. Fibers which encircle an organ are called *circular* or *transverse fibers;* they may be cut across or split lengthwise according to the plane in which the organ is sectioned. The same is true of the *longitudinal* fibers, which run lengthwise of the organ.

The formation of smooth muscle may be studied advantageously in the œsophagus of pig embryos and its development in this position has been carefully described by Miss McGill (Internat. Monatschr. f. Anat. u. Physiol., 1907, vol. 24, pp. 209–245). A part of a longitudinal section of the œsophagus of a 27 mm. pig is shown in Fig. 118. In such a section the developing longitudinal smooth muscle fibers or *myoblasts* are cut lengthwise (*s.l.*); and the circular fibers which form a layer internal to the longitudinal fibers are cut across (*s.c.*). The loose mesenchymal network, from which these fibers arise, is continuous with them above and below. A third thin layer of muscle fibers is forming at *m.m.*, and at the top of the figure, the entodermal epithelium which lines the œsophagus has been included, together with the basement membrane beneath it.

In becoming smooth muscle cells, the mesenchymal cells change from a stellate to a spindle-shaped form and come closer together, but

they do not lose their protoplasmic connections with one another. At the edges of the cells coarse *border fibrils* or *myoglia fibrils* are produced, which are similar to and believed by some to be indentical with the fibroglia fibrils of connective tissue. These peripheral myoglia fibrils may extend from one cell territory to another for long distanees. They may be strikingly demonstrated in sections stained with Mallory's phosphotungstic acid hæmatoxylin or with his aniline-blue connective tissue stain.

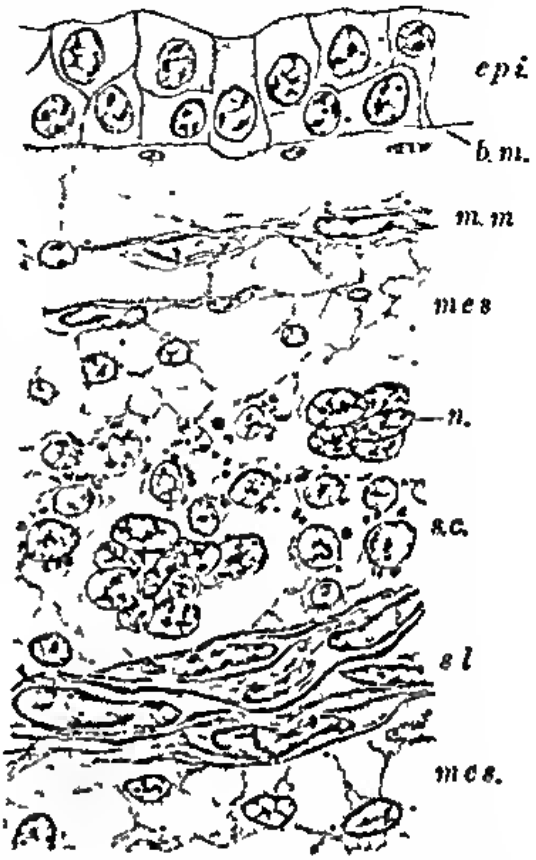


FIG 118.—FROM A LONGITUDINAL SECTION OF THE OESOPHAGUS OF A 27-MM PIG EMBRYO. × 700. (McGill)

b. m., Basement membrane; epi., epithelium, mes., mesenchyma, m. m., muscularis mucosæ; s. c., nerve cells, s. c., circular smooth muscle cut across, s. l., longitudinal smooth muscle cut lengthwise. Border fibrils at surface of cells.

The coarse fibrils shown by Miss McGill in both the circular and longitudinal muscle layers in Fig. 118 are "often found lying in part near the surface of the cell, resembling the border-fibrils of Heidenhain." She states that they are produced by a coalescence of granules within the cytoplasm, forming at first spindle-shaped bodies which later join end to end, making varicose fibers. Subsequently they become smooth. They may split into fine fibrils and usually they decrease in number as the embryo grows older. "In places they may be entirely absent in the adult tissue; rarely they are abundant." Benda[1] considered these coarse fibrils as supporting structures, like fibrils in the neuroglia or supporting tissue of the brain and suggested for them the name myoglia fibrils. Besides the peripheral myoglia fibrils, the cytoplasm of smooth muscle cells contains fine longitudinal fibrils, which have been described as the active agents in muscular contraction. Thus Miss McGill finds that in the contracted portions of the muscle fibers these fine *myofibrils* show "a distinct increase in caliber." She states that the fine myofibrils do not arise until the pig embryo reaches a length of about 30 mm. They are apparently homogeneous from the beginning and are distributed uniformly throughout the cytoplasm. Some of them are shown in the muscle layer *m.m.* in Fig. 118. Ordinarily these fibrils are indistinguishable in the close-grained, deeply staining cytoplasm which characterizes the muscle

[1] BENDA, 1902b.

cells. It may be that in fresh muscle, the myofibrils and the ground cytoplasm have nearly the same index of refraction. They become, however, usually visible after fixation of smooth muscle in acid solutions. The relation between the border or myoglia fibrils and the myofibrils, which may stain quite differently with selective stains, has been much discussed. Miss McGill believes that they arise as two different formations, while Häggqvist[1] regards the myofibrils as produced by splitting of border fibrils.

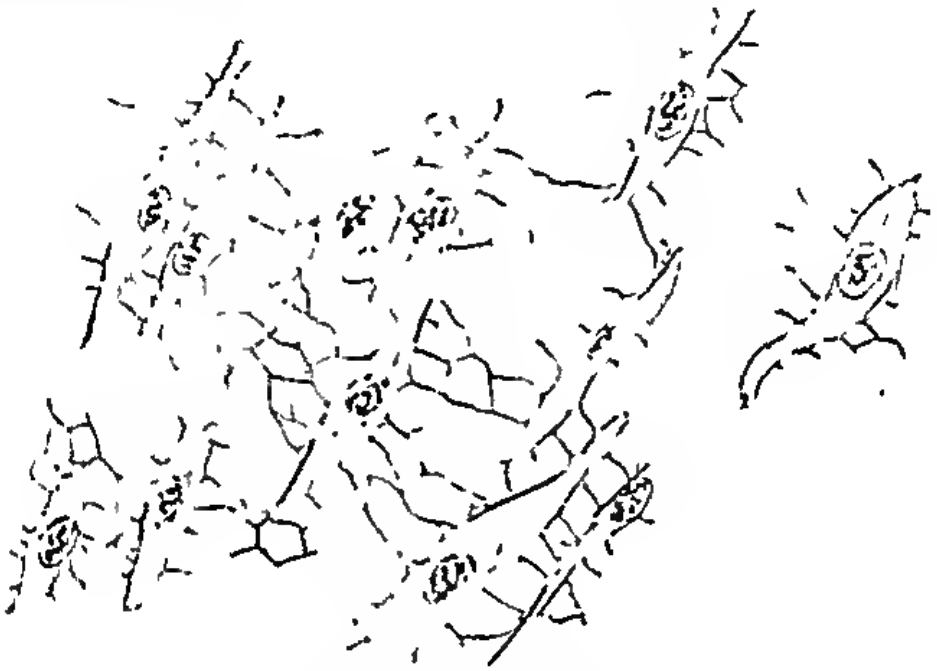


FIG. 119.—FETAL SMOOTH MUSCLE CELLS, UMBILICAL CORD. SOME OF THE CELLS SHOW MYOGLIA FIBRAE (BLEI). PHOSPHOTUNGSTIC ACID HÆMATOXYLIN

Along the sides of fetal muscle fibers there are at first protoplasmic processes which bind them together. Later these seem to be replaced by reticular fibers, like those of ordinary reticular tissue. They form a network investing the muscle cells, which is usually well shown in the walls of the blood vessels in the umbilical cord (Fig. 119). This intermuscular reticulum has been stated to be produced directly from the muscle fibers and to some extent, according to Miss McGill, from special mesenchymal cells within the muscle layer which develop into connective-tissue cells. In many layers of smooth muscle, however, connective-tissue cells are difficult to demonstrate. Finally it should be noted that elastic fibers are found between the muscle cells and vary greatly in number, being especially abundant in the walls of arteries.

Smooth muscle fibers in the adult are fusiform, cylindrical or slightly flattened cells, varying in length from about 0.02 mm. in some small blood vessels to approximately 0.5 mm. in the pregnant uterus. In the

[1] HAGGQVIST, 1931

intestine they are said to measure about 0.2 mm. Their diameter through the widest part is from 4–7 μ. Because of the length of these fibers and the fact that they are not perfectly straight, they are seldom wholly included in a single section. Moreover they are usually so closely packed that their outlines are hard to follow. They may be isolated, however, by treating fresh tissue with a 35% aqueous solution of potassium hydroxide,

FIG. 120.—ISOLATED SMOOTH MUSCLE FIBERS FROM THE SMALL INTESTINE OF A FROG. × 240

or 20% nitric acid. The fibers when shaken apart appear as in Fig. 120. Branching fibers have been isolated from the aorta and the endocardium and are said to occur also in places where the muscle fasciculi are arranged in the form of a network as in the pleuræ, ductus deferens and the urinary bladder.

FIG. 121.—A SPIRAL NUCLEUS FROM A CONTRACTED SMOOTH MUSCLE FIBER, INTESTINE OF NECTURUS. (McGILL.)

The fibers when sectioned longitudinally somewhat resemble connective tissue, from which they may be distinguished by the staining and texture of their cytoplasm and the position of their nuclei. With hæmatoxylin and eosin the muscle substance takes a deeper stain than the connective tissue fibers and it is not so refractive. In doubtful cases Mallory's aniline blue connective tissue stain may be used, which colors the muscle substance red and the collagenous tissue blue.

The nuclei of smooth muscle fibers are elliptical or rod-shaped bodies, containing a characteristic chromatic reticulum, with small granules frequently peripherally placed and sometimes several nucleoli. When the muscle fiber contracts, the nucleus shortens and broadens, but according to measurements made by Miss McGill,[1] there is no change in its volume. She finds, however, that the chromatin tends to collect at the poles of the contracted nucleus and that "the nucleus appears to take an active part in the process of contraction." Frequently spirally twisted or bent nuclei are found in layers of contracted muscle (Fig. 121) and they have been regarded as occupying contracted fibers. It is probable, however, that the spiral nuclei occur in relaxed fibers, which have been crumpled together by the contraction of adjacent fibers. Along side of the nucleus a centrosome may be found, occupying a shallow indentation in the nuclear membrane.[2]

At the poles of the nuclei there is often a condensation of granular cytoplasm, which is sometimes pigmented. The fibrils diverge to pass

[1] McGILL, 1909*b*. [2] WALTER, 1926*b*.

by the nucleus and the granular cytoplasm occupies the conical non-fibrillated space which is thus produced. Smooth muscle fibers contain mitochondria and a small Golgi net may be displayed at either one or the other pole of a nucleus. Granules of glycogen have been observed in the cytoplasm.

The surface of the smooth muscle fibers is sometimes thrown into transverse wrinkles by the contraction of a fiber. This appearance, suggesting a peripheral membrane, is probably caused by the unequal contraction of the fiber and its fine protoplasmic processes or the elastic tissue fibers encircling it. There has been much discussion on whether smooth muscle fibers are covered by a membrane comparable to the

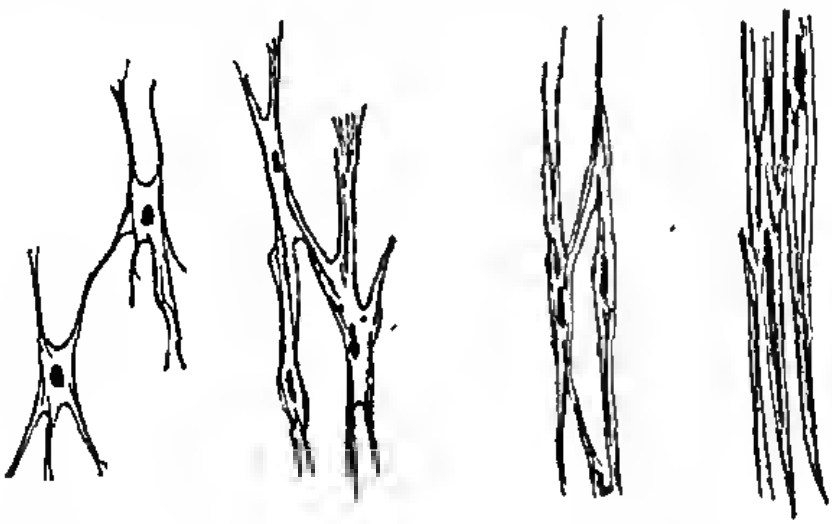


FIG. 122.—A DIAGRAM ILLUSTRATING THE TRANSFORMATION OF A LOOSE FIBRILLAR NET INTO THICK PACKED MUSCULATURE WITH SPINDLE CELLS IN A SYNCYTIUM (Benninghoff)

sarcolemma of skeletal muscle. Most histologists deny the existence of such a membrane, although Heidenhain regards the fibers as having a border sheath of hardened sarcoplasm. The subject has been discussed anew by Häggqvist, who does not support the idea of a membrane.

In transverse sections the fibers present rounded or polygonal outlines (Fig. 123). They vary in size since some are sectioned through the tapering extremity and others through the thick central part which contains the nucleus. When cut just above or below the nucleus, the section through the cell appears as a ring, the shiny edge representing the fibrils massed to surround the nucleus and the inner part the conical area of granular cytoplasm. A distance from this conical area the cytoplasm contains many punctate dots—the transverse sections of the myofibrils. The elongated nucleus is round in cross section and looks darker than in longitudinal view from the accumulation of the many tiny granules of chromatin.

Adult smooth muscle does not always retain its original syncytial nature. There may be present a complete syncytium with definite

protoplasmic connections between the cells; an incomplete or 'partial syncytium' where some cells are independent and others connected; or all cells may be separate elements. In the intestine, with the normal accumulation of food, the stretching of the tube reduces the thickness of the circular layer in a manner that can only be explained by a rearrangement of the cells. The muscle cells appear to slip by one another and to form a layer only a few fibers thick. When the intestine retracts, the cells assume their original arrangement of many rows and here it

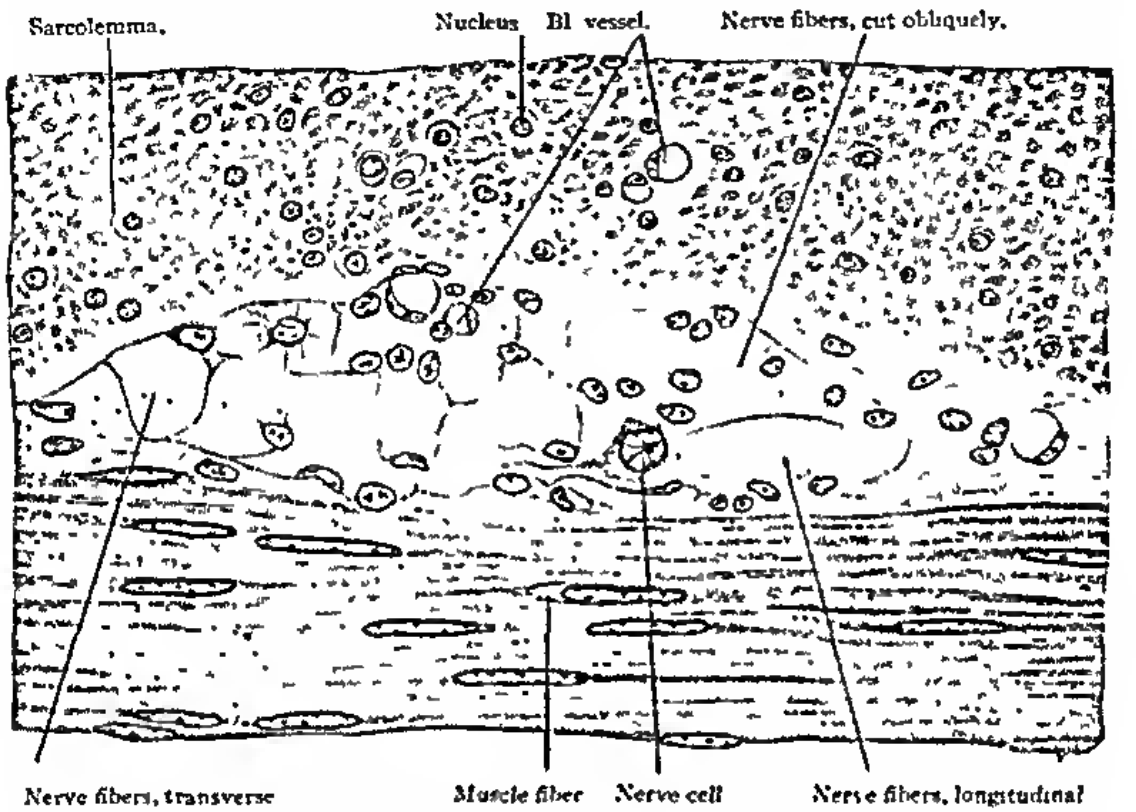


FIG 123.—SMOOTH MUSCLE FROM SMALL INTESTINE OF CAT, MUSCLES ARE CUT TRANSVERSELY ABOVE, LONGITUDINALLY BELOW, SOME MAY BE FOLLOWED LONG DISTANCES
Between the two layers a section of the nerve plexus (See p 355)

appears that they may be separate entities. W. H. Lewis[1] *explains the* action of these disconnected cells by attributing to them the property of 'stickiness,' so that the power of the muscle sheet depends on the adhesion of the individual muscles, which after stretching, return to their resting relations with other cells by 'capillary attraction.' Presumably the elastic and collagenous fibers, when present as in the walls of arteries, aid in restoring the normal caliber. With extreme contraction, however, the elastic and collagenous fibers no longer aid the muscles, but become crumpled into coarse folds, as seen frequently in contracted arteries. The enlargement of such muscular tubes as the vessels and intestines appears to be passive and due respectively to the pressure of the blood or food within. After extreme contraction the elastic tissue probably serves to

[1] LEWIS, W. H., 1922*b*.

dilate the tube to a certain extent. As to the cause of the contraction of the individual muscle fibers no agreement has yet been reached. The fibers shorten, but do not seem to become sufficiently wider to retain their full volume. Meigs[1] concludes that they have lost fluid during contraction. On the other hand when only certain portions of the individual fibers are contracted, as occasionally happens in fixation, the contracted parts are distinctly wider than the uncontracted. According to Miss McGill,[2] these deeply-staining nodular thickenings indicate a normal form of contraction in which the fiber does not contract as a whole, but a wave of contraction often communicated to neighboring fibers passes over it (Fig. 124).

FIG. 124.—A SMOOTH MUSCLE FIBER FROM THE STOMACH OF A SPARROW
Note the transverse contraction nodes and the myofibrils thickened in passing through them. (Noll.)

Smooth muscle is nourished by capillary blood vessels which tend to follow the course of the fibers and it is innervated by slender branches of the sympathetic nervous system. Lymphatic vessels are seen in the larger connective-tissue septa separating bundles of smooth muscle fibers and in the wall of the stomach and intestine they form plexuses between the layers of the musculature.

When smooth muscle is injured, the wound heals by scar formation (connective tissue). It is not known whether adult smooth muscle cells lack the capacity to divide or there are present other factors which prevent the reconstruction of the original condition. During pregnancy there is a great increase in the mass of smooth muscle in the uterus, which is caused by the elongation and thickening of the existing muscle cells. While mitoses have been seen here, it is impossible to tell whether these dividing cells are the fore-runners of smooth muscle cells (myoblasts) or are fibrocytes between the muscle cells.

SKELETAL MUSCLE

The skeletal muscles develop primarily from the mesodermic somites, which have been briefly described in a previous section (p. 59). The transformation of a portion of each of these blocks of tissue into layers and masses of skeletal muscle fibers was studied by Williams.[3] In Fig. 125, A, the core of the somite has fused with the ventral and medial walls of the mass, and the tissue thus formed is streaming over the aorta and toward the notochord. This tissue, the *sclerotome*, becomes mesenchyma and gives rise to smooth muscle and various other mesenchymal deriva-

[1] MEIGS, 1908. [2] MCGILL, 1909*a*. [3] WILLIAMS, L. W., 1910.

tives. In the part of the somite left in place, near the groove 'x,' the striated muscle fibers begin to develop. The cells here elongate at right angles with the plane of the figure, and thus lengthwise of the embryo.

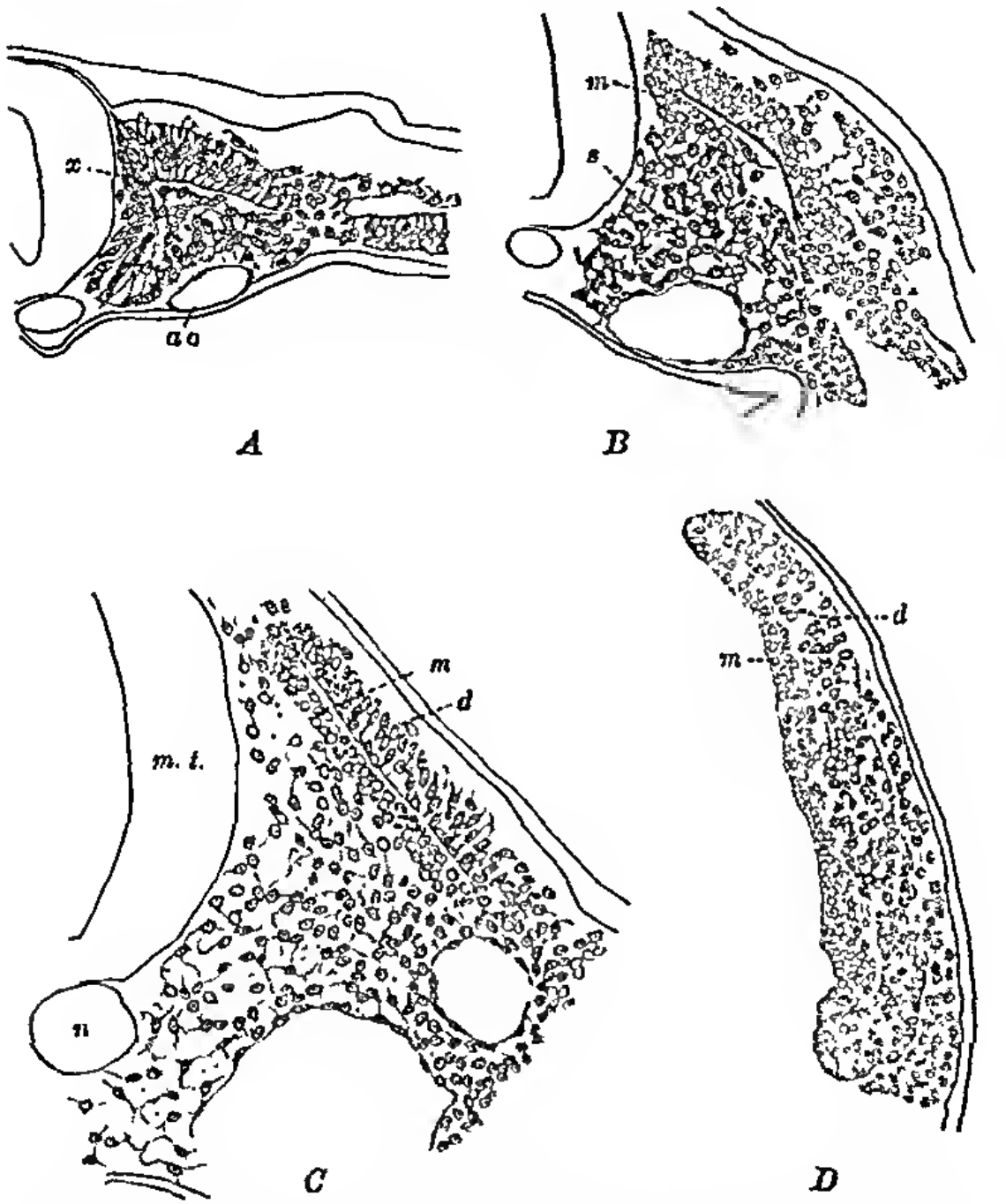


FIG. 125.—TRANSVERSE SECTIONS THROUGH THE MIDDLE OF CERTAIN SOMITES IN SUCCESSIVELY OLDER CHICK EMBRYOS. A, B, AND C, THROUGH ONE OF THE SECOND PAIR OF SOMITES IN EMBRYOS OF NINE, FIFTEEN, AND TWENTY-FIVE SEGMENTS RESPECTIVELY, D, THROUGH ONE OF THE FORTY-FOURTH PAIR IN AN EMBRYO OF FIFTY-TWO SEGMENTS. × 230. (Williams)

ao., Aorta; d, dermatome; m, myotome; m t., medullary tube, n, notochord, s, sclerotome, x, angle at which the myotome develops.

In an older stage (Fig. 125, B) these myoblasts have multiplied and have begun to form a plate of muscle tissue, the *myotome*, which extends ventrally as shown in C and D. The dorsolateral wall of the somite has meanwhile become a plate of tissue, the *dermatome*, which, with the myo-

tome, associated with it, is often called the *dermo-myotome*. The dermatome, according to Bardeen,[1] produces only striated muscle fibers; Williams finds that it forms only dermal connective tissue, and others consider that it gives rise both to muscle and connective tissue. The myotome is 'entirely transformed into muscle fibers.' The way in which the myotomes extend ventrally and break up into the ventrolateral trunk and neck musculature, and the longitudinal fusion and splitting of the dorsal part of the myotomes to produce the deep back muscles of the

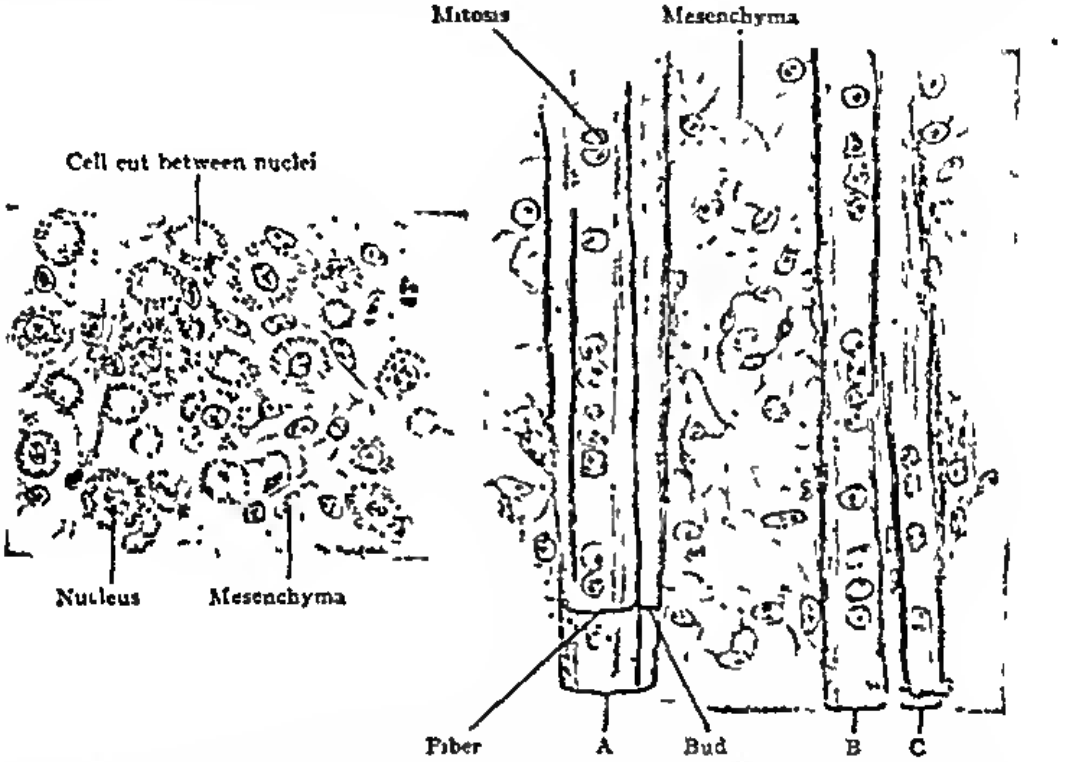


FIG. 126.—CROSS AND LONGITUDINAL SECTIONS OF MYOBLASTS FROM 60 MM. PIG EMBRYO

A, B, and C, three young muscle fibers, A is producing a bud from the main fiber, as yet without nuclei. Note the wrinkled sarcolemma on B and C.

trunk and neck, have been described by W. H. Lewis.[2] The skeletal muscles of the limbs have usually been described as arising from cells which have migrated into the limbs from the ventral part of the myotomes. If this takes place, the cells which migrate become indistinguishable from mesenchymal cells, but Bardeen and W. H. Lewis consider that 'the myotomes play no part whatever in the origin of the musculature of the limbs.' Moreover, Lewis states that 'the idea that myotomes play a rôle in the origin of the muscles of the head must be abandoned.' A radical difference in the source of smooth and striated muscle has therefore not been demonstrated, but the two forms of muscle develop very differently.

The myoblasts which produce striated muscle are found in the midst of a mesenchymal or connective tissue network, which surrounds them

[1] BARDEEN, *1900*. [2] LEWIS, W. H., *1910*.

and binds them together. During their growth, the myoblasts become greatly elongated, cylindrical structures with rounded or blunt ends. A row of pale centrally placed nuclei, resulting from repeated mitotic nuclear division, extends throughout the length of each cell. In the peripheral part of the myoblasts coarse myofibrils run lengthwise encircling the nuclei and the axial core of cytoplasm. The entire myoblast is surrounded by a membrane, to the formation of which the adjacent mesenchyma contributes.

The central position of the nuclei in myoblasts in pig embryos was clearly described by Schwann, in the second part of his treatise which established the cellular structure of animals (1839). He believed, however, that the myoblasts were formed by the coalescence of primary round cells arranged in a row. The gradual and nearly complete transformation of the protoplasm into longitudinal fibrils was correctly observed. Schwann found that the secondary cells, or mature fibers, were completely enclosed in structureless membranes, which were clearly seen in shrunken fibers.

Fig. 126 shows transverse and longitudinal sections of myoblasts developing in mesenchyma. The young muscle fibers are so long that only a portion of them is included in the longitudinal view; the nuclei are centrally placed. The coarse myofibrils appear as lines when seen longitudinally and as irregular dots when cut directly across.

As the cells grow older they become filled with fibrils, presumably through a longitudinal splitting of the coarse or primitive myofibrils; the nuclei, each surrounded by a small amount of granular cytoplasm, migrate to the periphery and rest just beneath the membranous investment. Occasionally a nucleus is found which has not reached the surface. Toward the ends of the young muscle fibers, the nuclei are more numerous and may retain their central positions. Centrally situated nuclei occur characteristically in the muscle fibers of some lower vertebrates. The growth of the fiber in length is supposed to take place at the extremities. In the formation of certain skeletal muscles, the myoblasts may increase in number by lateral budding. These buds elongate and become separated as new myoblasts along the side of the main fiber. Branched muscle fibers are found in the tongue and face of some adult animals.

Every striated muscle fiber is completely invested by a thin membrane named the sarcolemma (σάρξ, flesh; λέμμα, husk or shell). This term was introduced by Bowman[1] who described the membrane as 'a tubular sheath of the most exquisite delicacy, investing every fasciculus (or fiber) from end to end, and isolating its fibrillæ from all the surrounding structures.' He confirms Schwann's statement that it is not a fibrous structure derived from the surrounding connective tissue, and he states that the nuclei of the muscle come to lie 'at or near the surface but within the sarcolemma.' He adds, however, that he has seen similar nuclei in the sarcolemma itself. Since Bowman's time there has been pro-

[1] Bowman, 1840.

longed discussion as to the nature of this membrane. The outer portion, which may occasionally contain nuclei, appears to be of connective tissue origin, and is comparable with a basement membrane. The inner part, or true sarcolemma, is a structureless membrane closely applied to the surrounding connective tissue. It appears to be much more definite than any membrane which invests smooth muscle fibers, to which the term sarcolemma has been extended by Heidenhain and others. By microdissection[1] it is found to be a viscous elastic sheath, which can be pulled away from the fibrils, but returns almost to its original position when released.

Cross sections of skeletal muscle fibers have rounded or polygonal outlines formed by the sarcolemma and the surrounding fibrous membrane (Fig. 127). Often in histological preparations, the fibers are seen shrunken from the membrane. The nuclei occupying a little bulge beneath the sarcolemma, appear in section as ovals, some curved in conforming to the surface of the fiber. Within the fibers the cut ends of the myofibrils (muscle columns or sarcostyles) appear as minute but clearly distinguishable dots, or as short thin rods if the sections are slightly oblique. When examined with a lens of high power, the cut myofibrils may be seen uniformly distributed or marked out by fine lines of sarcoplasm into small polygonal areas,[2] called Cohnheim's areas or fields.[3] The lines of sarcoplasm between the columns are often noticeable in longitudinal sections. Although the general appearance suggests shrinkage, some authors hold that Cohnheim's areas are preformed during life. Some fibers stain more darkly than others, owing to the varying differences in the character of the sarcoplasm.

The most conspicuous characteristic of skeletal muscle, which has caused it to be called *striated*, is found in longitudinal sections. The myofibrils, which run lengthwise, are composed of alternating light and dark portions and are so arranged that the dark parts of one fibril are beside the dark parts of the adjacent fibrils. As a result of the close crowding of the fibrils, alternating light and dark transverse bands appear to pass from one side of the fiber to the other and these are the striations. They are illustrated in Fig. 129. When cut obliquely, the light and dark areas are seen superposed and confused, although the longitudinal fibrils are still observed distinctly.

Bowman (1840) stated that 'a decisive characteristic of voluntary muscle consists in the existence of alternate light and dark lines, taking a direction across the fasciculi.' He added that Leeuwenhoek had described the striæ repeatedly, believing in the earlier years of his inquiry that they were circular bands around the fibrils, but later regarding them as of spiral arrangement, comparable with an elastic coil of wire, and in some way capable of retraction. Bowman recognized that they were caused by the 'coaptation of the markings of neighboring fibrillæ.' He found that the muscle fibers can readily be split into longitudinal fibrillæ with transverse markings, but that 'in other cases their

[1] Kite, 1913. [2] Cohnheim, 1865. [3] Kölliker, 1867.

natural cleavage is into discs, and in all instances these discs exist quite as unequivocally as the fibrillæ themselves.' The discs are produced when the ends of a muscle fiber are pulled apart. Bowman regarded each disc as a plate of agglutinated segments, receiving a single segment from every fibrilla which crossed it. These segments he named *sarcous*

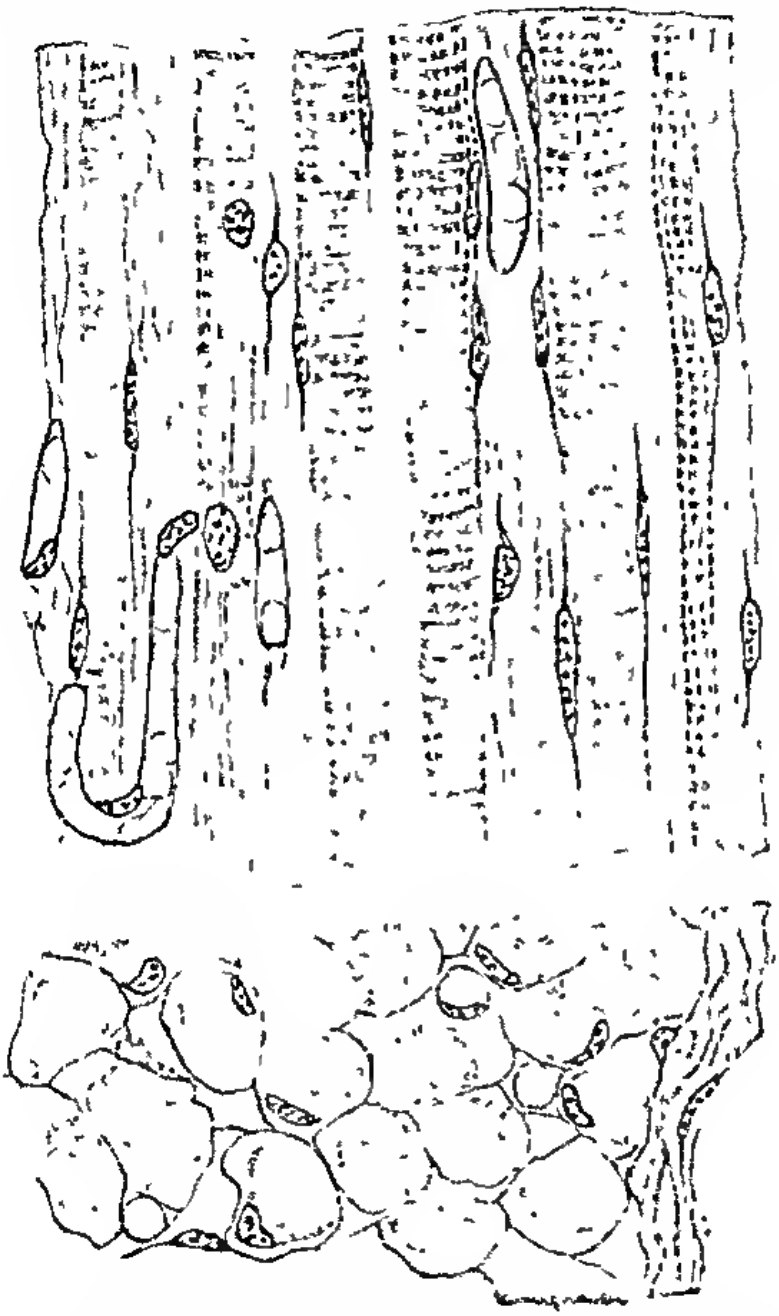


FIG. 127.—STRIATED MUSCLE, CHILD, LONGITUDINAL AND CROSS SECTIONS.

elements; they are united endwise to form the myofibrils and crosswise to form the discs. Usually the longitudinal cohesion is much greater than the lateral, and in the wing muscles of insects, according to Schäfer, the fiber 'never, under any circumstances, cleaves across into discs.'

The finer structure of the fibrils is illustrated in the diagram, Fig. 128, which represents a part of seven myofibrils, including three dark bands and portions of four light bands. Under polarized light the dark bands

are doubly refractive or *anisotropic*, and the light ones are singly refractive or *isotropic*. The names band and disc are often used interchangeably but band should be reserved for the appearances seen in fibers in longitudinal section and disc to represent the condition in three dimensions and appearing as a band when observed on edge. The striations are often designated by letters. The dark band is called Q (the initial letter of *Querscheibe*,

Fig. 128.—Diagram of Muscle Striations. (Heidenhain.)

The fibrils consist of alternating dark bands, Q, and light bands, J. J is traversed by the ground membrane Z, and Q by the median membrane M. In the right of the three muscle segments shown in the figure, the bands, N, have been drawn

or transverse disc) and the light band is called J (in German $J = I$, and was applied to a subdivision of the isotropic layer). The light band is bisected by the ground membrane,[1] or *Krause's membrane*, which appears as a very slender dark line, Z (*Zwischenscheibe*, or intermediate disc). The lines Z have been considered to represent continuous membranes which divide the fiber into muscle segments or *sarcomeres*. Between Z and Q in the highly developed striated muscles of insects, a band N has been described (*Nebenscheibe*, or accessory disc). The dark disc Q becomes gradually lighter toward its central part (thus forming h or Qh, 'h' standing for 'heller' = lighter), and in its central part it is sometimes seen to be crossed by Hensen's median membrane, M (*Mittelscheibe*). The latter is believed to be similar to Krause's membrane, but more delicate. Like the other bands it may appear dark or light according to the focus. In the muscle fibrils shown in Fig. 130, the bands Q, J, and Z may be readily identified; M appears as a rather broad white line which may include Qh.

Fig. 129.—A Section of a Muscle Fiber from the M. levator ani of Man

The section is cut obliquely and shows a separation of fibrils with anisotropic and isotropic discs. × 1450. Zenker fixation, hæmatoxylin and eosin

Much of the knowledge of the subdivisions of the bands in striated muscle has been gained from the study of lower vertebrates and insects. In human or mammalian muscle the main dark and light bands can be seen with the low powers of the microscope, and in favorable material and higher powers the Z band and Qh are detectable. When a fresh muscle is stretched almost to the breaking point, the alternating dark and light bands can still be seen in histological preparations, although they are farther apart. The light or isotropic discs are said to contain more water and less salts, while the dark or anisotropic discs have less

[1] Krause, 1869.

water and a greater concentration of salts. Macallum,[1] applying histochemical methods, found the dark discs to be richer in both phosphorus and potassium. In microincineration preparations, Scott[2] observed heavier ash deposits in the dark discs—the light discs being practically free of ash. With the electron microscope, Scott and Packer[3] identified Ca-Mg localized in the ash of the 'contraction bands.'

The cytoplasm of the muscle fiber is called the *sarcoplasm*. It varies both in amount and consisteney in different muscles and contains besides the myofibrils and the nuclei, refractile *interstitial granules* or *sarcosomes*, fat droplets, lipoids, glycogen, pigment and other substances. The granules have been carefully studied by Bullard,[4] who discussed their staining reactions and probable composition. The significance of the interstitial granules could not be determined. The fat droplets are regarded as reserve food material and they vary in abundance according to the quantity of fat in the food; while the glycogen is especially concerned with the phenomenon of contraction. Besides 'wear and tear' pigment some granules contain an iron bearing pigment—*myoglobin*. It is related to hæmoglobin and Morner[5] gave to it the name muscle hæmoglobin or myochrome. The amount of muscle hæmoglobin varies in different muscles and in the same muscle in different individuals. Since it is bound in the sareoplasm, muscle fibers rich in sarcoplasm are redder than those poor in ground substance. The mitochondria in skeletal muscle fibers are mostly filamentous, although granular or spherical forms occur; they are found more numerous near the nuclei than between the myofibrils.

It has been generally assumed that the shortening of skeletal muscle fibers takes place through contraction of their longitudinal fibrillar elements. Since these fibrils are not seen in living muscle, some authors consider that they do not exist as definite structures. Kite was able tu move a microdissecting needle freely through the light discs of the fibers, meeting no obstructions and to cut the firmer dark substance into smaller bits which could be readily dislodged. In tissue cultures the transverse bands appear in young muscle cells with no sign of longitudinal fibrillation. The inability to see fibrils in living muscle and to detect them with the method of microdissection are not sufficient criteria to invalidate their existence. Fibrils and the ground substance in which they are embedded have nearly the same index of refraction in living muscle and there is a wide difference between the magnitude of what can be detected by microdissection and the finest of fibrils. The 'ultimate' fibrils are of molecular proportion (p. 14), and bundles of them when of sufficient size are called myofibrils, muscle columns or sarcostyles.

[1] Macallum, 1905. [2] Scott, 1933. [3] Scott and Packer, 1939.
[4] Bullard, 1912. [5] Mörner, 1897.

Myofibrils can be seen extending lengthwise of the muscle fibers in preparations after fixation and staining. The present state of the question—how chemical energy is transformed into mechanical energy in contract-

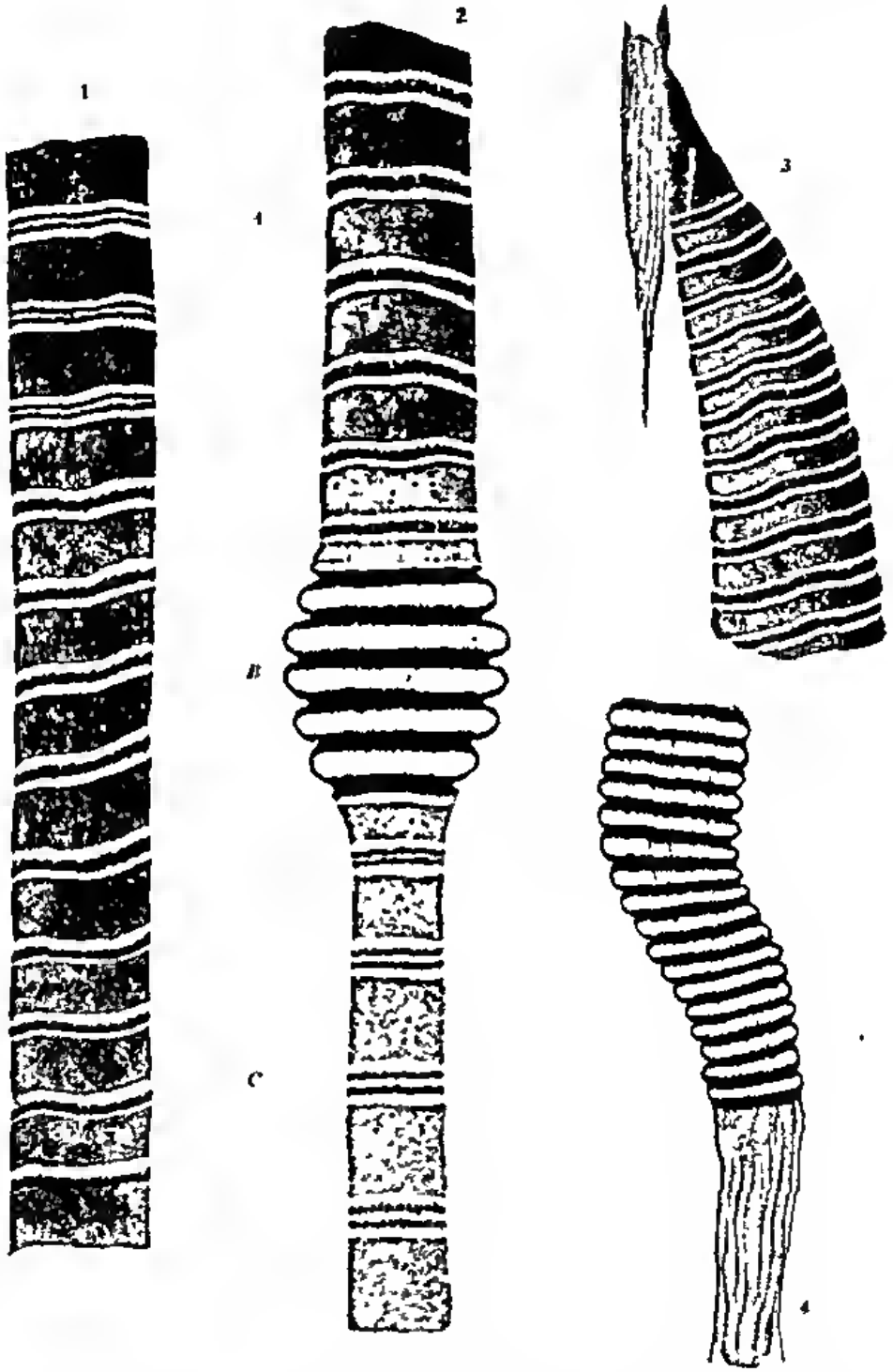


Fig 130.—Structure of Striated Muscle Fibers in the Leg Muscles of a Water Beetle.
1. Muscle fibers at rest, 2 fiber at rest at A, contracted at B, and stretched at C, 3 fibers with tendon attachment, 4. fiber teased in glycerine (Thomas Dwight, *Proc Boston Soc Nat Hist*, Vol. 16, 1873)

ing muscle is discussed by J. Sacks, 'Changing concepts of the chemistry of Muscular Contraction,' Physiological Reviews, Vol. 21, pp. 217–241, 1941.

In many animals, as in the rabbit, two sorts of striated muscles may be recognized —red muscle (*e.g.*, the M. semitendinosus and M. soleus); and pale or white muscle (*e.g.*, the M. adductor magnus). Correspondingly there are two sorts of fibers. First, there are dark fibers with abundant sarcoplasm, well-defined longitudinal striation, and poorly-developed transverse markings, having in general a small diameter; these occur in red muscles, which are also said to contain more myoglobin or muscle hæmoglobin. Secondly, there are pale fibers, with less sarcoplasm and better-defined transverse striations, having a greater diameter. These are the more highly differentiated fibers. Although in some animals these two sorts of fibers are found in separate muscles, in others, as in man, they are mingled in single muscles. In general the most constantly active muscles (cardiac, ocular, masticatory and respiratory) contain the most fibers with abundant sarcoplasm. The muscles having many fibers with scanty sarcoplasm contract more quickly but are exhausted sooner. The pale fibers are never so preponderant in man as to make pale muscles

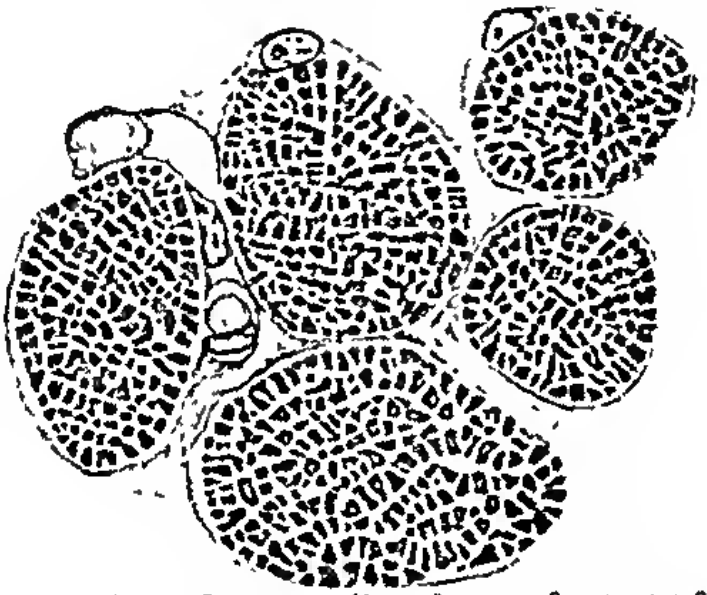


FIG. 131.—CROSS SECTIONS OF MUSCLE FIBERS IN THE HUMAN NEW-BORN SHOWING THE SHAPES OF THE MUSCLE COLUMNS OR COHNHEIM'S FIELDS (Weigel)

Adult muscle is composed of fibers arranged in compact bundles, shown in cross section in Fig. 132. Around all the larger muscles there is a connective tissue sheath, or *external perimysium*, which extends into the muscle in the form of septa, thus subdividing it into bundles or *fasciculi*. These septa constitute the *internal perimysium*, and the connective tissue extends from them around the individual muscle fibers, blending with the sarcolemma. In the interstitial connective tissue of the diaphragm, the muscles of the eye, the face and tongue, elastic fibers are abundant; but the muscles of the extremities are poor in elastic tissue, containing only fine, chiefly longitudinal fibers, found especially in the perimysium externum.

The size of the muscle fibers is subject to considerable variation. They are said to enlarge at a uniform rate throughout the body until birth, when their diameter is about twice as great as in embryos of four months. After birth the fibers of certain muscles become much coarser than those in others. Thus the gluteal muscles have large fibers (av. diam. 87.5 μ)

and the ocular muscles have small ones (av. diam. 17.5 μ), as determined by Halban.[1] He finds that the diameter of the adult fibers in general is about five times greater than at birth. As a result of exercise the diameter of muscle fibers in rats may show an average increase of 25% according to Morpurgo.[2] He states that the enlargement of the muscle takes place without an increase in the number of its fibers, but merely through the thickening of existing elements. The fibers which grow most are those which originally were thinnest, and which act as a reserve material with

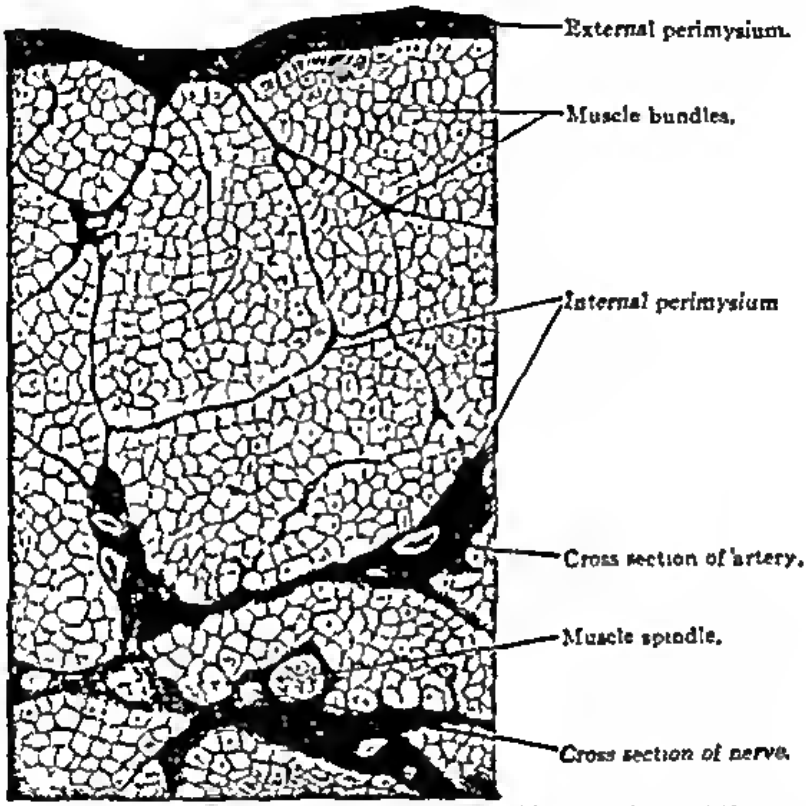


FIG. 132.—FROM A CROSS SECTION OF THE OMOHYOID MUSCLE OF MAN. × 60

great capacity for growth. The enlargement of fully formed fibers apparently takes place through an increase in the sarcoplasm, without multiplication or thickening of the fibrils. In the lower vertebrates (fishes and amphibians) the fibers are generally larger than in mammalian muscles. As to the length of skeletal muscle fibers, there is some disagreement. Kölliker[3] stated that a fiber extended the whole length of a muscle, *e.g.* from the tendon of origin to the tendon of insertion. Several later authors have given measurements, according to the length of a muscle, of 1 to 40 mm. to 'not more than 15 cm.' Lockhart and Brandt[4] isolated a fiber 34 cm. long from a human sartorius muscle 52 cm. long and noted that the fiber was abruptly broken in its length. In a fetal sartorius muscle 5 cm. long a single fiber was teased from the whole length of the

[1] HALBAN, 1894. [2] MORPURGO, 1897. [3] KOLLIKER, 1852.
[4] LOCKHART AND BRANDT, 1938.

muscle. Likewise, in adult human eye muscles, the fibers were as long as the muscles. The fibers were said to be separate, that is without a syncytial arrangement.

After injury skeletal muscle in the higher vertebrates gives slight evidence of regeneration. A proliferation of nuclei toward the injured ends of the muscle fibers has been recorded, but repair is chiefly through the production of a connective tissue scar. Speidel in living frog tadpoles has observed a regeneration of some muscle fibers. He found that while badly injured fibers degenerate, less severely injured ones may undergo a dedifferentiation with almost complete loss except some nuclei and sarcoplasm. From these, myoblasts arise which grow to form new fibers.

FIG 133.—THE UNION OF SKELETAL MUSCLE FIBERS AND TENDON FROM HIPPOCAMPUS (THE SEA-HORSE)

Formaldehyde fixation. Photographed in polarized light. ×630. (W. J. Schmidt.)

Striated muscle fibers have been seen in which some of the myofibrils or muscle columns are arranged spirally instead of parallel to the longitudinal axis of the fiber. Fibers with this arrangement are observed in the tongue of the toad and chameleon, the uvula of man, and the eye muscles of man, dog and rabbit. Each fiber shows a close central core where the fibrils are less twisted and scattered than at the periphery. As a result of the twisting the sarcolemma becomes indented, especially near the nuclei and the fiber uneven. A yellow pigment is said to occur characteristically in the sarcoplasm around the nuclei. The significance of the spiral arrangement is unknown; but Thulin[1] suggests that it probably allows for the lightening protrusion of the tongue in the toad and chameleon.

The way in which muscle fibers connect with tendon has been studied with conflicting results. There is a very close union and two principal opposing views: 1. the *apposition theory*—the muscle fiber is completely enveloped by sarcolemma and the tendon fibers are attached to its outer surface; 2. the *continuity theory*—the myofibrils and tendon fibrils are continuous each with the other through the sarcolemma. In both cases the tendon sheath blends with the fibrous investment of the sarcolemma. Baldwin[2] finds that the ends of the muscle fibers are primarily conical and are covered with sarcolemma; and the tendon fibrils connect with the sarcolemma at the apices of the cones. Processes of sarcolemma are thus primarily 'dovetailed' into the

[1] THULIN, 1908. [2] BALDWIN, 1912.

tendon. Secondarily the cones may blend to form a thickened flat layer to which perichondrial or periosteal fibers are attached. In no case is the sarcolemma penetrated by muscle fibrils or tendon fibrils and therefore there is no continuity between them. Goss[1] sees that the muscle fibers and the tendon are separated by an interval of as much as 100 μ, bridged by argyrophil fibrillæ. When a muscle with its attached tendon is put into hot water, the ends of the muscle fibers retract from the sarcolemma while the tendon remains connected. It has been claimed that this experiment shows a discontinuity between myofibrils and tendon fibrils, but it can be interpreted also, that the muscle and tendon shrink at different rates and the muscle fiber pulls away from the sarcolemma. Schultze[2] states that at the end of the muscle fiber the myofibrils are no longer differentiated into light and dark bands, but pass directly into the tendon fibrils, with which they are continuous—'muscle fibrils and tendon fibrils are parts of a single structure.' The sarcolemma then can be compared to a microscopic sieve through which the fibrils pass. From studies on the histogenesis and repair of muscle it has been asserted that a direct continuity occurs between muscle and tendon fibrils. Speidel[3] considers such a continuity in studying regenerating muscle in the tails of living tadpoles. A third, or *sarcolemma theory* is presented by Péterfi,[4] and others who think that contraction consists of the widening and shortening of each sarcomere and that the force is imparted to the telophragmata or Z discs and from them to the sarcolemma. Both these structures are related to fibrous connective tissue, at least in staining reactions and the sarcolemma connects directly with the fibrils of the muscle tendon or periosteum. The pull is transmitted not through the muscle fibrils but peripherally through the sarcolemma. The problem of muscle-tendon union and the literature on the subject is discussed at some length by both Wassermann[5] and Häggqvist.[6]

Muscles are abundantly supplied with blood vessels. The arteries enter the muscle, branch and rebranch forming primary and secondary networks and finally capillary plexuses with elongated rectangular meshes between the fibers. It has been suggested that the number of capillaries depends in part on the size of the muscle fibers; the capillaries are close together in birds and mammals where the fibers are small and farther apart in cold-blooded animals. Krogh[7] counted the capillaries in a large number of equal-size areas in sections from the same muscle and found a remarkable regularity in distribution—there being about 1350 capillaries to the sq. mm. in the gastrocnemius muscle of the horse.

[1] Goss, 1943. [2] Schultze, 1912. [3] Speidel, 1939. [4] Péterfi, 1913.
[5] Wassermann, 1929. [6] Haggqvist, 1931. [7] Krogh, 1919.

Veins draining the capillaries follow the course of the arteries and even the smallest venous tributaries are provided with valves. Because of the abundance and the arrangement of the arterial and venous networks, the vascular system adapts itself to changes brought about by muscular contraction, even when some of the capillaries are closed temporarily. The lymphatic vessels end in the intermuscular septa without extending

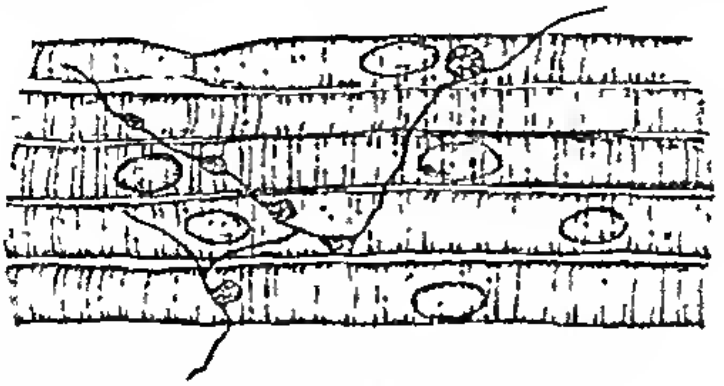


FIG 134.—DEVELOPMENT OF MOTOR END-PLATES IN THE TONGUE OF AN EMBRYO OF TALPA. × 2100. BIELSCHOWSKY'S METHOD. (Boeke.)

among the individual muscle fibers. They are numerous, however, in the connective tissue sheath of muscles and in tendon.

The motor nerves are chiefly of large size and branch repeatedly, each to supply an individual muscle fiber. On reaching a fiber they lose their medullary sheath and the axis cylinder enters the fiber to terminate in a specialized end-plate. An occasional 'accessory' non-medullated nerve fiber was thought at one time by Boeke[1] to be sympathetic in

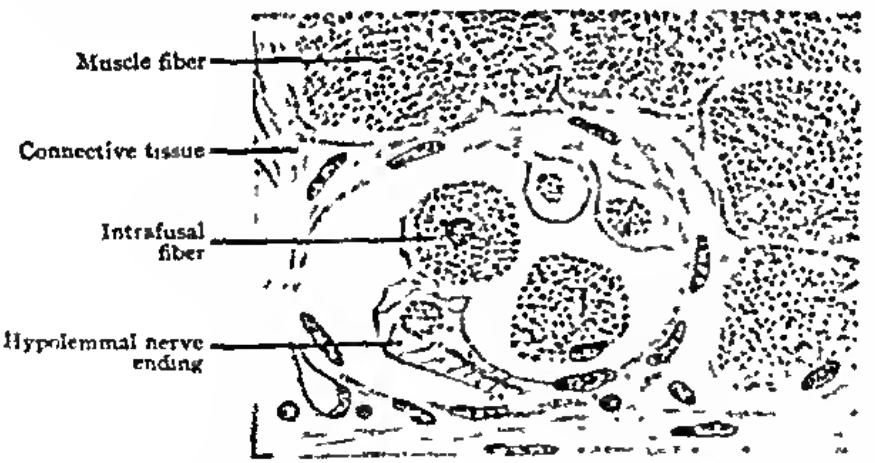


FIG 135.—MUSCLE SPINDLE, TRANSVERSE SECTION. NERVE FIBERS MAKING HYPOLEMMAL NET.

origin, but it is now generally agreed that it is a collateral motor fiber. Sympathetic nerve fibers, as far as known supply only the blood vessels.[2] Sensory nerves encircle the muscle fibers to terminate in the *muscle spindles*. The spindles are slender bundles of poorly developed fibers, generally situated near the septa formed by the internal perimysium, as seen in Fig. 135. Around these muscle cells nerve fibers wind spirally,

[1] BOEKE, 1909. [2] TOWER, 1931.

both structures being embedded in loose connective tissue, the whole surrounded by a denser connective tissue sheath. (See further p. 209.) All the muscle spindles are formed during embryonic life,[1] and their abundance and distribution in the various muscles in embryos have been studied by Gregor.[2] They have not been found in all muscles, and in certain muscles they are regularly more numerous than in others. Thus they have been reported as absent from the muscles of the eye, face, pharynx, small muscles of the larynx, the *Mm. ischiocavernosus* and *bulbocavernosus*, and certain others, including a large part of the diaphragm. They are numerous in the distal muscles of the limbs, and in certain muscles of the neck. The finer structure of the nerve terminations, both motor and sensory, will be considered with the nervous system.

CARDIAC MUSCLE

Cardiac muscle develops exclusively from the mesenchymal syncytium surrounding the endothelial heart tube. Its nuclei are found in the axial part of the protoplasmic strands, at varying intervals from one another. Peripherally a few banded myofibrils extend for considerable distances through the syncytium regardless of cell areas. They multiply rapidly, and form a peripheral layer of fibrils surrounding the central nuclei and axial protoplasm. Thus as seen in cross section, the strands of cardiac syncytium and the myoblasts of skeletal muscle resemble one another. The fibrils exhibit alternating dark and light bands which are arranged as in skeletal muscle, and ground membranes (*Z*) develop across the fibers, bisecting the light bands (*J*). The striations, however, are not so regular nor so highly developed as in skeletal muscle. At the periphery of the fibers there is a thinner and less distinct covering than the sarcolemma of skeletal muscle. This covering is interpreted by some as a true sarcolemma and by others as only a thickening of the superficial sarcoplasm. In early stages the muscle fibers in many places rest close against the endothelium of blood vessels; later they are surrounded by more or less connective tissue. Sections impregnated by appropriate reduced silver methods show the muscle fibers enmeshed in reticular tissue.

In the adult the cardiac muscle fibers anastomose freely, thus retaining their original syncytial arrangement throughout the whole of the myocardium of both atria and ventricles (Fig. 136). They do not, however, form an irregular network, but are arranged in layers, in which the fibers tend to be parallel. The elliptical nuclei retain their central positions. At each pole of a nucleus there is a conical mass of sarcoplasm,

[1] Cuajunco, 1927. [2] Gregor, 1904.

occupying the interval left between the myofibrils as they diverge to pass by the nucleus. The sarcoplasm is granular and contains besides interstitial granules and mitochondria, a brown pigment near the poles of the nucleus. Significant amounts of this pigment are found in old age and in emaciated persons. It has a component which stains with the fat soluble dye Sudan *III* and it does not give the reaction for iron. Normal cardiac muscle of mammals displays a varying quantity of neutral fat droplets which are arranged in rows between the myofibrils and in the

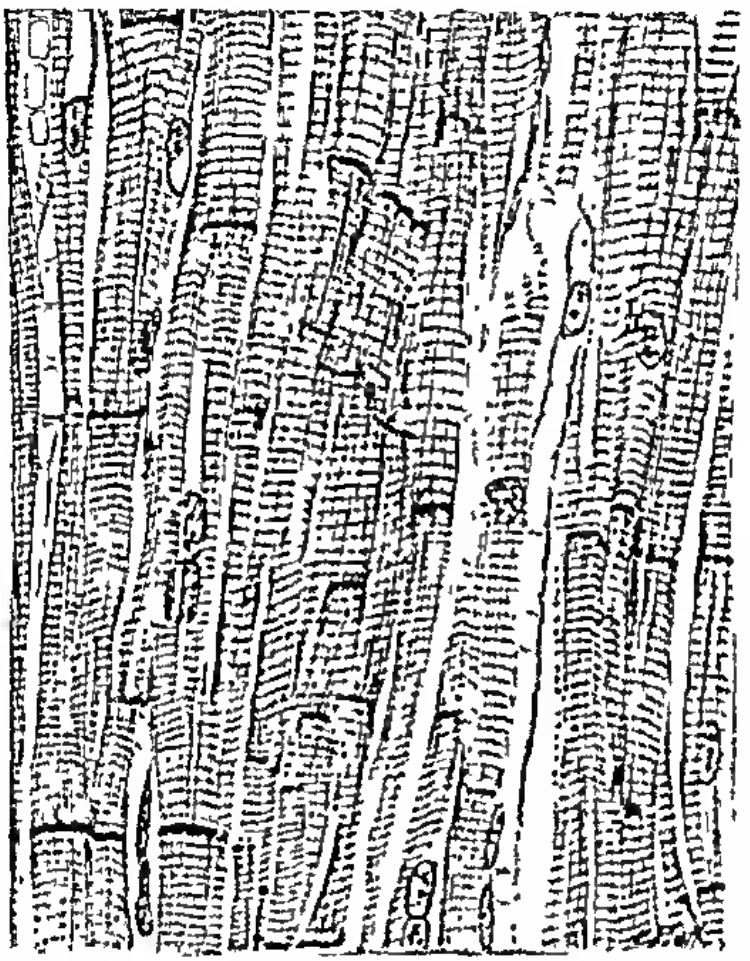


FIG. 136.—A SECTION OF HUMAN CARDIAC MUSCLE. (Drawn from a preparation by Prof. M. Heidenhain.)

perinuclear sarcoplasm. These fat droplets are present in the later weeks of fetal life and subsequent to birth their quantity fluctuates with the intake of food.

A feature of cardiac muscle which is unlike anything observed in smooth or skeletal fibers is the presence of *intercalated discs*. These are transverse lines across the fibers, occurring at variable distances from one another. Heidenhain[1] shows them as always connected on one side with the ground membrane ζ, and somewhat narrower than a sarcomere (*i.e.*, the distance between two successive ζ bands). They may cross an entire

[1] HEIDENHAIN, 1911.

fiber of the syncytium, or a part of it, or may form a series of steps from one side to the other. In ordinary histological preparations, the discs are usually inconspicuous, but they can be brought out clearly by silver nitrate or by staining with iron or phosphotungstic acid hæmatoxylin and other special staining combinations. There is no agreement as to the significance of these intercalcated discs. They have been considered as membranes dividing the syncytium into separate cells, because fresh cardiac muscle is said to separate at the discs when treated with reagents as solutions of sodium or potassium hydroxide, also pathologically the muscle fragments at the level of the discs. But in favorable preparations, the myofibrils are observed to pass across the discs from one part of the syncytium to another. Heidenhain regards them as the places where new sarcomeres form, thus providing for the growth of the heart, but they are late in development and relatively less abundant and simpler in the young than in adults. Jordan and Steele[1] among others, consider them evidence of local contraction. They have been interpreted also, as fine elastic tendinous inscriptions in the muscle and as thickened or altered Z discs. Because of their numerical increase with age, Cohn[2] thinks of them as occurring mechanically with stress. Carey[3] views the cross striations as the expression of pressure waves radiating from centers of nerve impulse, and the discs as points of interference where waves arriving by many anastomosing pathways fail to meet accurately. He finds them in other branched muscles also. A rather unique and speculative interpretation is given by Kato[4] who believes that the discs are a special nutritive contrivance of the heart muscle fibers. Transverse capillaries enter the superficial stratum of the muscle cell and then degenerate, leaving crevices in the protoplasm through which food and oxygen can reach the interior of the cell. They appear only when the fibers attain a large diameter and are thus small or absent in embryos. In the bird, the heart muscle fibers are very thin and can be nourished without this contrivance

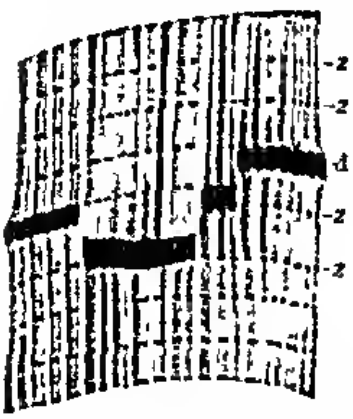


Fig 137.—Intercalated Disc (d) from Human Cardiac Muscle, Stained with Thiazin Red and Toluidin Blue (*Heidenhain*)
z, Krause's membrane

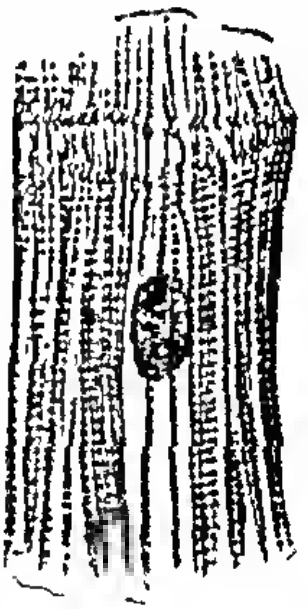


Fig 138.—A Portion of a Cardiac Muscle Fiber Showing the Continuity of Myofibrils across an Intercalated Disc (Przewoski)

[1] Jordan and Steele, 1912 [2] Cohn, 1932. [3] Carey, 1936. [4] Kato, 1928.

There are, therefore, three peculiarities of cardiac muscle through which it differs conspicuously from skeletal muscle, namely, its anastomosing fibers, central nuclei, and intercalated discs.

The origin of the visible striations in both skeletal and cardiac muscle is still not clear. They do not appear to be necessary for contraction, for the embryonic heart may beat before they can be detected. Goss[1]

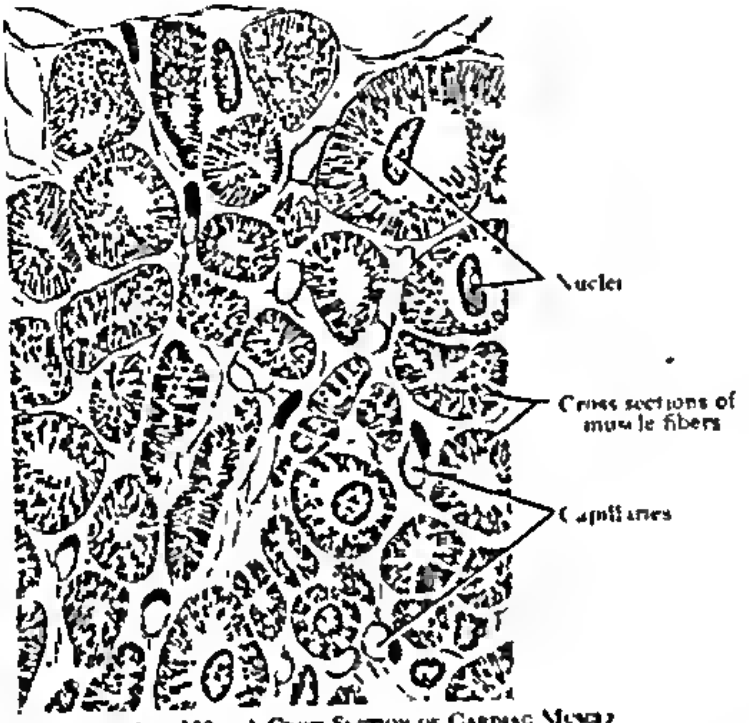


FIG. 139.—A CROSS SECTION OF CARDIAC MUSCLE

From a section through the wall of the right atrium at the entrance of the superior vena cava. × 500. Absolute alcohol fixation, Hansen's hæmatoxylin, eosin-phosphomolybdic acid-Methyl blue. (von Mollendorff.)

describes the movement of mitochondria previously scattered in the cells into more or less regular longitudinal and transverse rows, but states that no difference was perceptible in the contractions before, during or after the change. 'The only factor which was associated with the differentiation was that of mechanical tension in the cells.' After fixation, a very few fibrils have been found in the hearts of chick embryos of 10 somites, when pulsations first begin.

On the other hand Wolbach,[2] from the study of muscle tumors, brings forward the view that the striated myofibrils arise by the multiplication and alignment of diploid granules, derived from the centrosome or centriole, which then become joined by fibrils. This would link the muscle fibrils with the cilia, or with the flagella of protozoa, both of which are connected with basal bodies supposedly of centriole origin, and thus strengthen the prevalent theory of the kinetic rôle of this body within the cell. No embryological evidence has, however, supported this finding.

The rhythm of the heart beat is presumably preserved by the syncytial nature of the heart muscle as a whole, one portion affecting another with-

[1] Goss, 1932. [2] Wolbach, 1928.

out the necessity of nerves. But W. H. Lewis,[1] in the study of tissue cultures of heart muscle, finds that isolated portions of cells may beat independently often with a different rhythm from that of the main mass, and questions whether heart muscle is actually syncytial and not rather an 'adherent reticulum.' In the adult heart the duty of transmitting the impulse for contraction seems to devolve in part on certain specialized cardiac fibers found under the endocardium in the '*sino-atrial*' and '*atrio-ventricular bundles*' and their connections. These are the '*Purkinje fibers*' which differ from ordinary cardiac muscle fibers in being thicker and showing fewer myofibrils. They are confined near the periphery or irregularly scattered in the sarcoplasm and the cross striations are indistinct. While the fibers are arranged in apparently separate bundles, they may be seen to merge with cardiac fibers at the finest branches of the bundles. Purkinje fibers may be considered then as a muscular syncytium which has retained perhaps somewhat more of the ability to conduct nervous impulses by being less differentiated for contraction. They are smaller in the human heart than in the sheep and ox where they are relatively enormous.

Injured cardiac muscle fibers so far as known do not regenerate. Scars (connective tissue) of varying sizes from microscopic to large macroscopic masses are seen closing the defects with threads of connective tissue intermingled with the ends of the muscle but without any visible sign of new formation of muscle.

The actual cause of the shortening of muscle fibers, whether smooth or striated, is as yet undetermined The older theories that fluid passed from the intercellular spaces into the fibers, causing them to swell and shorten, or from one portion of the striated fibril to another, have been gradually abandoned as inadequate from the physical point of view. Contraction is accompanied by the alteration of glycogen to lactic acid, and the present tendency is to regard the presence of this acid as responsible for a change in surface tension between fibrils and sarcoplasm sufficient to cause movement, or for a change in the molecules of the fibrils from a 'liquid crystal' to a more solid form, with accompanying shortening. Beyond the fact that striated muscle contracts as a rule more rapidly and more strongly than smooth muscle, the very meaning of the striations is not known. W. H. Lewis suggests that 'since myofibrils are artifacts or transitory structures, and since muscle-cells in which no fibrils or cross-striations can be detected, either before or after fixation, beat rhythmically, theories of muscle contraction can not be based upon visible fibrils or cross-striations with any assurance of possible validity. The conditions which are found in cultures practically force us to base our theories on the physical and chemical conditions of the contractile molecule itself. The configuration of such gigantic colloidal molecules may well be that of elongated flexible crystals or threads arranged with their long axes parallel to the direction in which contraction takes place. Contraction then might result from the shortening and broadening of these unstable molecules by either physical or chemical alterations in their structure.'[2] As a result of reactions

[1] Lewis, W. H., 1919. [2] Lewis, W. H., 1926.

taking place in the side-chains, the ends of the long molecules are brought closer together in the contraction and farther apart in the relaxation of a muscle.

Carey,[1] by increasing the pressure within the bladder of a puppy, obtained rhythmic contractions of the organ over a period of 48 days, and found that the original smooth muscle fibers had changed to striated fibers, resembling heart muscle. He concludes that the type of muscle depends upon the extrinsic stresses which it encounters in the position in which it develops, and not on any inherent quality of the muscle cell itself. This experiment has not been confirmed

NERVOUS TISSUE

General Features. In nervous tissue the protoplasmic functions of irritability and conductivity attain their highest development. Irritability is that property which enables the cell to react to various stimuli, such as pressure or light; and through conductivity the effects of stimulation are

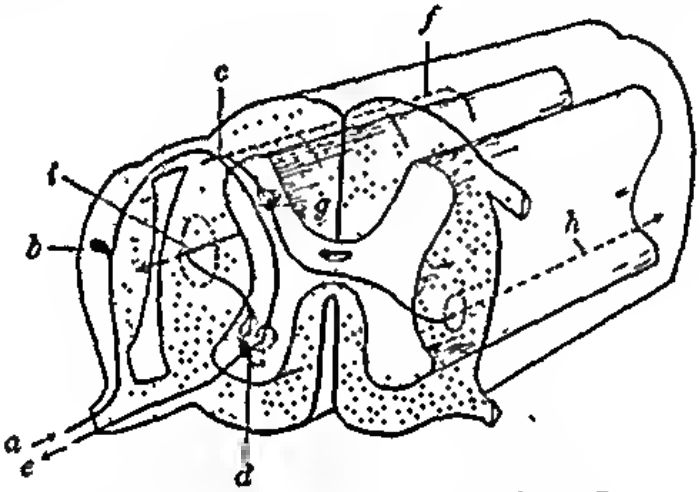


FIG. 140.—DIAGRAM OF THE SPINAL CORD SHOWING A SENSORY FIBER, a; A MOTOR FIBER, e; AND THE FIBERS WHICH CONNECT THEM WITH EACH OTHER AND WITH THE BRAIN

transmitted to distant parts of the cell, or to adjacent cells. In all animals the cells of the outer or ectodermal layer are those most exposed to stimulation, and the ectoderm accordingly gives rise to the entire nervous system. In some animals all the ectodermal cells have been described as equally responsive to stimulation, and the name 'sensory layer' has been applied to the ectoderm as a whole. Usually, however, the sensory cells become specialized in definite and limited areas of the ectoderm. M. Schultze (1862) showed that the sensory cells of the nose and eye are epithelial elements, the bases of which are prolonged into filaments which serve as nerves to convey sensation. He taught that the specific functions of the sense organs depend on their respective epithelial cells, which accordingly may be designated as olfactory, gustatory, auditory or visual cells.

Not only does the ectoderm produce sensory *neuro-epithelial cells*, the nucleated bodies of which remain in the epithelium, but it gives rise to

[1] CAREY, 1921.

more deeply placed *nerve cells*, which connect with the epithelial cells and place them in communication with the muscles. In simple forms of animals this connection is very direct, and the response of the muscle to epithelial stimulation is quite automatic. In the higher animals there are both direct and indirect paths from the sensory endings to the muscles, and muscular action may be inhibited or initiated by certain of the centrally placed nerve cells.

The centrally placed cells in vertebrates constitute the spinal cord and brain, which together form the *central nervous system*. The bundles of fibers, which convey impulses to and from the central nervous system, together with the cells associated with them, constitute the *peripheral nervous system*.

In the olfactory epithelium of vertebrates there are neuro-epithelial cells which send fibers directly into the central nervous system, but in other cases the nucleated bodies of the sensory cells are not found in the epithelium. They occur in circumscribed masses or *ganglia*, from which fibers extend both into the central nervous system, and outward to various sensory structures, where they terminate in contact with cells which stimulate them. Thus the stimulus which gives rise to a tactile sensation is received by the terminal ramifications of a nerve fiber in the skin. The stimulus is conveyed along this fiber (Fig. 140, *a*), through the spinal ganglion *b*, into the spinal cord, where it produces several branches (at *c*). One of these branches passes to a motor cell, *d*, to which it transmits its stimulus. The motor cell sends a fiber outward (*e*) to terminate in contact with a striated muscle, which is thereby stimulated so that it contracts. This direct path from the sensory ending to the muscle provides for reflex or unconscious action, such as is taken when the hand is suddenly withdrawn from a painful contact. In such a case a considerable group of muscles may contract together, since the sensory fiber sends branches up and down the cord (*f*), and these in turn give off *collateral branches* which pass to motor cells at different levels.

The cell which conveys the tactile sensation from the skin to the spinal cord gives rise to branches which terminate in contact with other cells in the spinal cord, as shown in Fig. 140, *g*. From these cells processes cross to the opposite side of the cord and pass up to the brain (*h*), where they connect with nerve cells through which the sensations become conscious. These brain cells presumably become permanently modified by the sensations which they receive, so that they store experiences. As a result of the sensation transmitted from the skin, certain cells in the brain may send stimuli downward to the motor cells of the cord, which then cause the muscles to act voluntarily. The descending fiber crosses to the oppo-

site side during its descent, and occupies the position in the cord shown in Fig. 140, *i*. A branch is shown passing to the motor cell, *d*.

From this sketch of the constitution of the nervous system, it is seen that it consists essentially of cells, made up of *cell bodies* and of *fibers;* the fibers are prolongations of the cell bodies. The cells are *sensory*, or *afferent*, conveying impulses toward the central nervous system, or if in the spinal cord, toward the brain; and *motor*, or *efferent*, conveying impulses away from the brain or spinal cord. The sensory cell bodies lie either in the ganglia or in the dorsal part of the cord or brain; the motor cells lie in the ventral part of the cord. The groups of these cells in the cord constitute the *dorsal* and *ventral horns*, or *columns*. Within the cord these cells connect with others, forming *ascending* and *descending tracts*, or bundles of fibers passing toward the brain and away from it, respectively. Fibers

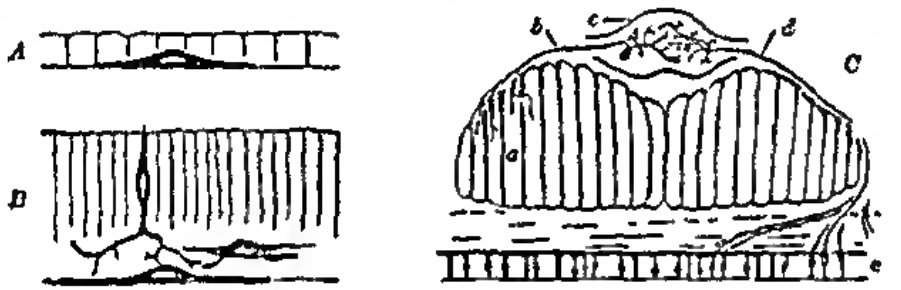


FIG. 141.—A, DIAGRAM OF THE MUSCULAR MECHANISM IN A SPONGE (Parker.) B, DIAGRAM OF THE NEURO-MUSCULAR MECHANISM IN A MEDUSA (Parker, after Hertwig.) C, DIAGRAM OF THE VENTRAL NERVOUS CHAIN (c) AND ADJACENT STRUCTURES IN AN EARTHWORM (Parker, after Retzius.)

a, longitudinal muscle, b, motor fiber, d, sensory fiber, e, epithelium on the under surface of the body containing neuro-epithelial cells

which serve to connect different levels of the cord with one another are known as *association fibers;* those which connect the opposite sides are *commissural fibers.*

Certain features in the development of the nervous system in lower animals, of interest in connection with the mammalian nervous system, are shown diagrammatically in Fig. 141. In sponges, according to Parker, there is no nervous tissue of any sort, but beneath the thin epithelium he finds elongated, contractile cells which 'resemble primitive smooth muscle fibers' (Fig. 141, A). They have been regarded as modified epithelial cells. Parker finds that they are stimulated directly, as a result of changes in the sea-water, so that they slowly contract and close the orifices around which they are situated. Since the sponges are lower than any animals which are known to have nerve cells, Parker concludes that muscular tissue arose independently of nervous tissue, and is the more primitive.[1]

In the medusæ, neuro-epithelial cells, nerve cells, and both smooth and striated muscle fibers are present. According to Oskar and Richard Hertwig, the muscle cells are derived from the deep part of the ectodermal epithelium, and from the first they are connected with nerve cells or neuro-epithelial cells (Fig. 141, B). In other words, in the medusæ muscle and nerve develop in primary communication with one another.[2]

[1] PARKER, 1910 [2] HERTWIG AND HERTWIG, 1878.

In the earthworm (Fig. 141, C) neuro-epithelial cells in the ventral body wall send fibers to a cord of nervous tissue which constitutes a central nervous system. From cells in this cord, processes extend to the muscles, as shown in the diagram. Thus the neuro-epithelial cell does not stimulate the muscle directly; it conveys an impulse to the motor cell which in turn acts upon the muscle. In addition to the cells shown in the diagram the cord contains ramifying association and commissural cells. Thus stimulation at one point on the surface of the animal may cause coördinated muscular contractions in different parts of the body. As Retzius has pointed out, if the neuro-epithelial cells should withdraw into the interior of the animal, leaving their branching process in the epidermis, the conditions in vertebrates would be closely paralleled.

The development and adult anatomy of the central nervous system, and the intricate courses of the fibers within it, are the subject of special

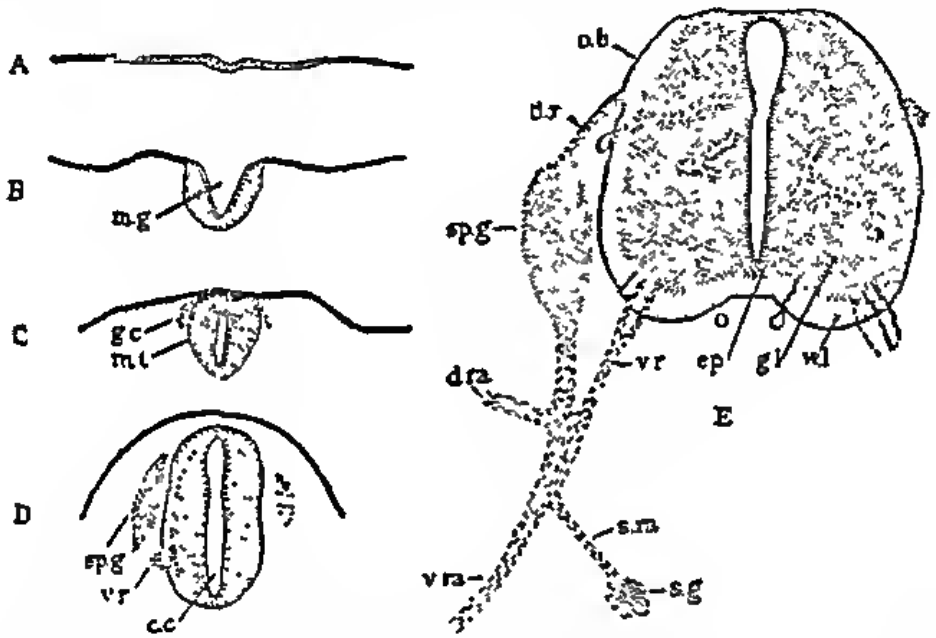


Fig. 142.—The Development of the Nervous System as seen in Cross Sections of Rabbit Embryos: A, 7½ Days. B, 8½ Days C, 9 Days. D, 10½ Days. E, 14 Days

c. c., Central cavity, d. r., dorsal root, d. ra., dorsal ramus, ep., ependymal layer, g. c., ganglion cells; g. l., gray layer, m. g., medullary groove, m. t., medullary tube, o. b., oval bundle, s. g., sympathetic ganglion, sp. g., spinal ganglion, s. ra., sympathetic ramus v. r., ventral root, v. ra., ventral ramus, w. l., white layer

text-books and will not be dealt with here. It is considered sufficient to give a brief description of the spinal cord as the place of origin of the spinal nerves, and as containing types of nerve cells and nerve fibers. The following account deals first with the development of the spinal nerves and the sympathetic system; and secondly with the adult structure of these parts, including the nerve cells of the cord and ganglia, the nerve fibers, and the nerve endings.

Development of the Spinal Nerves. The formation of the medullary groove (or neural groove) as a longitudinal trough in the ectoderm, and its conversion into the medullary tube by the coalescence of its dorsal edges, have been described in a previous section (p. 56). The anterior part of the tube expands to form the brain; the posterior part becomes the relatively slender spinal cord.

Very early in development, the cells of the medullary tube form a syncytium. Those nuclei of the syncytium which border upon the lumen of the tube, or *central canal*, divide repeatedly by mitosis, and many of them are forced outward laterally, so that the sides of the tube become greatly thickened. In the floor and roof of the tube a corresponding thickening fails to take place, as shown in Fig. 142.

The lateral walls of the tube very early become divisible into three layers. The inner layer consists of germinal or proliferating cells and is wide only in the embryo. In the adult it becomes reduced to a single layer of inactive cells, which surround the central canal like a simple epithelium and constitute the *ependyma* (Gr. ἐπένδυμα, a cloak). The middle layer is composed of cells derived from the germinal layer, and in the adult it constitutes the *gray substance* of the cord. Its cells early differentiate into two types—the supporting cells, or *neuroglia*, and the *nerve cells*. The neuroglia cells develop long processes, extending at first radially from the cavity to the external limit of the tube. With their smaller side branches they form a meshwork in which the nerve cells are held. The outer layer is at first entirely free from nuclei, and later it contains only a few, belonging to the neuroglia and to the endothelium of vessels which penetrate the cord. It contains no nerve cells, but the nerve fibers which develop from them extend in it in various directions, chiefly up and down the cord. As these fibers become medullated or myelinated (*i.e.* become coated with a fatty substance, see p. 185), the layer becomes white macroscopically, and it forms the *white substance* of the adult cord. In preparations in which myelin is deeply stained, the white substance appears darker than the gray substance. From what has been said, it appears that the medullary tube early becomes divisible into inner, middle, and outer layers, which give rise to ependyma, gray substance and white substance respectively.

In the gray substance certain cells become pear-shaped by the growth of a process usually directed toward the periphery of the cord. This marks the cell as destined to be a nerve cell, and such cells are called *neuroblasts*. The process elongates and is known as the *axon*, or *neuraxon*, or nerve fiber. Later other processes may grow out from the neuroblasts, but these are of different character from the axon and are called *dendrites*. The latter receive nervous impulses, the axon transmits them from the cell body. From the ventral horn of the gray substance the axons of some of the neuroblasts penetrate the limiting membrane of the cord and grow through the surrounding mesenchyma. Bundles of these form the ventral roots of spinal nerves, one pair for each body segment. The fibers are efferent or motor, since they convey impulses from the cord. The axons of other neuroblasts remain within the cord, running in the outer layer

to higher or lower levels of the central nervous system. The nerve cells are gathered in groups in the gray substance according to their function.

At about the time when the *medullary tube separates from* the epidermal ectoderm, some cells become detached from the medial

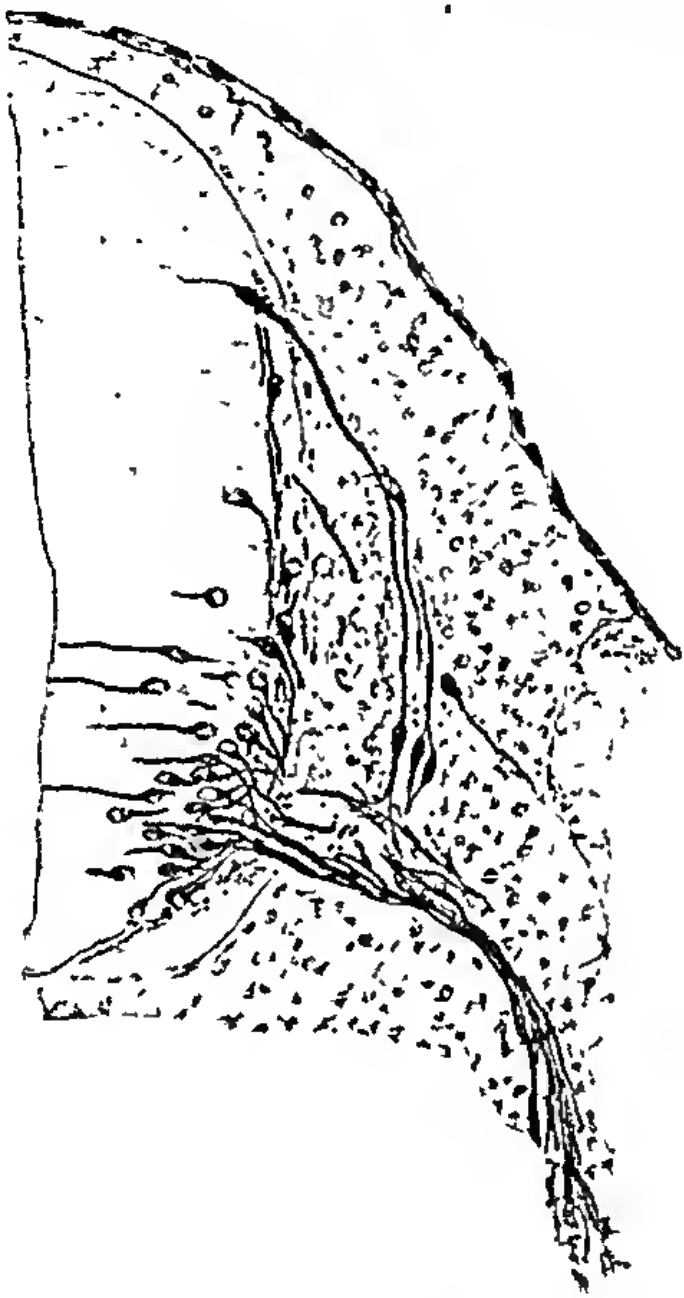


Fig. 143.—A Cross Section of a Portion of the Medullary Tube of a One or Two Pig Embryo Showing the Formation of Neuroblasts (Held).

dorsal portion of the tube and pass down on either side of it, as shown in Fig. 142, *C* and *D*. These cells constitute the *neural crest*. They multiply by mitosis and accumulate in paired masses, corresponding in number with the segments of the body. Thus they form the *spinal ganglia*. A typical cell of a spinal ganglion is at first round, but later becomes bipolar by sending out two processes, one toward the periphery and the

other toward the medullary tube. These processes grow out from opposite ends of the cell (Figs. 144 and 145). With further growth the nucleated cell body passes to one side of the prolongations, with which it remains

FIG. 144.—UNIPOLAR AND BIPOLAR NEUROBLASTS

A and B, unipolar neuroblasts from the medullary tube of a 3 day old duck embryo, C, a bipolar neuroblast from the same, D, a bipolar neuroblast from the trigeminal ganglion of a 3 day old duck embryo (Held)

connected by a slender stalk. Such T-shaped cells are characteristic of the spinal ganglia. The fibers which grow toward the medullary tube constitute the dorsal roots of the spinal nerves. They enter its outer part and then bifurcate, sending one branch toward the brain and the other down the cord. These longitudinal fibers form distinct *oval bundles* just within the cord, one on each side (Fig. 142, *E*). Since these bundles receive accessions of fibers from every spinal ganglion, they enlarge as they approach the brain. The fibers of the oval bundle branch freely at their terminations, and along their course they give off *collateral branches*, which enter the deep substance of the cord. The peripheral fibers from the spinal ganglia grow outward through the mesenchyma, and terminate in sense organs or sensory endings, which will be described presently. The fibers of the spinal ganglia are essentially

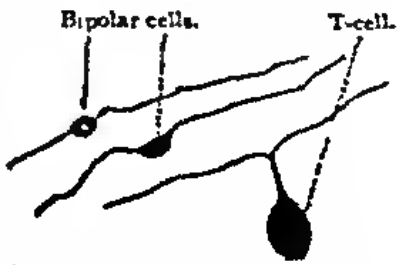


FIG. 145.—SPINAL GANGLION CELLS
The bipolar forms are from a chick embryo incubated six days

sensory or *afferent*, conveying impulses from the periphery toward the cord, and up the cord toward the higher nervous centers.

Peripherally the ventral root joins the bundle of fibers growing outward from the spinal ganglion, and the two together form a spinal nerve. Every spinal nerve consequently has a dorsal (sensory) root and a ventral (motor) root. The fibers from the two roots travel in the same connective tissue sheath, but otherwise they remain entirely distinct. The peripheral fibers from the cells in the ganglia are really dendrites, since they convey impulses toward the cell body. Histologically, however, they are in no way different from the axons.

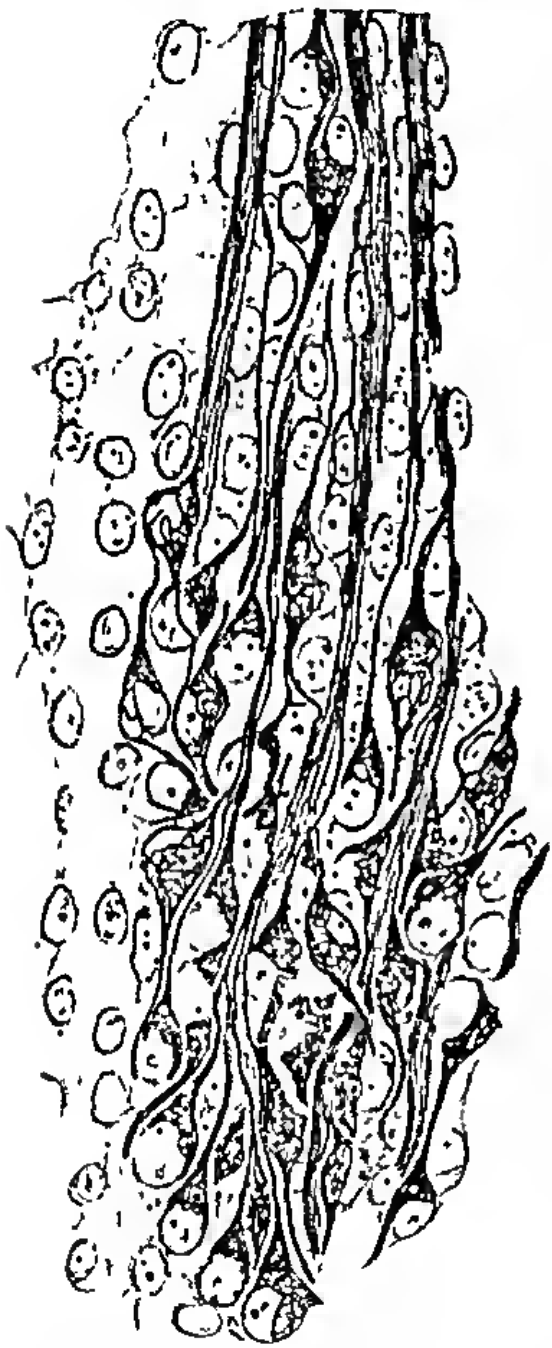


FIG. 146.—A LONGITUDINAL SECTION OF A SPINAL GANGLION OF A 17 MM PIG EMBRYO

To the left connective tissue cells are seen enveloping the surface of the ganglion Cajal's method (Held)

The fundamental facts which have just been reviewed eluded anatomists for centuries. The nerves, extending from the brain and cord to all the important organs, were regarded as tubes, conveying a vital fluid necessary for organic activity; when this supply was cut off the organs ceased to perform their functions. Thus if nerves to the skin were destroyed, the skin became insensible; or if those to muscles were cut, the muscles could not contract. The possible existence of sensory and motor nerves with different functions was debated and generally rejected, until Charles Bell proved conclusively that 'nerves entirely different in function extend through the frame; those of sensation; those of voluntary motion; . . . these nerves are sometimes separate, sometimes bound together; but they do not, in any case, interfere with or partake of each other's influence.' This brilliant discovery was verified by physiological experiments to determine 'whether the phenomena exhibited on injuring the separate roots of the spinal nerves corresponded with what was suggested by their anatomy.' Bell found that such was the fact [1]

It was at first supposed that the nerves grew out from the cord and brain and acquired connections with their end-organs; but the apparent difficulty which the fibers would

[1] BELL, 1824.

have in reaching them, and the fact that the connections must be established before the nervous system can be functional, have led to the idea that the nervous and muscular systems are connected at all stages of their development. In tadpoles, however, Harrison[1] has shown that such connection is not an indispensable requisite for the normal development of the muscles, since they are formed in a normal manner after the medullary tube and neural crest have been removed from the entire posterior portion of the body. He finds further that nerves grow out into the adjacent tissues from transplanted portions of the medullary tube. Therefore he concludes that the nerves normally grow out to their end-organs and unite with them, but that this takes place very early in development,

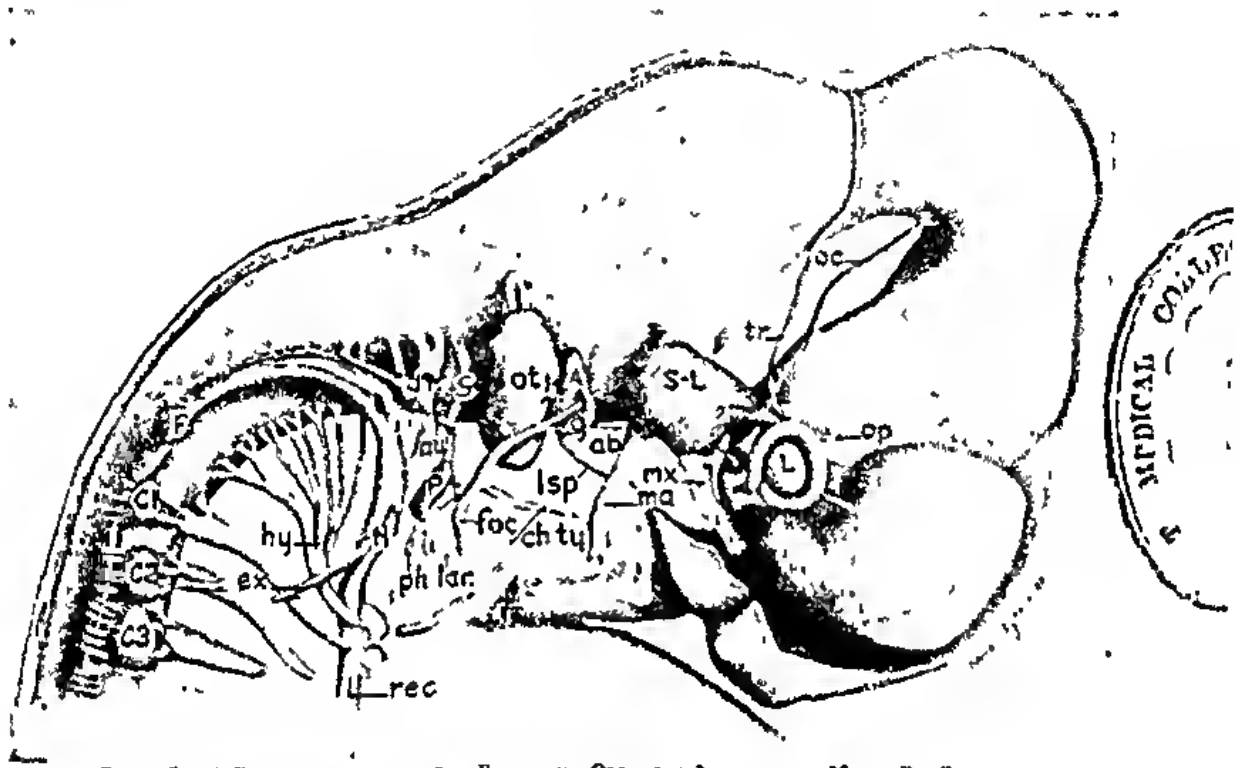


FIG. 147.—A RECONSTRUCTION OF THE BRAIN AND CEREBRAL NERVES OF A 12 MM. PIG EMBRYO

Olfactory (not shown), *optic* (fibers in the stalk of the eye, the lens of which is marked L), *oculomotor*, oc; *trochlear*, tr; *trigeminal*, semilunar ganglion, S-L; ophthalmic, op; maxillary, mx; mandibular, ma; *abducent*, ab, *facial* geniculate ganglion, g; large superficial petrosal, lsp; chorda tympani, ch. ty; facial, fac; *acoustic*, a; supplying the otocyst, ot; *glossopharyngeal*, superior, S and petrosal, P ganglia, tympanic, t; hyoidal, for; pharyngeal, ph; *vagus*, jugular, J and nodose, N ganglia, auricular, au; *laryngeal*, rec, being the recurrent nerve, the main stem proceeds to the abdomen, *accessory*, internal ramus joining the vagus and the external ramus, ex; *hypoglossal*, hy; Froriep's rudimentary hypoglossal ganglion, F sometimes sends fibers to the hypoglossal nerve. C1, C2, C3, first, second and third cervical ganglia. Reconstruction made by Dr. F. T. Lewis.

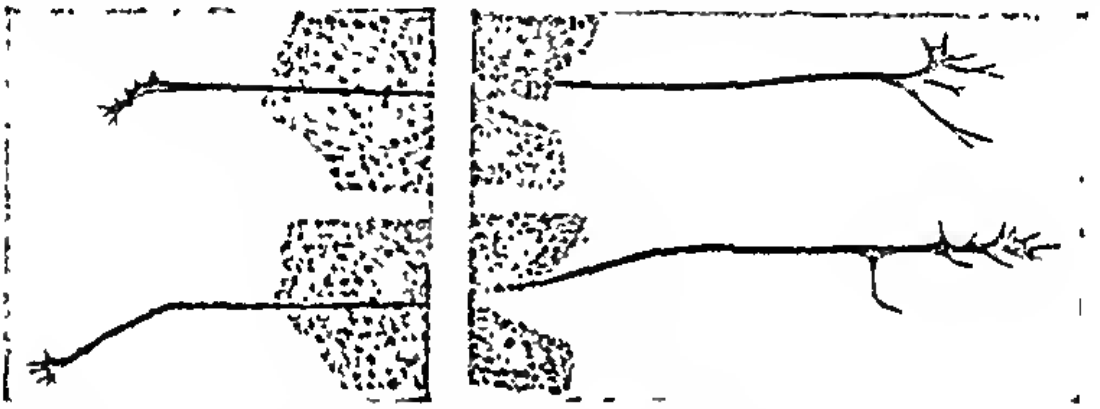
when the paths are quite direct. Subsequent growth of the body causes the muscles to shift about and become widely separated from the central nervous system, so that the nerves become greatly elongated and follow irregular courses.

The participation of the mesoderm in the formation of nerve fibers has repeatedly been asserted, and some authorities considered that the long fibers passing from the spinal cord to distant muscles were formed from chains of cells, either mesodermal or ectodermal. Certain of Harrison's experiments were designed to show whether the nerve fibers are formed by peripheral cells or grow out from the central nervous system. In tissue cultures, made by placing fragments of the medullary tube of tadpoles in lymph, at a stage when the tube consists entirely of round cells, he observed the actual growth of the fibers. Examined after a day or two of cultivation, in a considerable number of cases they were seen extending out into the lymph clot (Fig. 148).

[1] HARRISON, 1904 and 1906.

Harrison concludes that the nerve fibers begin as an outflow of hyaline protoplasm from the nerve cells. The protoplasm is actively amœboid, and, as a result of this activity, it extends farther and farther from its cell of origin, retaining its pseudopodia at its distal end. Similarly enlarged 'cones of growth,' provided with spiny processes, have been observed in preserved tissue by Cajal; and His, from embryological studies, had long maintained that the nerve fibers grow out from neuroblasts in the central nervous system and spinal ganglia. Harrison[1] concludes that his experiments 'place the outgrowth theory of His upon the firmest possible basis.'

Development of the Spinal Sympathetic System. *In mammalian embryos measuring* 10–12 mm., each of the thoracic spinal nerves exhibits a branch directed toward the *aorta, and ending in a rounded mass of ganglion cells. This is the sympathetic or visceral ramus,* terminating in a sympathetic ganglion (Fig. 142, E). Formerly it was generally



A B

Fig. 148.—The Growth of Nerves in Tissue Cultures (Harrison.)

A, Two views of the same nerve fiber taken twenty-five minutes apart, during which time the fiber has grown 20μ. B, Two views of another fiber, at lower magnification, taken fifty minutes apart.

believed that all the nerve cells in the sympathetic ganglia migrated outward from those in the spinal ganglia, but Kuntz[2] derives some of them from the spinal cord direct, by way of the ventral roots. Thus both motor and sensory elements are included.

From each sympathetic ganglion fibers grow to join neighboring ganglia, above and below, thus connecting all these ganglia of one side *into the ganglionated trunk of the sympathetic nerve, or sympathetic* chain. A second set of fibres grows from the ganglia back to the spinal nerves, along which they pass to be distributed peripherally to trunk and limbs, for the innervation of blood vessels, skin glands and perhaps for the sympathetic innervation of skeletal muscles (see p. 167). Thus each sympathetic ganglion is connected with its spinal nerve by two *rami communicantes*, known as the white and gray rami respectively, the white rami consisting chiefly of those fibers passing outward from the cord. A third group of fibers grows out from each ganglion ventrally to supply blood vessels and viscera.

It is characteristic of these branches that they unite with one another freely, forming net-like sympathetic plexuses, within which there are

[1] Harrison, 1908 [2] Kuntz, 1926 and 1934.

many scattered nerve cells. When the nerve cells in these ganglionated plexuses are particularly abundant, the structure is called a ganglion, though generally retaining a plexiform character.

In 1664, Willis published a remarkably clear account of the nerve 'commonly called intercostal because it rests against the roots of the ribs.' This nerve, which is the ganglionated trunk of the sympathetic system, had generally been supposed to descend from

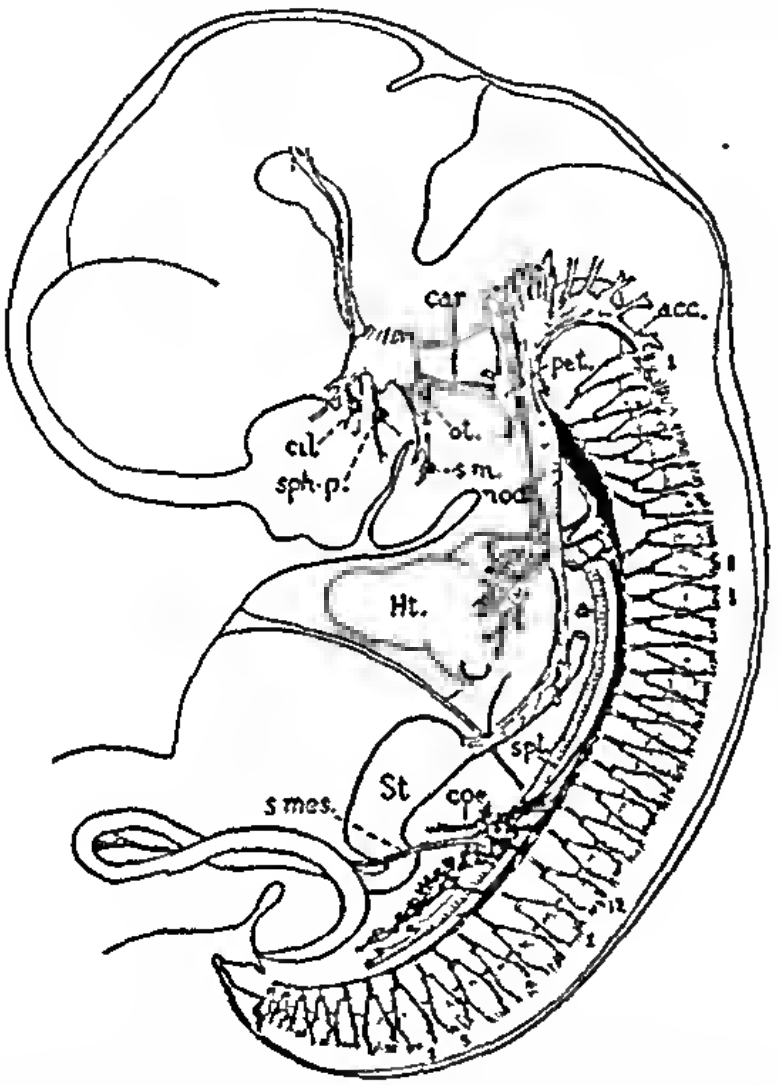


Fig. 149.—The Sympathetic System in a 16-mm Human Embryo. (Streeter.)

The ganglionated trunk is heavily shaded. The first and last cervical, thoracic, lumbar, sacral and coccygeal spinal ganglia are numbered. a, aorta, acc., accessory nerve; car., carotid artery, cil., ciliary ganglion. coe., cœliac artery, Ht., heart, nod., nodose ganglion, ot., otic ganglion; pet., petrosal ganglion, s-m., submaxillary ganglion, s. mes., *superior mesenteric artery*, sph-p., sphenopalatine ganglion, spl., splanchnic nerve; St., stomach.

the cerebral nerves. Willis described its connections with these nerves and, through each intercostal space, with the spinal cord. He noted the cardiac branches, and stated that the great mesenteric plexus, placed in the midst of the others, like a sun, sent its nerve fibers like rays in all directions (hence it came to be called the 'solar plexus'). Willis found that this nerve sent branches to all the abdominal organs below the stomach. He considered that its function was to place the heart and viscera in connection with the brain so that they should act in harmony (Anatome cerebri, *Amstelodami, 1664*). Because of their frequent communications with other nerves, Winslow (1732) called the ganglionated trunks the *Nervi sympathetici maximi*.

Bichat (Anatomie générale, 1802, translated by Hayward 1822) subdivided the nervous sytem into two parts 'essentially distinct from each other, the one having the brain and its dependencies for its principal center, and the other having the ganglions.' The latter is 'almost everywhere distributed to the organs of digestion, circulation, respiration, and secretion.' 'Each ganglion is a distinct center, independent of the others in its action, furnishing or receiving particular nerves as the brain furnishes or receives its own . . . The continuous thread that is observed from the neck to the pelvis is nothing but a series of communications . . . these communications are often interrupted, without any inconvenience in the organs to which the great sympathetic goes.' That the sympathetic system acts independently of the central nervous system, at least to a great extent, is its most prominent physiological characteristic.

Thus the sympathetic system merits to some extent the terms organic, visceral, or vegetative system, which have been applied to it. Burdach (1819) stated that it might be called the 'automatic system,' and the term 'autonomic system' has more recently been used, but Burdach preferred *sympathetic system*, which has been internationally adopted by anatomists.

STRUCTURE OF NERVOUS TISSUE

From what has been said it follows that nerve cells may be found not only in the central nervous system but also in the ganglia, spinal and sympathetic, and in many of the organs, while their processes or nerve fibers extend throughout the whole body. Owing to the extent of the ramifying processes characteristic of nerve cells, it is rare that an entire cell, even a small one, is included within a single section. A motor cell, such as sends its fibers from the cord to distant muscles, has never been seen as a complete, isolated structure. From what is known of its several parts, however, a diagram of such a cell may be put together, as shown in Fig. 150. At the top of the figure is the nucleated cell body, which in different nerve cells varies in diameter from 4–150 μ. Frequently this nucleated portion is referred to as the nerve cell in distinction from the processes which grow out from it. The processes include the relatively short and irregularly ramifying *dendrites*, which convey impulses toward the cell body, and a single fiber, the axon or *neuraxon*, chemically and physically different from the others, which conveys impulses away from the cell body. If the various processes radiate from the cell body in several directions, the cell is described as *multipolar;* if the neuraxon is at one end of the cell and a single dendrite at the other, the cell is *bipolar* (Fig. 144); sometimes the nerve cell has only one process and is *unipolar*, as in the mature cells of the spinal ganglion which have a T-shaped process, and in other cells in which dendrites have not developed. The dendrites have the granular structure of the protoplasm from which they grow out, and were therefore originally named 'protoplasmic processes.' The neuraxon seems quite distinct from the cell body. At its origin it often appears as a clear slender cone, free from granules, implanted directly upon the cell body, or upon the root of one of the larger dendrites.

It tapers as it passes outward. Beyond the apex of the cone, which is a place where the neuraxon is easily broken, the fiber enlarges. The neuraxon may send out *collateral branches*, which are usually at right angles with the main fiber.

As the neuraxon passes out from a motor cell, it is at first free from any surrounding sheath (Fig. 150, a). In the outer layer of the spinal cord it becomes coated with a layer of the refractive fatty substance known as *myelin*. This is formed in the cord or *medulla spinalis*, and fibers which have this sheath are said to be *medullated fibers*. On leaving the cord, the neuraxon is still surrounded by the myelin sheath, but the latter is invested by a membrane called the *neurolemma* or sheath of Schwann (Fig. 150, c). At quite regular intervals along the course of the fiber, the myelin sheath is constricted or interrupted, forming the *nodes of Ranvier*. These are 0.08–1.00 *mm.* apart, being closer together in growing fibers, and in the distal part of adult fibers. Midway between two nodes there is a nucleus, which may be found at any point in the circumference of the fiber, just within the neurolemma; it occupies a depression in the myelin. Toward its distal end the fiber usually branches, and the branches are given off at the nodes. The myelin sheath then becomes thin and at last ceases, so that the fiber is surrounded merely by neurolemma (Fig. 150, d), and finally this ends. The naked axis cylinder then breaks up in its terminal arborization, forming the motor organs attached to striated muscle fibers. In comparison with the size of its cell body, the neuraxon shown in the diagram is too short; in extreme cases, as in the neuraxons extending from the spinal cord to muscles in the foot, it may be actually more than a meter long, or several thousand times the diameter of the cell body from which it comes.

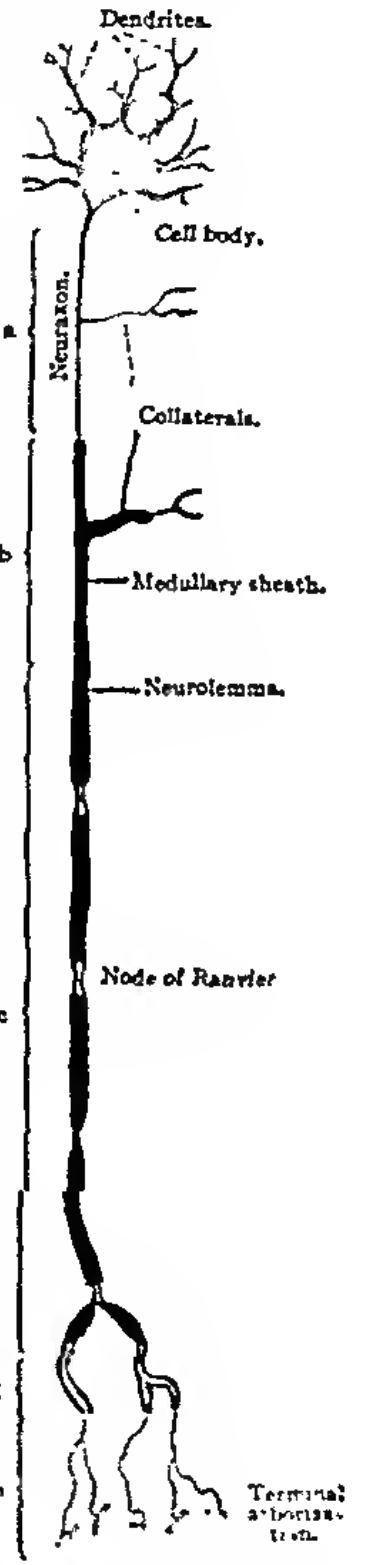


FIG. 150.—DIAGRAM OF A NERVE CELL.

The medullated nerve fibers were the first parts of the nerve to be studied microscopically, and were referred to as 'cylinders;' the central fiber was called the *axis*

cylinder. Remak[1] was the first to describe non-medullated nerves, which are still known as 'Remak's fibers,' but their nervous nature was not readily admitted. Moreover, Remak recognized that nerve fibers proceed from cells. Deiters[2] supplemented these observations by showing that all 'ganglion cells' (referring to nerve cells within the spinal cord and brain) are centers for *two systems of true nerve fibers*, (1) the generally broader and always single and undivided *axis cylinder process;* and (2) the *protoplasmic processes* with their extensive system of *minute branches.* He discussed whether the nerve cells anastomose with one another, and concluded that all such anastomoses which had been reported were due to deceptive appearances. Thus the nerve cells were believed to communicate by contact and not by continuity.

The confused mass of interwoven fibers which sections of nervous tissue ordinarily present, is, therefore, not a general syncitium from which sensory and motor fibers run out, but an orderly arrangement of branching cells. Striking proof of this was afforded in Golgi's description of the olfactory bulb (1875). In the plate which accompanied his publication, the cells in the different layers, and their various processes, were drawn in black with absolute assurance, similar figures of 'Golgi preparations' are now seen in all treatises on the anatomy of the nervous system (Fig. 152). Golgi found that if fresh tissue is placed in a solution of potassium dichromate and osmic acid, and is later transferred to a solution of silver nitrate, a heavy black deposit occurs in certain nerve cells, extending throughout their minutest ramifications, whereas adjacent cells are wholly unaffected. The process must be carried out with great care, and even then it is capricious; but this method has afforded fundamental information in regard to the forms of individual nerve cells.

In order to emphasize that the nervous system is built up of separate cells, the term *neuron* has been widely used to designate a complete nerve cell, with all its branches. Fig. 150, therefore, represents a neuron, together with certain sheath cells. Owing to the great extent of neurons, it is usual to study their different component parts separately; the cell-body, the cell processes or nerve fibers, and the nerve endings will therefore be described in turn.

Nerve Cells. *Spinal Cord.* Nerve cells may be found in the central nervous system (brain and spinal cord), in ganglia, or in some organs irregularly scattered along the sympathetic nerves. The motor or efferent cells of the spinal cord are classical objects of study, and should perhaps be described first. They occur in the ventral columns (horns) of the cord (see Fig. 151) and are large enough to be readily visible with low power. They innervate directly the striated muscle. They are characteristically multipolar; the dendrites are broad, branching and tapering, as can be seen in isolated specimens, but in sections they usually can be followed only a short distance. The neuraxon begins as a slender fiber arising from a clear 'implantation cone or hillock' of rather distinctive shape; the nucleus is distinctively large, round, or oval, with little chromatin and a prominent nucleolus. The cytoplasm with common stains is dark and densely granular, but when the cell is specially

[1] REMAK, 1838 [2] DEITERS, 1865.

treated, as with gold chloride or pyridine silver, it is seen to contain an abundance of minute neurofibrils, running near the cell periphery and also forming a network about the nucleus. They pass out into the dendrites and into the axon, where they run in a close bundle of some hundred individual fibrils, and are continued into the collateral branches and terminal arborizations, making a striking characteristic of all nerve cells. Equally striking and characteristic of the nerve cell body is the chromophil substance or Nissl's bodies, best obtained by proper staining after fixation in some fluid containing alcohol.[1] These bodies are large,

FIG. 151.—GROUP OF MOTOR NERVE CELLS IN VENTRAL COLUMN OF SPINAL CORD, HUMAN. METHYLENE BLUE AND EOSIN.

block-like masses, rhomboid and angular in shape, evenly distributed in this type of nerve cell, and separated by clear paths of cytoplasm in which small granules of the same stainable material may be present. They are not found in the nucleus nor in the implantation cone or axon, but extend into the bases of the dendrites. They have been thought of as between the neurofibrils, and the striped pattern they sometimes exhibit has given them also the name of tigroid bodies. Their significance will be discussed later.

Other constituents of the nerve cell are the mitochondria, which are usually in the form of short filaments or granules, and the Golgi apparatus, which is a net surrounding the nucleus and extending into the larger dendrites. All the constituents mentioned are subject to variation with the different functional states of the cells.

[1] EINARSON, 1932.

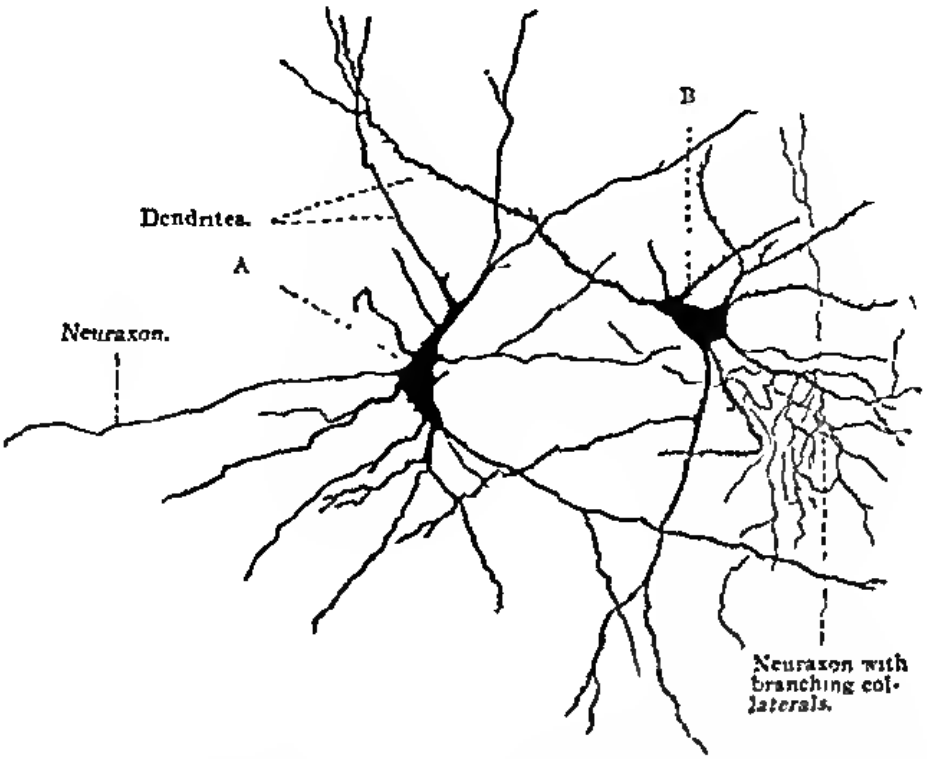


FIG. 152.—TWO NERVE CELLS FROM THE CENTRAL NERVOUS SYSTEM. GOLGI PREPARATION. × 200.
A, Cell of Deiter's type, having a neuraxon ending at a considerable distance from the cell body, B, cell of Golgi's type having a neuraxon with many branches ending near the cell body.

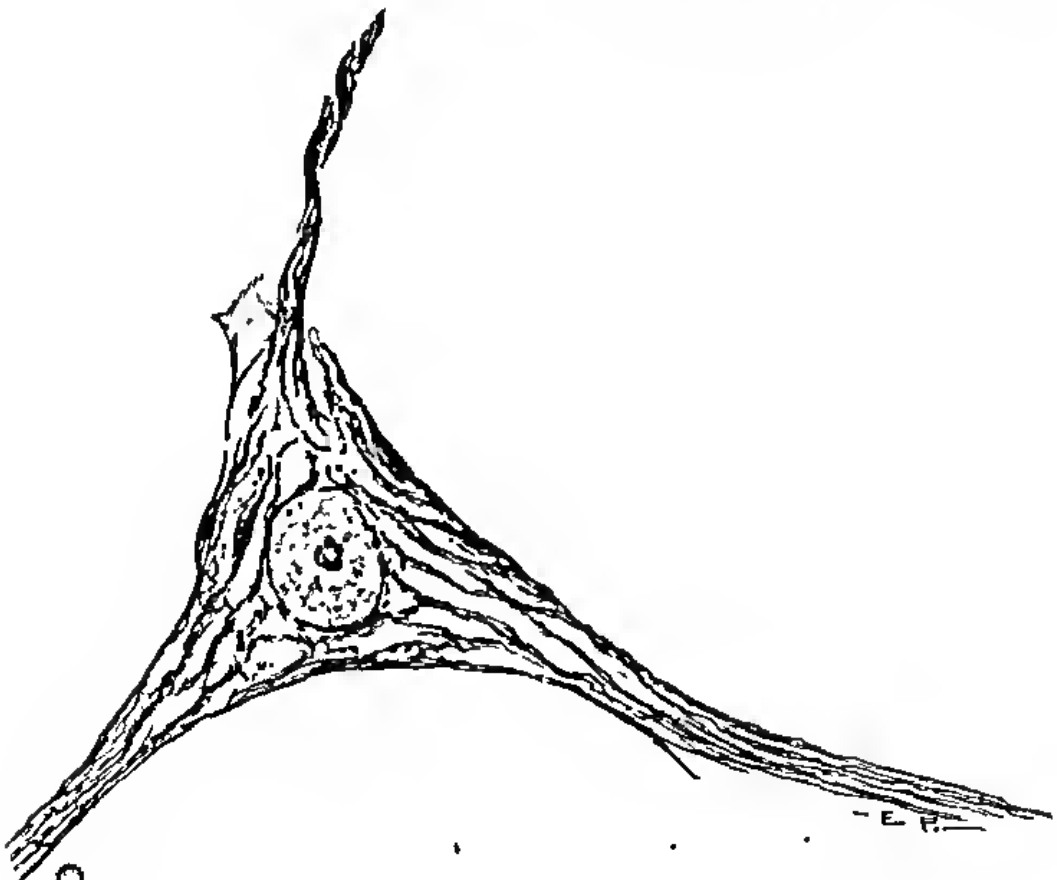


FIG. 153.—A NERVE CELL IN SPINAL CORD OF A RABBIT SHOWING A NEUROFIBRILLAR NETWORK.
Formaldehyde fixation. Bodian impregnation.

The cells appear large in cross sections of the spinal cord, but their actual size is much greater. Lhermitte and Kraus[1] point out that they are elongated lengthwise of the cord, so that they are usually cut in their smallest diameter. The vertical extent of these cells explains also why, in sections, the axon is so seldom seen leaving the cell, as it may arise from any part of its surface, even from the base of a large dendrite.

A second type of motor or efferent cells is found, also in the spinal cord, in the lateral column of the gray matter. These are the visceral efferent cells, sending their axons by way of the white rami to the sympathetic ganglia to initiate action in smooth muscles and glands either directly or by stimulating the cells of these ganglia. They are usually smaller than the ventral column cells and less branched. Their chief difference, however, is in the character of the Nissl bodies, or chromophil substance. In the somatic motor cell this is chiefly in the form of large, dense granules, sharply circumscribed (Fig. 155); in the visceral cells it is less prominent, more granular, apt to be arranged near the cell periphery. The two types are found also in the brain, where cell groups, whose axons run to striated muscle or are connected indirectly through other neurons with striated muscle, show block-like Nissl bodies, while cells in the visceral chain exhibit granular chromophil substance. These characteristics, however, are not definite; in any group of cells of one type there will be great variation. Yet in general the distinction may be accepted. Malone[2] has even recognized, in the cells of the vagus nerve that supply heart muscle, a type intermediate between the motor cell to striated muscle and the visceral cell to smooth muscle and glands, as characterized by an intermediate arrangement of the chromophil substance.

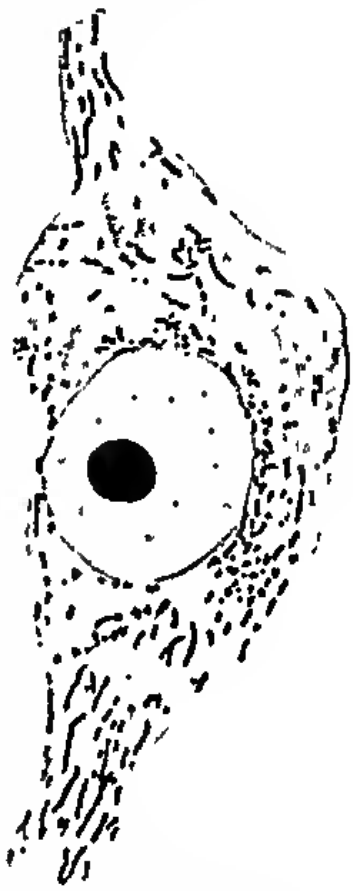


FIG. 154.—MITOCHONDRIA IN A NERVE CELL-VENTRAL HORN OF THE SPINAL CORD OF A WHITE MOUSE

Formaldehyde fixation, potassium dichromate, acid fuchsin-Methyl green (Nicholson—courtesy of Wistar Institute)

The character of the chromophil substance in nerve cells deserves special attention because of its variability. It may be reduced after prolonged activity and exhaustion of the cell, or after the injury of part of the neuron. For example, if a group of axons is cut at one region of the brain axis, the cell bodies of which they were a part soon show a change of the Nissl substance, or 'chromatolysis,' and thus may be definitely recognized in another part of the central nervous system, though the path of the axons could not have been found by dissection. Experimentation based on this fact is often

[1] LHERMITTE AND KRAUS, 1925. [2] MALONE, 1913.

used to show the course of tracts in the brain. But in order to evaluate the results properly, one must know the normal pattern of the substance for any given group of cells, for the normal visceral cell might be mistaken, as far as its chromophil substance goes, for a motor cell showing chromatolysis.

Sensory ganglia. Another type of nerve cell, having only one or two processes is found in the spinal ganglia. Although a ganglion is characterized by the accumulation of the bodies of nerve cells, it is traversed by many fibers, as seen in the section of a spinal ganglion (Fig. 156). Under high magnification the cell bodies appear as in Fig. 157. The nuclei are large vesicular structures, round or oval in outline, containing

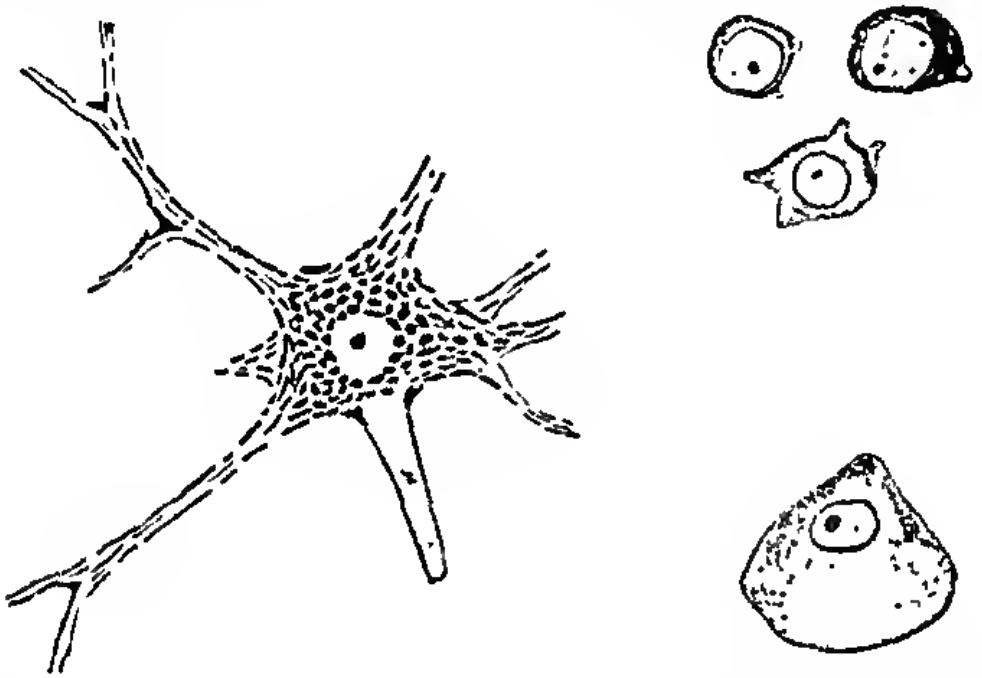


FIG. 155.—NERVE CELLS, NISSL STAIN. MOTOR FROM SPINAL CORD, SYMPATHETIC, AND SENSORY (von Möllendorff and Malone.)

a characteristically prominent nucleolus. They are surrounded by abundant, darkly staining, finely granular protoplasm, which exhibits its fibrillar structure and Nissl's bodies only with special methods. The chromophil substance of these cells, and of other sensory cells within the brain, is usually diffusely distributed as fine granules, but, in the larger cells, the granules are loosely aggregated in places to form bodies of medium size, not dense nor sharply differentiated from the surrounding diffuse granules.[1] Frequently the protoplasm contains pigment granules. Fine-meshed reticular networks have been found covering the exterior of the nerve cells, and they have been ascribed both to the terminal ramification of nerve fibers and to branches of the supporting tissue. A ganglion cell is often surrounded by a capsule or structureless membranous sheath with nuclei on its inner surface continuous with

[1] MALONE, 1932.

the neurolemma sheath of the associated fibers. Connective tissue, containing small blood vessels, passes between the ensheathed cells of the ganglion.

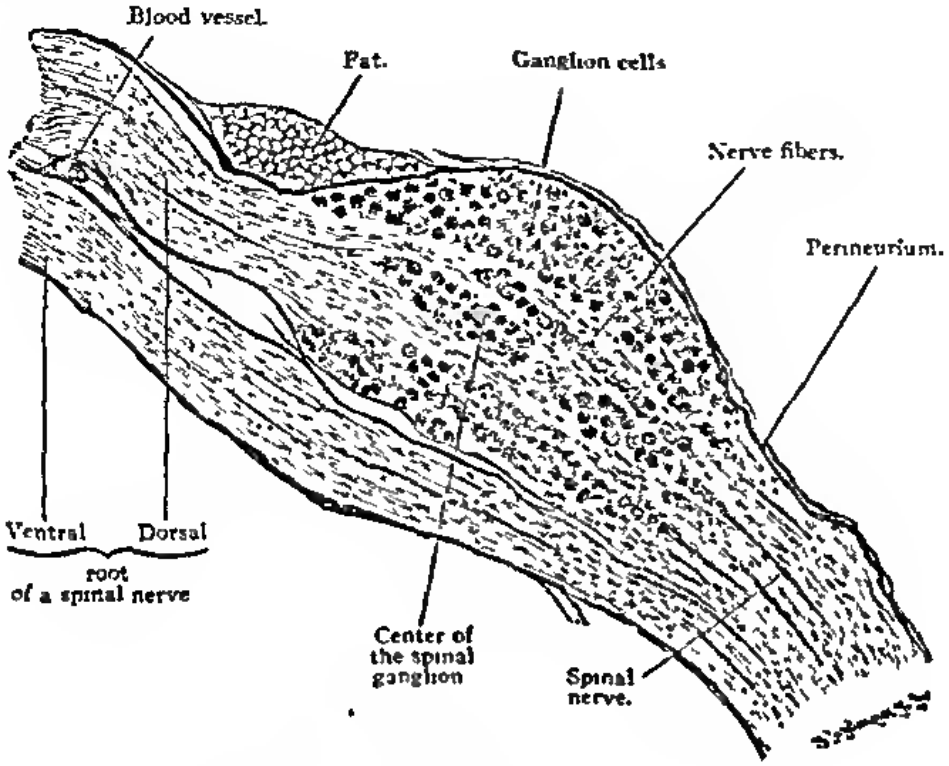


FIG. 156.—LONGITUDINAL SECTION THROUGH A SPINAL GANGLION OF A CAT. × 18.

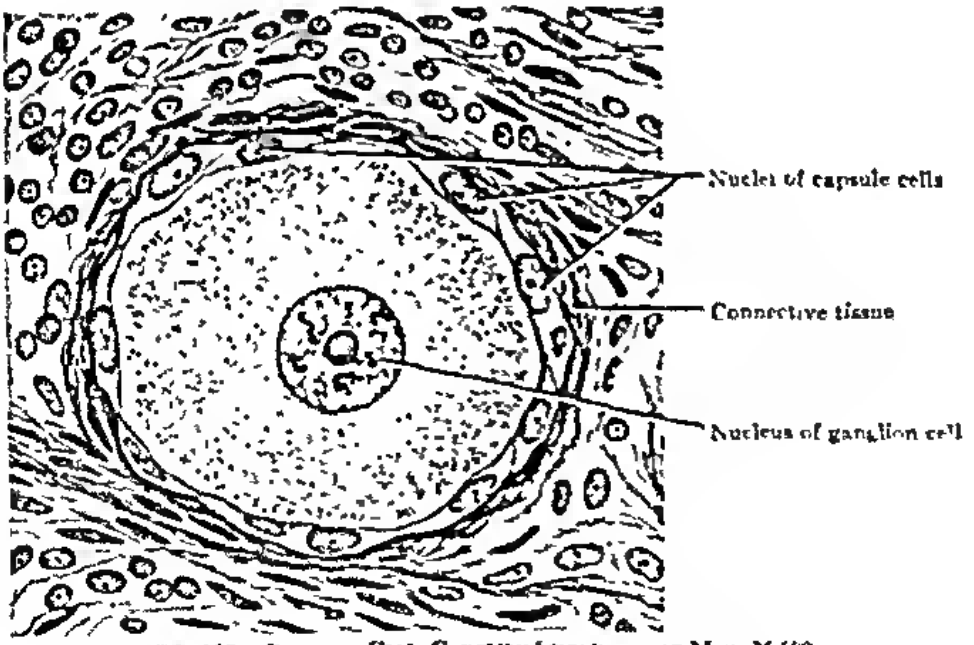


FIG. 157.—GANGLION CELL GANGLION SPIRALE OF MAN. × 600. Flemming fixation. Azan (von Mollendorff.)

Sympathetic Ganglia. The sympathetic ganglia consist chiefly of multipolar nerve cells, smaller than those of the spinal ganglia, though a few unipolar and bipolar cells are also present. They resemble other

nerve cells in the character of their nuclei and protoplasm, and often contain pigment. The neurofibrils are slender and arranged in a fine net[1] and the Nissl substance is granular with small, ill-defined bodies (Fig. 155). Like the cells of the spinal ganglia the sympathetic cell is enclosed in a capsule, the nuclei of which may be flattened and far

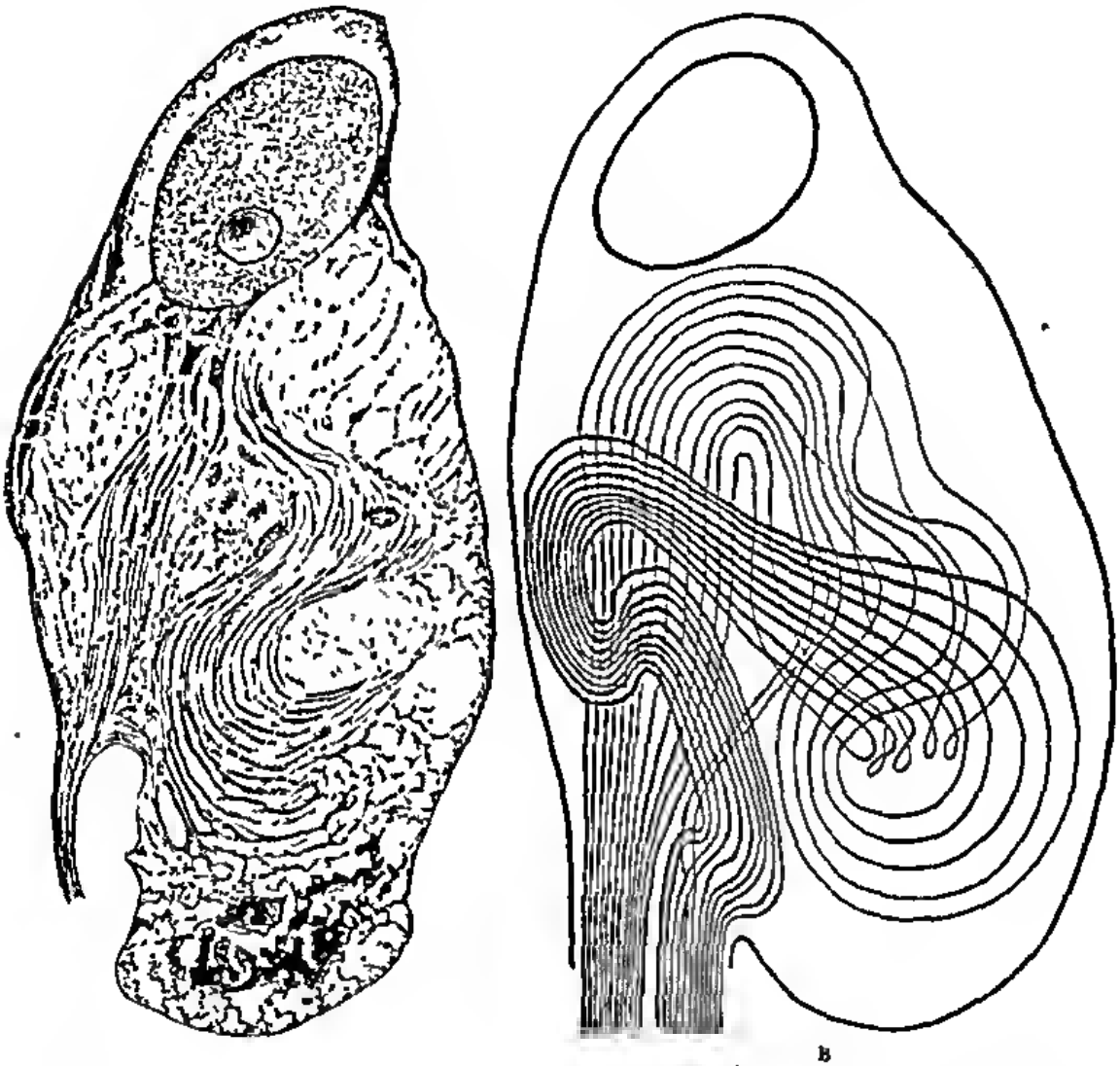


FIG. 158.—SPINAL GANGLION CELL OF A FROG

Sublimate—osmic acid fixation, Vanadium hæmatoxylin. A, Middle section from a series of 12—shows neurofibrillæ and protoplasmic network. B, Schematic representation of fibrillæ from a series of 12 sections. (Heidenhain.)

apart, especially in the more peripheral ganglia. In some instances the dendrites ramify beneath this capsule, where they form an open network either uniformly distributed or grouped at one side of the cell. These dendrites are in relation with fibers from other cells which pierce the capsule, and the mass of interlacing dendrites and fibers is known as a 'glomerulus.' Dendrites from other ganglion cells may run long distances. The axons are usually unmyelinated.

[1] KUNTZ, 1932.

The nature of neurofibrils has been the subject of controversy for many years. First recognized in living tissue and in the invertebrates, the neurofibrils in these lower forms have been traced from cell to cell, making a true nerve net, in which the individual neurons formed merely a pathway. Until recently neurofibrils had not been seen in the living cells of vertebrates but structures most suggestive of them have been observed in tissue cultures. Yet the neurofibrils are a striking characteristic of all neurons, and the tendency to consider them the definite conducting elements of the nervous system is strong, though modern physiological calculations indicate that the conducting substance of a nerve must be in a liquid or at least a semiliquid condition, which points to the

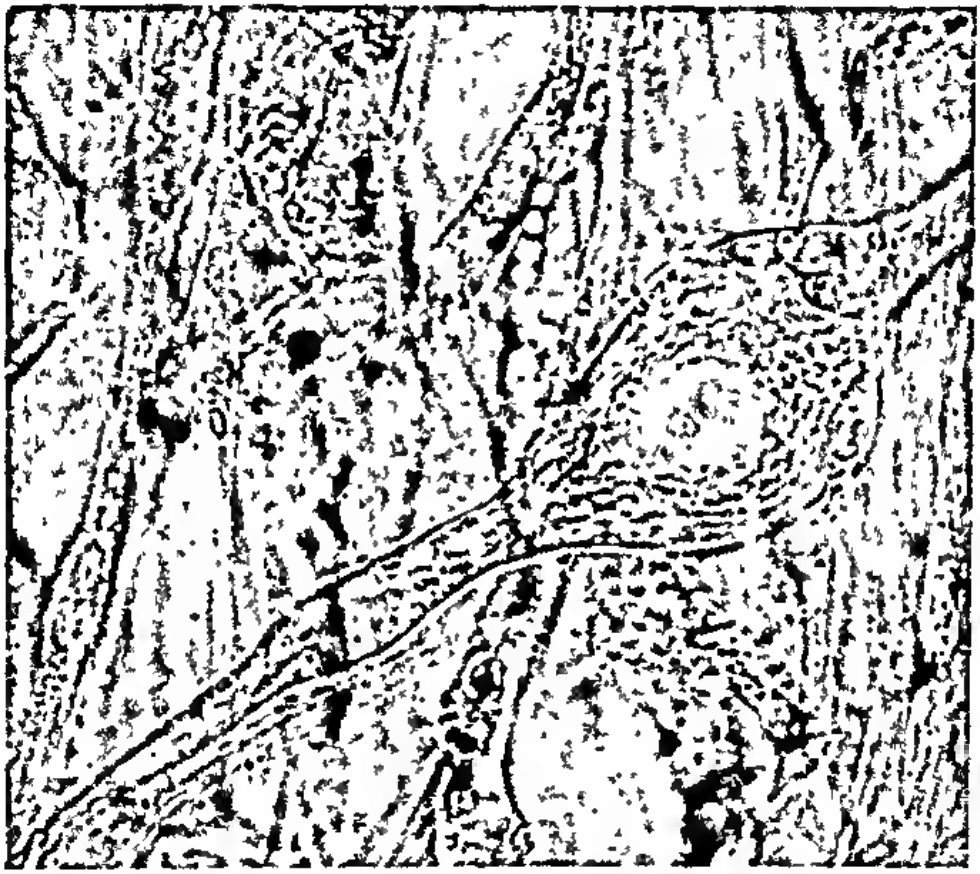


FIG. 159.—NERVE CELLS IN TISSUE CULTURE. INDICATION OF NEUROFIBRILLAE. (Murmahan.)

protoplasm itself as the conductor. The nervous impulse passes from one nerve cell to the next by contact or contiguity of the terminal arborizations of the axon of one with the dendrites or cell body of the next, instead of by continuity of the neurofibrils from one cell area to another. The place of contact or contiguity is the *synapse*, and it has been proved that impulses may traverse a synapse in only one direction, though a nerve fiber may transmit impulses in either direction. For a discussion of the problem of the neurofibrils see *Parker*.[1]

By microdissection methods, de Rényi[2] has shown that the cytoplasm of spinal ganglion cells is dense and rubber-like, so that when the cell is torn apart by two needles, each part retains its shape; whereas in the motor cells the cytoplasm is rather soft, gelatinous, and extremely plastic. The bearing of this on the true nature of the neurofibrils and Nissl bodies is not clear. They may be artifacts of fixation or staining. The same may be true also of the chromophil substance. Darkfield illumination shows granules in the living nerve cell cytoplasm, but they do not agree well with the chromophil par-

[1] PARKER, 1929. [2] DE RÉNYI, 1932.

tern. Ultraviolet photography gives better results, but the rays may be injuring the cells and causing coagulation of previously fluid substance.

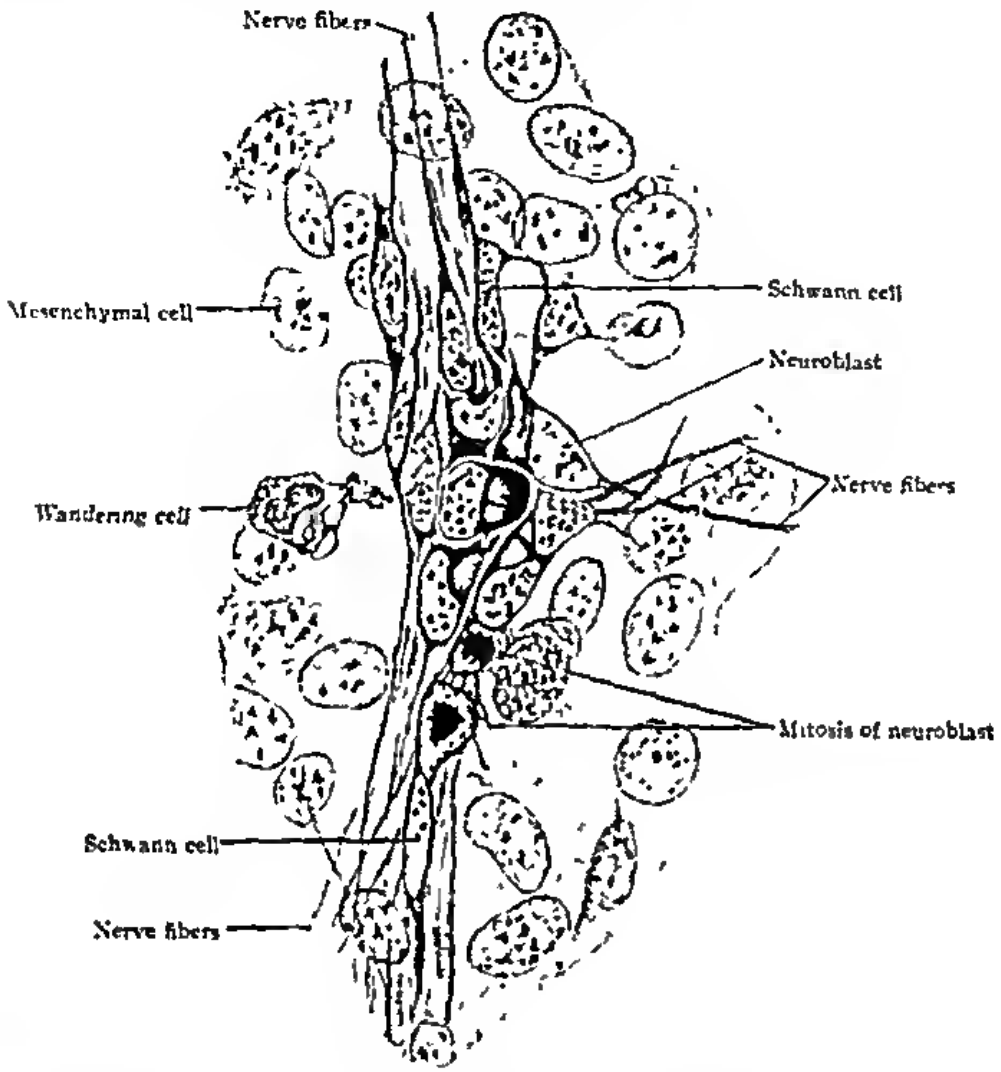


FIG. 160.—SYMPATHETIC NERVE CELLS WITH NERVE FIBERS AND SCHWANN CELLS. CULTURE OF RAT EMBRYO. (Maximow.)

Nerve Fibers. Nerve fibers include the axons of motor cells, and the axons and the peripheral processes of sensory cells, which later, though conveying impulses toward the cell body, and hence true dendrites, are similar to axons histologically. Though present in the gray matter of the brain and spinal cord and as individuals in the tissues, they are best studied in the white matter of the cord, where they run longitudinally in bundles (tracts), or in the peripheral nerves.

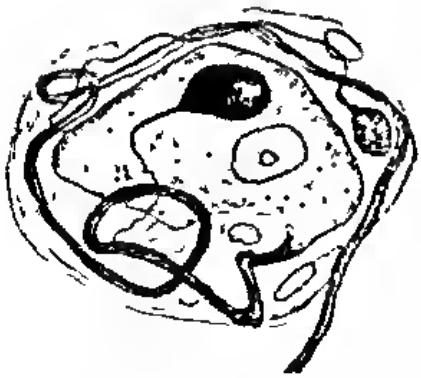


FIG. 161.—CELL FROM THE GANGLION NODOSUM VAGI WITH END-PLATES. HUMAN. (Cajal.)

In the diagram of a neuron (p. 185) the axon is shown encased in its myelin sheath for the greater part of its course. This is not essential, however, and many nerve fibers remain

'non-medullated,' or 'unmyelinated.' Isolated fibers, running alone through the connective tissue near their destination, would be very difficult to detect histologically without special stains. In ordinary preparations they are recognized only as they occur in nerves.

Nerves are bundles of nerve fibers passing between the central nervous system and the various parts of the body; they are so widely distributed that they may be found in sections of most of the organs and tissues. When examined fresh, in reflected light, nerves are seen to be of two sorts, formerly known as white and gray nerves, respectively. Similarly, sections of the brain and spinal cord are formed of white substance and gray substance. The obvious distinction in color is due to the presence or absence of microscopic sheaths of myelin around the individual fibers. Nerves which contain a large proportion of myelinated or medullated fibers are white; and those which have few are gray. All nerve fibers when first formed are non-medullated, and most of the sympathetic nerves remain in this condition.

Non-medullated nerves can readily be found between the circular and longitudinal layers of smooth muscle in any part of the digestive tube. They are circumscribed bundles of fine fibers running through the coarser connective tissue (Fig. 163). Many of them contain nerve cells of the sympathetic type unmistakably characterized by large, round or oval, vesicular nuclei, having a prominent nucleolus. Around the nucleus is dense protoplasm, starting out in branching processes, all but the roots of which are usually cut away in sectioning. Other cells are found having relatively small nuclei and very indefinite or wholly imperceptible protoplasmic bodies. These are supporting cells; they produce a syncytial framework in which the nerve cells and their very delicate ramifications are embedded. The framework tends to form septa, subdividing the nerve into smaller bundles. When cut directly across, a bundle of non-medullated nerve fibers can be recognized as small discrete dots, with rather deep protoplasmic stain, which remain distinct with changing focus, and if slightly slanting will shift somewhat in position. Seen in

FIG. 162.—NERVE CELL CONTAINING PIGMENT. SUPERIOR CERVICAL GANGLION HUMAN. BY FERROCYANIDE METHOD. × 500. (P. Stöhr Jr.)

horizontal position they are fine parallel lines. Obliquely cut, they are hard to recognize, but the arrangement of the connective tissue forming compartments within a bundle is sufficient for identification.

Some non-medullated fibers, but by no means all, are closely invested by sheath cells. According to Schäfer, the nuclei of these cells appear to be interpolated in the substance of the fiber, and it is impossible to demonstrate a distinct sheath. Similarly Bardeen has stated that it is 'mainly a matter of judgment to decide whether the fibrils are surrounded by or embedded within the sheath cells.' They correspond with the neurolemma cells of medullated nerves.

Fig. 163. — Three Non-medullated Nerve Fibers with Schwann Cell Nuclei. Cat (Cajal.)

Along the course of many non-medullated nerve fibers there are seen at various intervals small oval swellings or varicosities.[1] In most histological preparations impregnated with reduced silver salts, these varicosities appear as solid bodies, but in favorable specimens they are observed as swellings with the neurofibrils spread out in passing through them. The cause of the varicosities is unknown. Some authors regard them as artifacts, probably the result of a local imbibition of fluid.

Medullated Nerves. The larger sympathetic nerves contain a considerable number of medullated fibers, and the splanchnic nerves are described as white. In trunks of the spinal nerves, however, the medullated fibers attain their maximum development, though even here they are always accompanied by others without myelin derived in part from the gray rami, in part from the spinal ganglia.[2] Examined with low magnification, such a nerve is seen to consist of round cords embedded in loose connective tissue (Fig. 164). This loose tissue, which surrounds the entire nerve and its several cords, is the *epineurium;* its connective tissue bundles are chiefly longitudinal, and are associated with abundant elastic tissue and frequent fat cells; it contains the blood vessels which supply the nerve. Each cord is surrounded by a dense lamellar layer of connective tissue, which contains flattened cells in contact with one another so that they form more or less continuous membranes. This layer is the *perineurium. It is continuous with the outer* membranes covering the cord, and contains cleft-like spaces which are said to communicate with the subdural and subarachnoid spaces, but which do not connect with lymphatic vessels in the epineurium. Prolongations of the perineurium extend as septa into the larger nerve

[1] Nemiloff, 1910. [2] Ranson, 1911.

bundles and constitute the *endoneurium*, which may penetrate between the individual nerve fibers, forming the so-called 'sheaths of Henle,' just outside the neurolemma.

Peripheral nerves except the terminal ramifications are provided with intraneural blood vessels or *vasa nervorum*. Small nutrient arteries from nearby vessels pass into the epineurium and after making anastomoses form networks of arterioles in the perineurium from which are derived capillary plexuses with elongated meshes in the endoneurium. These capillary plexuses are drained by venules accompanying the arterioles and then in turn by small veins which usually leave the nerve with the

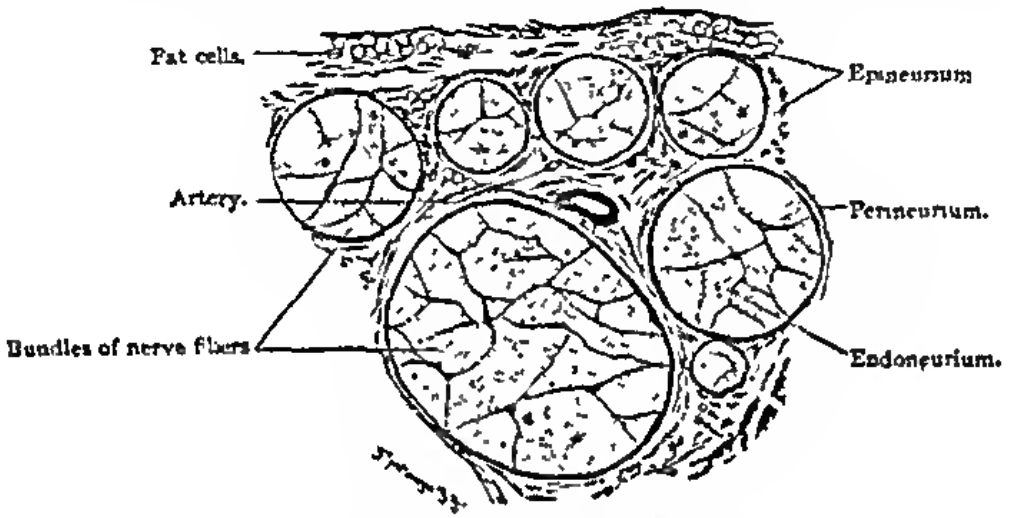


FIG. 164.—MEDULLATED NERVE. PART OF A CROSS SECTION OF THE HUMAN MEDIAN NERVE. × 20

incoming arteries. Vasa nervorum occur also in the sympathetic nerves. Fine non-medullated nerves (nervi nervorum) have been described in the epineurium and perineurium. Some of these are evidently sensory and terminate in end-bulbs, while others probably having a vaso-motor function supply the arteries. Precise knowledge on the nervi nervorum is still fragmentary. The ganglia of the spinal nerves are more richly vascular than the nerves and except for the trigeminal ganglion little is known about the blood vessels of the cerebral ganglia.[1]

Unlike the nerve fibers of the white matter of the spinal cord, all of which are straight and parallel, the fibers of most nerves take an irregular, wavy course, so that occasionally even in transverse sections some of the nerve fibers are cut lengthwise. The individual fibers are thus longer than the nerve as a whole, and may escape injury by stretching.

The individual nerve fibers vary in diameter, and the larger ones are probably those which have a longer course. The axon itself is a slightly refractive protoplasmic mass, frequently showing fine longitudinal marking, due perhaps to the neurofibrils, which may be brought out by appropriate staining. Occasionally these may occupy a central core.

[1] ADAMS, 1942.

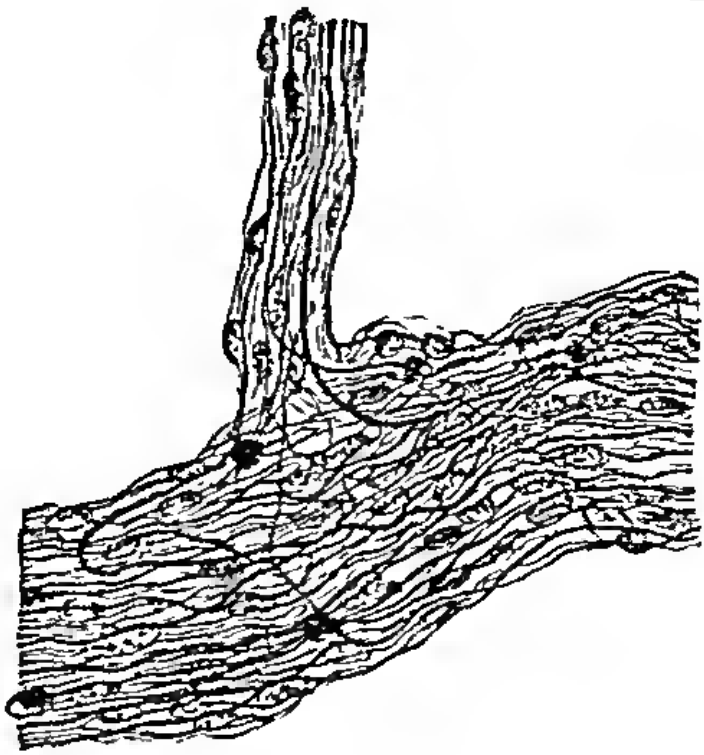


Fig. 165.—Branching of a Large Nerve Bundle. Human. Bielschowsky Method. (P. Stöhr Jr.)

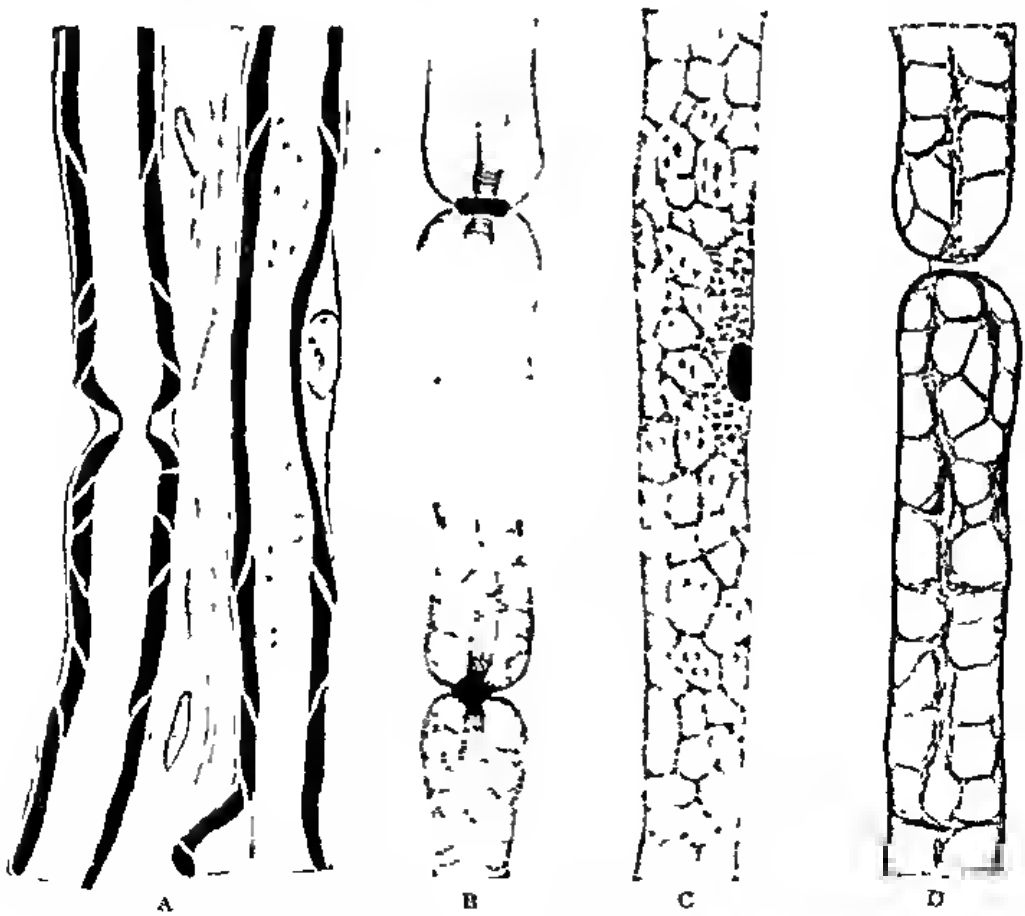


Fig. 166.—Nerve Fibers, with Myelin Sheath

A, osmic acid preparation, frog; B, silver nitrate, Guinea pig (after Schaffer, and below after von Möllendorff); C, methylene blue, cat (after Nemiloff); D, silver impregnation, cat (Cajal).

surrounded by a clear zone. The course of the axon is usually somewhat wavy within the myelin sheath, so that it may appear eccentrically placed in transverse sections. The myelin sheath which surrounds the fiber also varies greatly in thickness. In ordinary preparations it forms light zones around the dark fibers, suggesting the relation between protoplasm and nucleus; but the rod-like nature of the central fibers is evident on changing the focus, and of course the axon does not take a nuclear stain. The myelin is surrounded by the neurolemma, within which the single internodal nucleus is occasionally included in a given section. The pictures given in different histological specimens vary greatly with the treatment of the tissues. Myelin is a mixture of complex fats and lipoid substances, some of which are combined with sugar. In fresh nerves it is colorless, homogeneous, and refractile. Like fat, it is dissolved by alcohol or ether and blackens with osmic acid. In preserved specimens it may dissolve out, or the emulsion may break down, giving rise to various forms of shrinkage. A network which appears after fibers have been treated with alcohol and ether is said to be composed of *neurokeratin*, a substance insoluble in these reagents, which does not blacken with osmic acid. The pattern of the neurokeratin varies widely. It may be radially or concentrically disposed, the size of its mesh large or small; sometimes it seems to be absent, though this may be the result of poor preservation. In preparations blackened with osmic acid, the myelin is often traversed by oblique clefts, the *incisures* of Lanterman[1] (Fig. 166, A). The arrangement of these characteristic clefts may be pictured by imagining a succession of stemless funnels strung along the axis cylinder, not all of which are pointed the same way. The incisures are doubtless artificial, and their number is increased by pulling the nerve fibers apart; they appear to be empty or crossed by strands of myelin, but the neurokeratin framework may be so arranged as to correspond with these intervals.

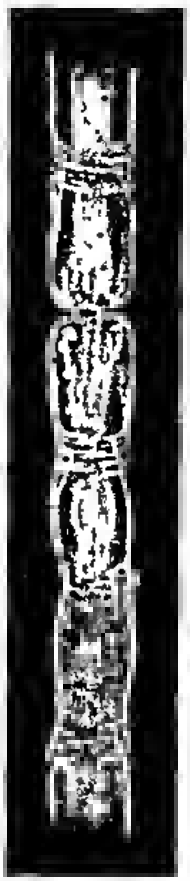


FIG. 167.—MEDULLATED NERVE FIBERS. FROM DARK-FIELD ILLUMINATION (Levi)

At the nodes the axon is continuous, while the myelin is interrupted. The axon may appear narrowed or enlarged, according to treatment. Silver nitrate blackens the fiber at this point and the color continues a

[1] These clefts are often called the incisures of Schmidt-Lanterman. H. D. Schmidt, 1874, of New Orleans observed them and A. J. Lanterman, 1877, of Cleveland described them as slits or incisures passing obliquely into the medullary sheaths. He found them in the medullated nerves of all classes of vertebrates—fishes, amphibia, reptiles, birds and mammals, and wrote that they were deeper in the frog than in man.

short distance in each direction, diminishing in intensity. At the same time a transverse band appears, making a cross (Fig. 166, B). This band has been interpreted as cement substance between the two contiguous neurolemma cells, or as a deposit on specially dense bands of the endoneurium, supposed to be present at this spot; but the actual conditions here are not well understood.

The relation of the myelin to the neurolemma or sheath of Schwann has long been of interest. It may be intracellular or extracellular. Harrison[1] proved that the sheath cells come from the neural crest by way of the dorsal roots, and that without their presence the peripheral myelin does not develop; and Speidel[2] has been able to watch these cells wander along the growing nerve fibers, and to recognize the first appearance of myelin about the fiber as a deposit near the nucleus of the sheath cell, spreading in both directions. A simple interpretation would therefore be that sheath cells wrapped themselves around a fiber and became loaded with fat in small droplets. The neurokeratin would be the altered cell protoplasm, the nucleus would be accounted for, the outer cell wall (neurolemma) and inner cell wall ('axolemma') would come together at the end of the cell, the node. But the axolemma is hard to demonstrate, and in the spinal cord, where myelin sheaths are present, there are no, or very few, cells comparable to the neurolemma. Hardesty[3] finds such cells present in numbers in the fetal cord while the myelin is being formed, but very scarce even at birth. The sheath cell can be stained differentially (Fig. 166, C, D), and it then appears as a fine network of protoplasm containing a large nucleus, each cell ending at the node; according to some authors this net is in three dimensions, but others maintain that it spreads only over the surface of the myelin as a lace-like veil, not penetrating toward the axon except at the node (Fig. 166, B). Bardeen[4] considers that the myelin is derived from the intercellular substance between the fiber and the sheath-cell and is 'due to influences exerted by the axis cylinder fibrils.' That the axis cylinder plays the chief part in its production is indicated by the fact that the myelin breaks down when the fiber degenerates.

By microdissection methods in the living nerve fibers of the frog de Rényi[5] found the axon of a jelly-like consistency, denser at the nodes where it is constricted in life to one-half its diameter, though these relations may be reversed in fixed tissues owing to the greater shrinkage of the softer gel. The axons can be slipped by the node and still retain its characteristics. By injecting fluid under slight pressure he was able to separate the myelin from the axon, though this seemed to entail perhaps the rupture of some protoplasmic connections. At the node the myelin resisted this separation, but when the resistance was overcome the fluid flowed out of the node and readily lifted the neurolemma from the outer surface of the myelin. The neurolemma is continuous past the node. The constriction at the node he considers due to a reinforcement there of the connective tissue fibers of the sheath of Henle. He found no indication of the axolemma. Schwann's cells are tubular and extremely thin, but whether they were organically connected end to end at the nodes, or joined by some cement substance, he was unable to decide. The neurokeratin he considers a coagulation artifact, because it can be reproduced by the action of fixatives on separate drops of myelin expressed from the sheath.

The production of myelin is said to begin at about the fourth month, at the central ends of the nerves. It begins at different times in different tracts and systems, and the

[1] Harrison, 1906 [2] Speidel, 1933 [3] Hardesty, 1905.
[4] Bardeen, 1903 [5] de Rényi, 1929

medullary sheaths of the spinal nerves are not all formed until two or three years after birth. This affords one method of tracing the course of nerve tracts through the brain and spinal cord. Another method is based on the fact that after section of a nerve fiber the distal portion (*i.e.*, that not remaining in connection with the cell body) suffers a degeneration of the myelin, the loss of which is readily detectable by the proper stains. This, with the concomitant chromatolysis of the cell body, may reveal the whole extent of the neuron. The sheaths continue to increase in thickness into adult life.

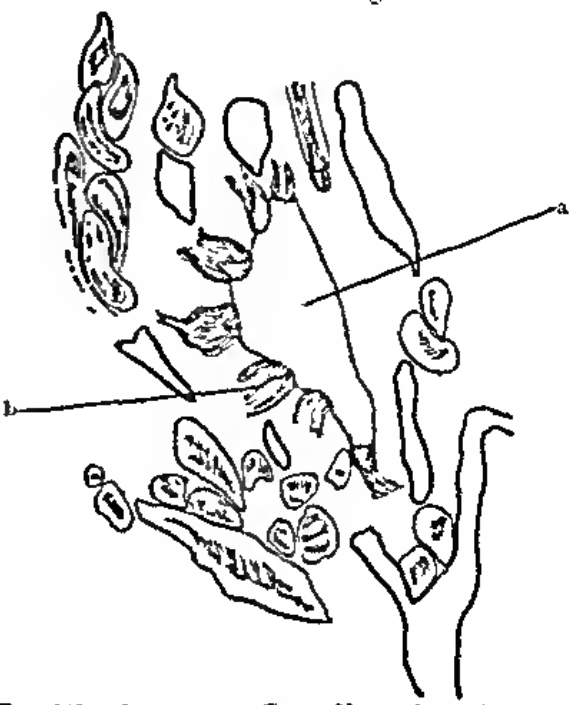


FIG. 168.—SYNAPSES ON GIANT NERVE CELL, AMPHIBIA. (Bartelmez and Hoerr.)
a, cell body, b, terminal processes of other cells.

Nerve Endings. *The Synapse.* The endings of nerve fibers in the central nervous system are in relation with the cell bodies or dendrites of other neurons. By impregnation methods or methylene blue stains the terminal branches, bereft of their myelin sheaths, can be traced as wavy, often beaded fibers, winding about the dendrites or ending in a coil about the cell, often with expanded, club-like end-bulbs; but the finer details, whether the fibers actually fuse with the protoplasm of the

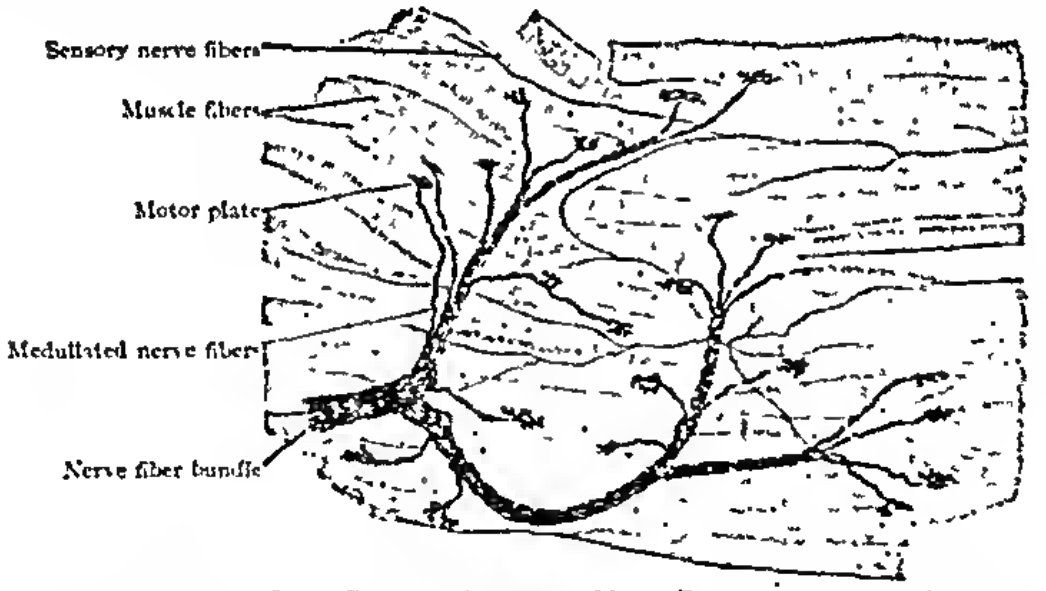


FIG. 169.—MOTOR NERVE ENDINGS OF INTERCOSTAL MUSCLE FIBERS OF A RABBIT. × 150.

other neuron or merely lie in contact with it or just near it, cannot be definitely traced by these methods. In the giant cells of the brain of the teleost *Ameiurus*, whose large size makes them especially favorable for such study, it has been possible[1] to trace the synapse between the club-

[1] BARTELMEZ AND HOERR, 1933.

ends of the VIII nerve and the dendrite of the giant cell (*Fig. 168*), as seen by a usual histological stain. Though the two elements of the synapse each show a limiting membrane where they are separate, at the junction only a single line can be detected, but the two neurons do not fuse, and neurofibrils presumably do not pass from one to the other.

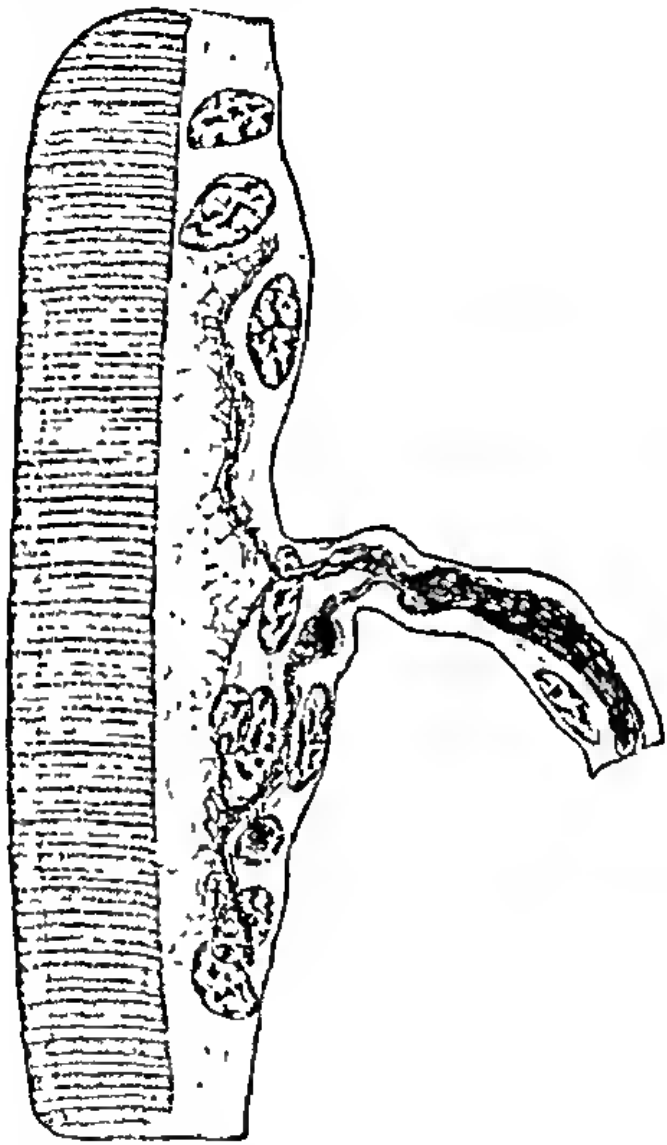


Fig. 170.—Motor Nerve Ending, Longitudinal of Muscle. (Bocke.)

Motor Endings. The motor nerve endings are the terminations of efferent nerves, in contact either with muscles, to induce motion, or with gland cells, to initiate secretion. Like all nerve endings they are difficult to study because of the unreliability of the staining methods necessary to bring out the fine nerve fibers as distinct from the tissues among which they run. Impregnation methods and supravital staining with methylene blue are most commonly used, but both require great care in interpretation of results.

Striated muscles are innervated by the neuraxons of the ventral roots, which grow out from cell bodies remaining within the central system, and form plexuses of medullated fibers in the perimysium, from which branching medullated fibers extend into the fasciculi (Fig. 169). Each muscle fiber receives one of these branches, or sometimes two placed near together. They are usually implanted near the middle of the muscle fiber. The connective tissue sheath of the nerve blends with the perimysium, and the neurolemma is said to be continuous with the sarcolemma. On the inner side of the sarcolemma the myelin sheath ends abruptly, and the nerve fiber ramifies in a granular mass considered to be modified sarcoplasm, which may contain muscle nuclei. This entire structure appears as a distinct, elevated area, estimated to average from 40 to 60 μ in diameter; it has been named the *hypolemmal motor plate*. The nuclei are thought to be in part continuations of the sheath of Schwann, in part those of the muscle cell. Beneath the fine nerve fibrils may be found a *periterminal net*, not stained by fiber stains, fading off to the sarcoplasm (Fig. 170).

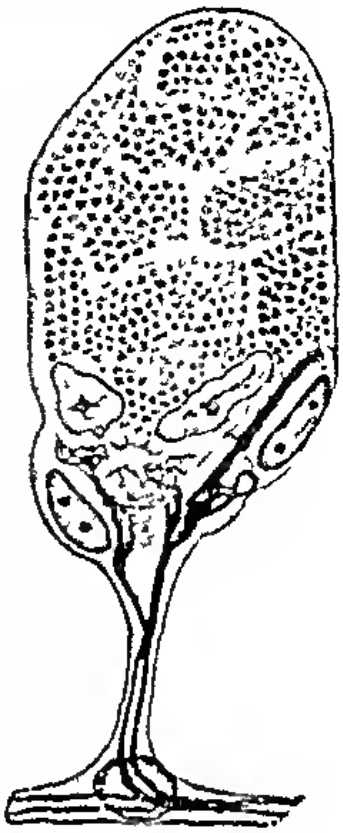


Fig. 171.—Motor Nerve Ending, Transverse of Muscle (Boeke).

In addition to the motor plates, striated muscle may receive much simpler motor endings, the *terminaisons en grappes*. The fine branches of the axon end in bulb-like enlargements, or small loops, either within the sarcolemma or outside of it (*epilemmal*). According to Hines[1] (Fig. 172)

Fig. 172.—Nerve Terminations on Striated Muscle. (Hines.)

A medullated nerve fiber (m.a c.) divides to form two fibers terminating as a motor end plate. Another fiber, non-medullated (n.a c.) contributes an accessory epilemmal ending (a c.) and another similar ending to the smaller muscle fiber.

these, at least in the case of the extraocular muscles of the rabbit, are not of sympathetic origin (see p. 167).

[1] Hines, 1931.

The nerves to smooth and cardiac muscle and to the glands (glandulo-excitor) are a part of the sympathetic system. They are non-medullated fibers which branch repeatedly, forming plexuses. From the plexuses slender varicose fibers proceed, in the case of smooth muscle cells, to end in contact with their surface in one or two terminal or lateral nodular thickenings or loops, or, according to Boeke,[1] to penetrate the fiber to end near the nucleus. Probably every muscle cell receives an ending, except in the case of those of the blood vessels where fibers have been traced along the vessel for some distance, apparently showing only a few endings. Except that the nerve endings in heart muscle are a little larger and often provided with a small cluster of terminal bodies, they are like those of smooth muscle.

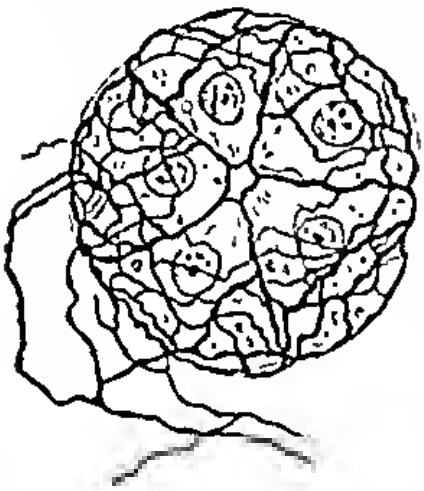


Fig. 173.—Intra-alveolar Nerves Submaxillary Gland of the Cat. Golgi Method. × 765. (Rossi and Mocchi.)

In the glands the fibers run to the bases or sides of the gland cells, where they may end freely or become flattened against the sides. Some believe the fibers enter the cells and expand in a terminal loop. Only a few cells in an alveolus seem to be in contact with nerve fibers, and in many glands the fibers have been traced only to the blood vessels.

Sensory Endings. The outward growth of nerve fibers from cells in the ganglia of the spinal and cerebral nerves has already been described. Near their terminations these fibers branch repeatedly at the nodes, lose

Fig. 174.—Nerve Endings in the Pleura Pulmonalis of a Dog. Methylene Blue. (Larsell.)

their myelin sheaths, and form terminal arborizations in contact with epithelial, connective tissue, or muscle cells. These are the *sensory endings*, and apart from those connected with the eye, ear, and other organs of special sense, they may be described as follows.

Free Endings. Sensory fibers to the epidermis and to the corneal and oral epithelia penetrate the basal layer, passing between the cells as unsheathed fibers, and ramify among the cells in the outer layers. The extremities of the fibers, which may be pointed or club-shaped, are in

[1] Boeke, 1932.

contact with the epithelial cells, but do not enter them. In the process of branching the neurofibrils become distributed in smaller and smaller bundles, which often anastomose, forming plexuses; but whether the interlacing constituent fibrils unite with one another so as to form a net has been questioned. At the ends of the branches, each fibril has become separate from the others; frequently it shows varicose enlargements.

Free sensory endings occur not only in stratified epithelia but also in muscle, tendon and connective tissue. In simple epithelia the free endings may be sensory, but in glandular epithelia they are often efferent fibers. The ultimate branches of the nerves are so delicate that they cannot be seen in ordinary preparations; they have been demonstrated chiefly by the methylene blue method, applied to living or very fresh tissue.

In the epidermis, as a modification of the free endings, fibers are found terminating in disc-shaped networks (*tactile menisci*) at the base of modified cells. These tactile cells may occasionally be seen in ordinary preparations.

FIG. 175.—BEGINNING OF DEVELOPMENT OF A MEISSNER'S CORPUSCLE IN THE SKIN OF A FINGER OF A TWO WEEKS OLD CHILD. Golgi II Method (Rachmaninlin.)

Terminal corpuscles are nerve endings consisting of a coarse nerve fiber, or knot of small branches, surrounded by a *semifluid* intercellular substance (which is granular in preserved tissue), and enclosed in a connective tissue capsule. The terminal ramifications of the nerve show irregular swellings or *varicosities*, and apparently they unite so as to make a network. Often more than one fiber enters a corpuscle, and it has been suggested that they include afferent and efferent fibers. Generally the connective tissue sheaths of the entering fibers blend with the capsule, and the myelin sheaths are lost just within it. Terminal corpuscles have been grouped as *tactile*, *genital*, *bulbous*, *articular*, *cylindrical*, and *lamellar*.

Tactile corpuscles (or Meissner's corpuscles) are elliptical structures, 40–100 μ long and 30–60 μ broad (Fig. 177). They are characterized by transverse markings, due to the corresponding elongation of the capsule cells and the tactile cells within. From one to five medullated fibers enter the lower end of a tactile corpuscle, losing their sheaths soon after entering. They pursue a spiral course through the corpuscle, giving off branches which end in enlarged terminal networks between and upon

the tactile cells. These corpuscles are found in some of the papillæ, or connective tissue elevations just beneath the epidermis, being especially

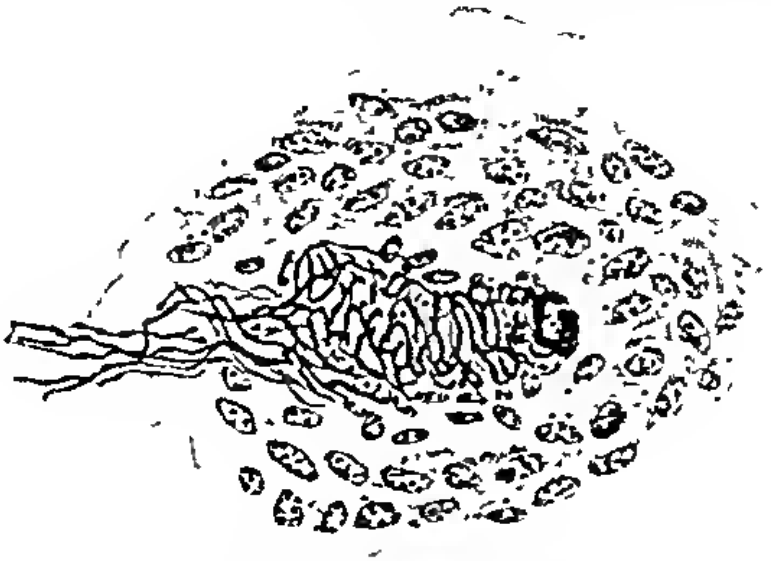


Fig. 176.—Meissner's Corpuscle in the Skin of a Finger of a Six Weeks Old Child. Golgi II Method (Rachmatullin)

numerous in those of the soles and palms (23 in 1 sq. mm.) and in the finger tips; they occur also 'in the nipple, border of the eyelids, lips, glans penis and clitoris.'

Fig. 177.—A Meissner's Corpuscle within a Dermal Papilla. Thick and Thin Medullated Fibers. Human Finger. Methylene Blue (Dogiel)

Genital corpuscles are large, round or oval bodies 60–400 μ long (Fig. 178) which may receive as many as ten nerve fibers. These ramify and send branches to neighboring corpuscles, and also to the epidermis.

The genital corpuscles are deeply placed beneath the epithelium of the glans penis, clitoris, and adjoining structures.

Bulbous corpuscles (of Krause) are smaller than the genital corpuscles, having a diameter of 20–100 μ. They are most numerous (1–4 in a sq. mm.) in the superficial connective tissue of the glans penis and clitoris. Similar structures, either round or oval, are found in the conjunctiva and 'edge of the cornea, in the lips and lining of the oral cavity, and probably in other parts of the corium.' They have thinner capsules and receive fewer nerves than the genital corpuscles, which they resemble. The articular corpuscles, found near the joints, belong in the same category.

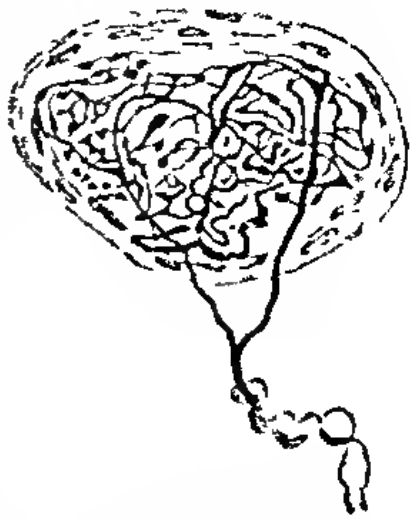


Fig. 178.—Genital End-bulb from the Glans Penis. Methylene Blue. (Dogiel.)

Cylindrical corpuscles (cylindrical end bulbs of Krause) contain a single axial nerve fiber with few or no branches, terminating in a knob-like or rounded extremity. The fiber is surrounded by a semifluid substance, sometimes described as an inner bulb, and this is enclosed in a few concentric layers of cells which are continuous with the sheath of the nerve. Cylindrical corpuscles are found in the mucous membrane of the mouth and in the connective tissue of muscles and tendons.

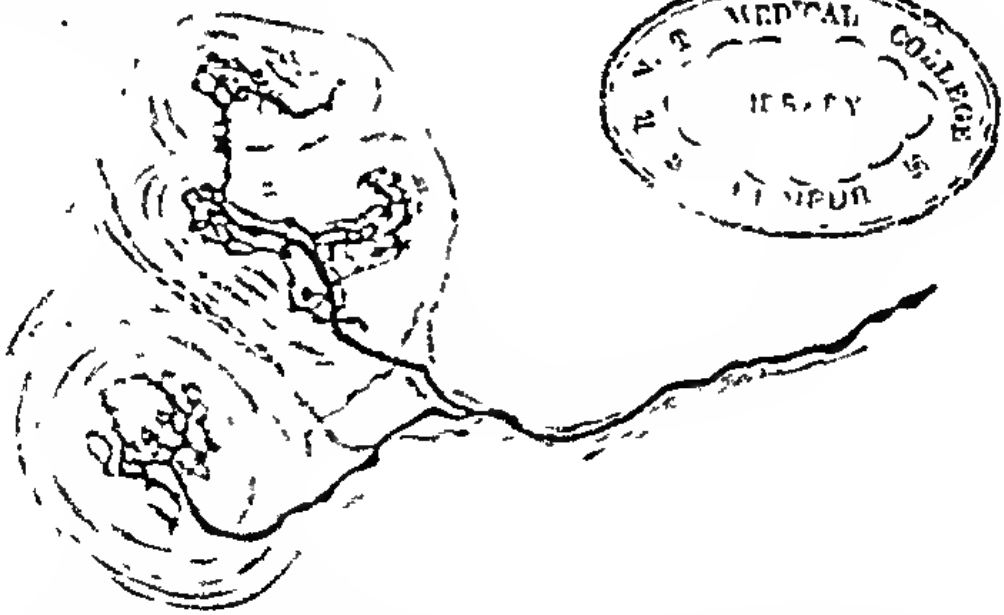


Fig. 179.—A Double End-bulb from the Human Peritoneum. Methylene Blue. (Dogiel.)

Lamellar corpuscles (or Pacinian corpuscles) are macroscopic elliptical structures 0.5–4.5 mm. long and 1–2 mm. wide (Fig. 181). They were first observed in dissections as minute vesicular bodies attached to the terminal branches of nerves. Microscopically they are striking objects,

suggesting an encysted foreign body. The axial core of the corpuscles is surrounded by concentric layers, sometimes as many as fifty, which represent a perineurium distended with fluid. A single large nerve fiber enters one end of the corpuscle and loses its myelin as it traverses the

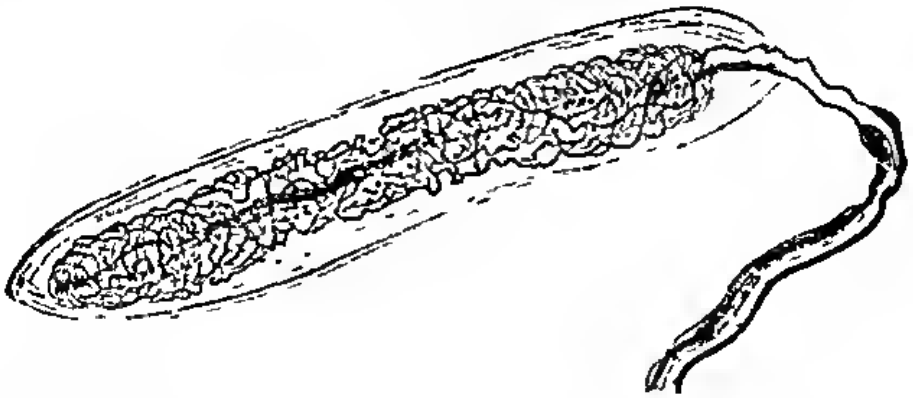


Fig 180.—An Encapsulated Nerve Apparatus from the Outer Connective Tissue Lamina of the Prostate of a Dog. A Thick and Thin Medullated Fiber, the Latter Forming the Terminal Apparatus. Methylene Blue (Timofeew)

lamellæ. It extends through the semifluid core without obvious branches, sometimes being flattened and bandlike; it may fork at its further end or form a coil of branches, and it has been observed to pass out and enter another such corpuscle. Usually the corpuscles are sectioned obliquely or transversely so that the concentric layers completely encircle the inner core.

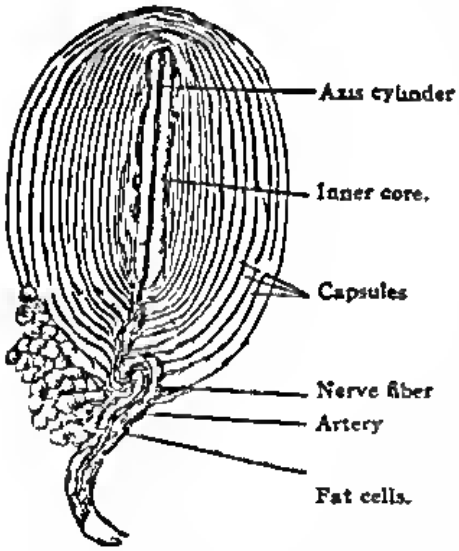


Fig 181.—Small Lamellar Corpuscle from the Mesentery of a Cat. × 50

The nuclei of the capsule cells appear as thickenings. The myelin of the nerve fiber may be traced to the inner core

Special methods have shown that the axial fiber may possess many short lateral branches ending in knobs, and that one or more delicate fibers may enter (or leave) the corpuscles in addition to the large one just described; they form a net surrounding the axial fiber. A small artery may pass into the corpuscle beside the nerve and supply the lamellæ with capillaries. Lamellar corpuscles are abundant in the subcutaneous tissue of the hand and foot and occur in other parts of the skin, in the nipple, and in the territory of the pudendal nerve; they are found near the joints (particularly on the flexor side) and in the periosteum and perimysium, in the connective tissue around large blood vessels and nerves, and in the tendon sheaths; also in the serous membranes, particularly in the mesenteries.

According to Schumacher,[1] the lamellar corpuscles become inflated when the blood pressure is increased, and 'their structure and distribution, together with the results of

[1] Schumacher, 1911

experiments, indicate that they are regulators of the blood pressure.[1] Others of the terminal corpuscles have been considered related to various types of sensation, as for instance the tactile bulb is so named because the nerve fibers might be compressed between the specialized connective tissue cells. Attempts to correlate the sensations noted in life with the histological study of the nerve endings in the skin have, however, led to no sure results.[1] The internal corpuscles serve to detect sensations not derived from

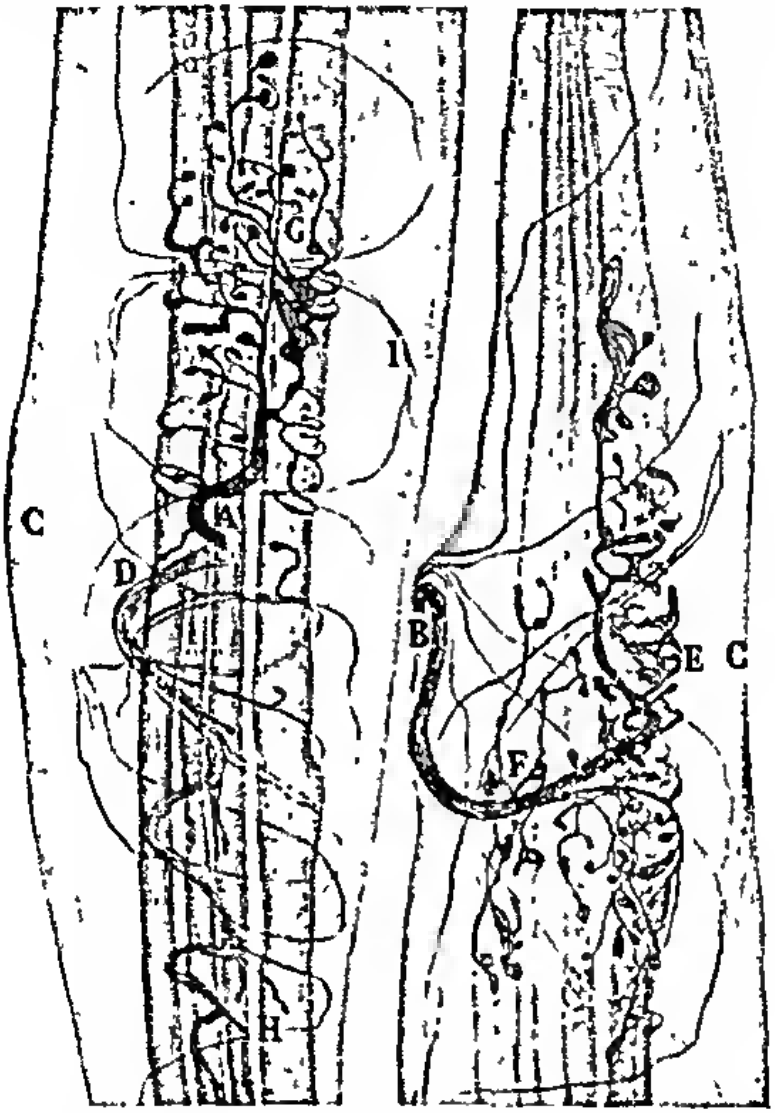


Fig. 182.—Neuromuscular Spindle of a Human Fetus of Six Months.
A, large ramifying fiber, in shape of claws, G, B, large hederiform termination E; C, connective tissue sheath, H, motor fibers, I, fine fibers (Fello.)

outside the body, and are therefore called proprioceptive. The best known are those giving notice of the position of the muscles and joints.

Muscle Spindles.[2] As seen in ordinary preparations a muscle spindle is shown in Fig. 135. They are slender groups of 3–20 muscle fibers, 1–4 mm. long and 0.08–0.2 mm. wide, around which nerve fibers terminate as shown in Fig. 182. The spindles are surrounded by a thick con-

[1] Bazett, McGlone, Williams and Lufkin, 1932.

[2] Kühne, 1863a and 1863b.

nective tissue sheath or capsule, continuous with the perimysium, and said to be divided into an inner and an outer layer by a space filled with fluid. The muscle fibers of the spindle are poorly developed. They are distinctly striated toward their tapering and very slender ends, but in their middle portions, sarcoplasm and nuclei are abundant and the

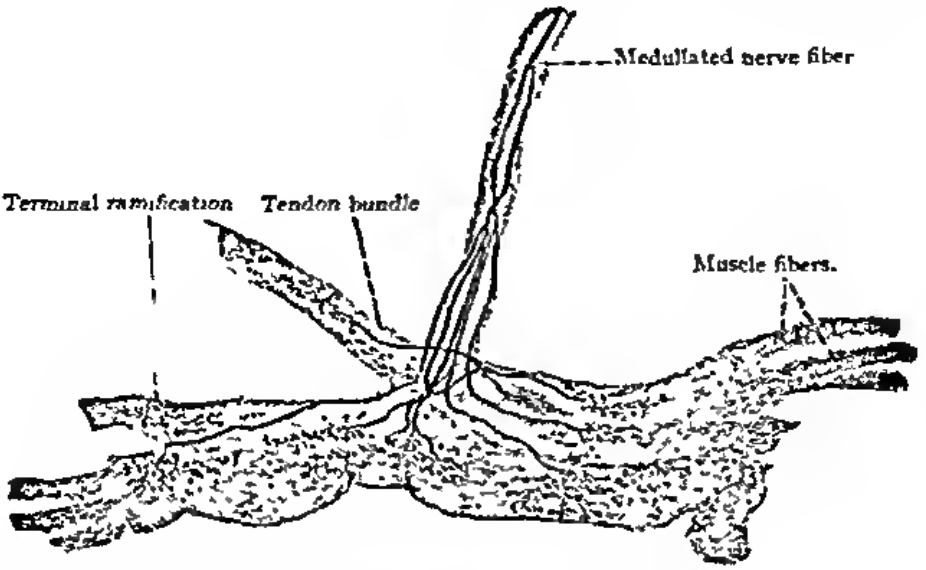


Fig 183.—Tendon Spindle of an Adult Cat × 80

striations ill-defined. Three or four nerves terminate in each spindle. Their connective tissue sheaths blend with the perimysial capsule, and they branch and lose their myelin as they pass through it to the muscle cells. Those at the middle of the muscle cells form an epilemmal spiral closely applied, and are considered sensory; those fibers running to the

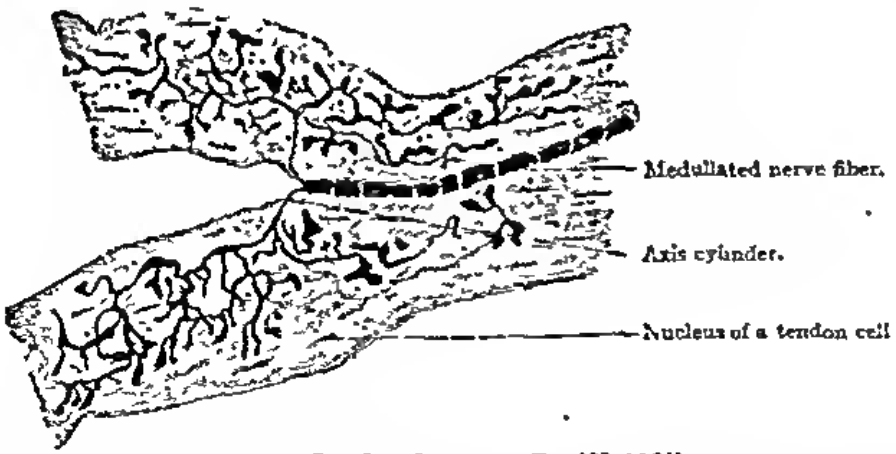


Fig 184.—The Left Portion of Fig 183 × 345

slender ends of the muscle cells terminate in modified hypolemmal motor plates.[1] In the smooth musculature of the bronchi of the lungs 'smooth muscle spindles' are described[2] in which the nerve fiber ends in short, knobbed branches on and between the muscle fibers.

[1] Hines, 1927. [2] Larsell and Dow, 1933.

Tendon Spindles. Tendons possess free sensory endings, together with the tendon spindles. These are small portions of the tendon, 1–3 mm. long and 0.17–0.25 mm. wide, enclosed in sheaths of connective tissue. They stain more deeply than the surrounding tendon.

The few nerve fibers which terminate in a tendon spindle lose their sheaths and branch freely, ending in club-shaped enlargements (Figs. 183 and 184). They are found in all tendons and serve to transmit the sensation of tension, being active in connection with coordinated movements.

In connective tissue the sensory nerves may have free endings. In addition to these the subcutaneous tissue near the coils of the sweat

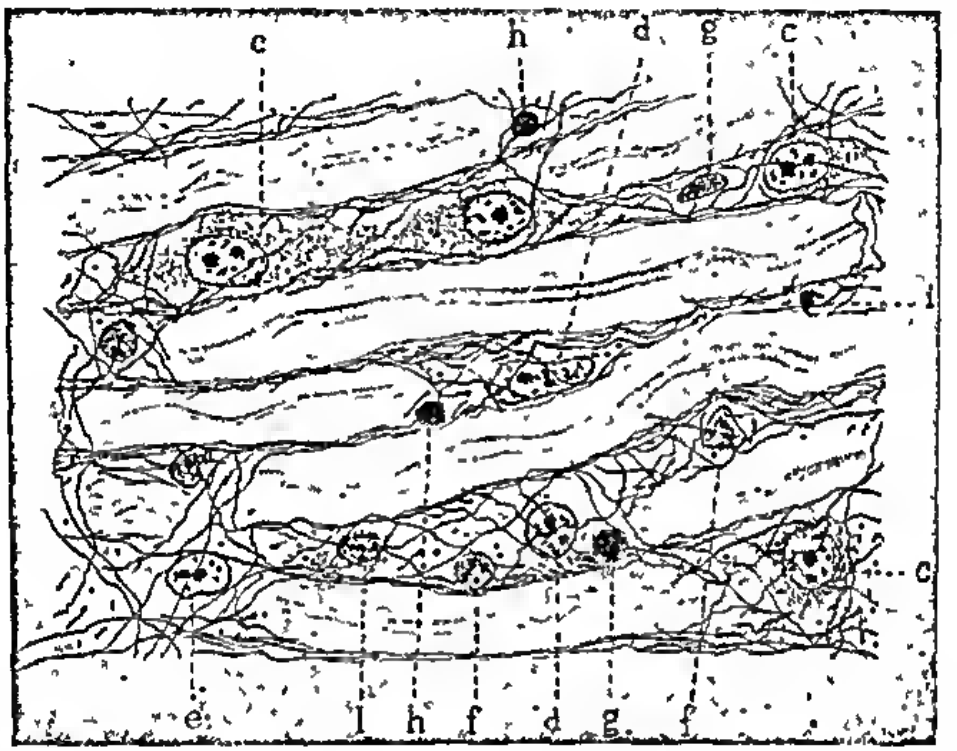


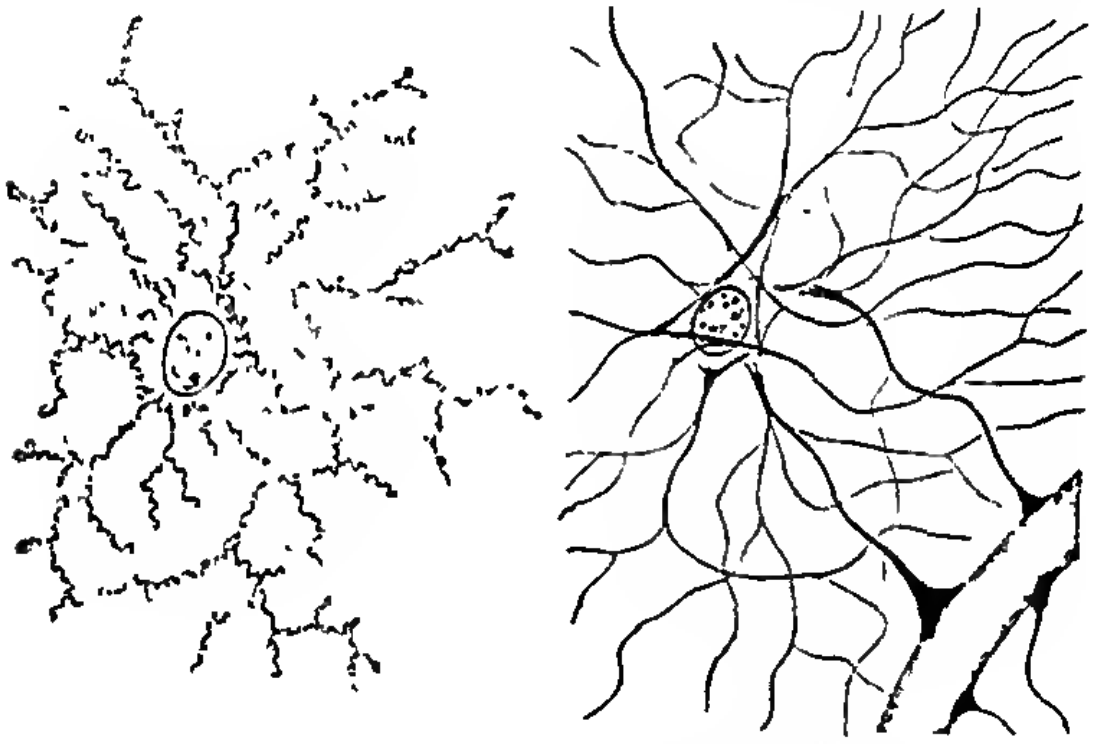
Fig 185.—Neuroglia Cells and Fibers from the Spinal Cord of an Elephant (Hardesty)
c–h, Successive stages in the transformation of neuroglia cells, ending with disintegrating nuclei (h); l, leucocyte. Benda's stain. × 940

glands, and in the corium of the fingers and toes, sometimes contains *terminal cylinders* (of Ruffini) which resemble tendon spindles in the way that their nerves ramify. These cylinders have less distinct capsules than those which characterize the nerve *corpuscles.*

Neuroglia. Neuroglia, developed from the ectoderm of the medullary tube, is the supporting tissue of the brain and spinal cord. Certain of the indifferent cells, instead of becoming neuroblasts, elongate radially, at first stretching from the cavity of the tube to the periphery. In a few regions they retain this original character, as in the median ventral wall of the cord and the retina of the eye, and in other regions the wall of the original tube remains thin, the cells forming a sheet of cuboidal epithelium, as in the chorioid plexuses and the chorioid coat of the eye.

In the thicker portions of the tube, however, they divide, some remaining as a lining for the cavity, the ependymal cells, some attached to the outer surface where their processes become felted into an external limiting membrane, and some occupying more intermediate positions. All have small bodies and longer or shorter processes, sometimes very numerous and finely branched.

The best known type of neuroglia cell is the *astrocyte*, so called because from its small body branching processes extend in all directions. There are two kinds of astrocytes, fibrous and protoplasmic. The first has



A B

FIG. 186. — ASTROCYTES, A, PROTOPLASMIC AND B, FIBROUS. (Hortega.)

a small cell body enclosing an oval or spherical vesicular nucleus, fairly large yet much smaller than that of a nerve cell, and many long slender processes, not much branched. It is especially characterized by containing, within the protoplasm of cell body and processes, stiff-looking fibers which may extend from one cell to another. These are only brought out by special stains such as phosphotungstic acid hæmatoxylin, the same used for fibroglia and myoglia fibers, which the neuroglia fibers closely resemble in form and arrangement. When the fibers are stained, it is usually impossible to distinguish the finer protoplasmic processes accompanying them, but the fibers are seen to form an intricate network, loosely surrounding nerve cells and nerve fibers, and extending both radially and longitudinally. A single process of each cell is said to reach and spread out on the surface of a blood vessel; this is supposed to derive

nourishment from the blood and is called the 'sucker foot.' The processes of these cells may be either long or short.

A second type resembles the first except that it never normally develops fibroglia fibers, and the processes are thicker, more branched, and contain vacuoles or granules. The protoplasm is differentiated into exoplasm and endoplasm. These are the so-called *protoplasmic* astrocytes. They have rather large oval nuclei, with many heavy chromatin granules near the membrane. They also have sucker feet. These cells are found only in the gray matter.

Oligodendroglia ('few dendrites'), called the 'third element' by Cajal, are recognizable in ordinary stains as apparently naked nuclei, spherical and vesicular, found as satellites to the larger nerve cells of the brain, and in rows along the fiber tracts of the brain and cord. With special stains

FIG 187.—NEUROGLIA CELLS FROM BRAIN OF RABBIT
Microglia (above) and oligodendroglia Stained by Penfield's method.

the protoplasm is found to have short, very slender and beaded processes, which branch at right angles. They may be analogous to the Schwann sheath cells and to the subcapsular satellites of the ganglia.

Del Rio-Hortega was able to distinguish two cell types among Cajal's 'third element,' the oligodendroglia just described, and *microglia*, which he calls the true 'third element.' The latter is morphologically and developmentally different from real neuroglia, as it is derived from the mesoderm (hence also called 'mesoglia'), appearing in the central nervous system only with the first advent of the blood vessels. The cells have small, dark, usually oval or bent nuclei, and slender many-branched processes. They are amœboid and phagocytic, 'the macrophages of the nervous system.' According to Russell[1] they may even become round cells, like ordinary macrophages. They are found especially near the nerve cells and blood vessels.

Because of the dense interlocking nature of the central nervous system, the shapes and relations of the different types of neuroglia cells cannot he

[1] RUSSELL, 1929.

distinguished without special impregnation methods. In ordinary histological material they can, however, be recognized by their nuclei. In the astrocytes, protoplasmic and fibrous, these are round or irregularly oval, smaller than those of most neurons, without the characteristic nucleolus, but with a rather small amount of chromatin. In the oligodendrocytes the nuclei are smaller, round, clear or with finely divided chromatin. The microglia nucleus is irregular in shape, also small, but much darker. The protoplasm of the last two is usually inconspicuous, so that they are often spoken of as 'naked nuclei.'

The knowledge of neuroglia has been advanced recently by Cajal and his pupils, del Rio-Hortega and Achúcarro, chiefly by the use of carefully devised stains. The original Spanish publications are not readily obtainable, but an excellent résumé is given by Penfield.[1]

VASCULAR TISSUE

Vascular tissue includes the heart, the blood vessels, and the lymphatic vessels, together with the blood and the lymph.

BLOOD VESSELS

General Features. The existence of blood vessels was well known to the ancient anatomists, and a distinction was sometimes made between pulsating and non-pulsating vessels. They were included by Aristotle under the term φλέψ (vein). He described the two great vessels at the back of the thorax, one of which is the vena cava; the other, as he states, 'by some is termed the *aorta*, from the fact that even in dead bodies part of it is observed to be full of air.' He added that 'these blood vessels have their origins in the heart, for in whatever direction they happen to run, they traverse the other viscera without in any way losing their distinctive characteristics as blood vessels; whereas the heart is, as it were, a part of them.' Subsequently the term *artery* was applied to the aorta and its branches, which were found partly empty of blood after death, and were believed to convey air; the windpipe was called the *arteria aspera*.

Vesalius described an artery as 'a vessel similar to a vein, membranous, round, and hollow like a pipe, by means of which vital spirit and warm blood, rushing impetuously, are distributed throughout the entire body; by the aid of these, and thus through the motion of the artery itself (which is by dilatation and contraction) the vital spirit and the natural warmth of the several parts are renewed.'[2] Vesalius described the arteries and veins as composed of coats (*tunicæ*) in which he found loose tissue and layers of fibers—circular, oblique, and longitudinal

The valves of the veins, consisting of thin membranes projecting into their lumens, were described and clearly figured by Fabricius, under whom Harvey studied at Padua.[3] Fabricius observed that the ostiola are found chiefly in the veins of the limbs and are

[1] Penfield, 1932. [2] Vesalius, 1543. [3] Fabricius, 1603.

'open toward the roots of the veins but closed below.' He considered that 'to a certain extent they hold back the blood, lest, like a stream, it should all flow together either at the feet, or in the hands and fingers.' He stated that the veins can be easily dilated and distended, since they are composed of a simple and thin membranous substance; and concluded that the veins have valves to prevent over-distention, but the arteries, because of the thickness and strength of their walls, do not require them.

In demonstrating the circulation of the blood (in 1628) Harvey contributed little to the knowledge of the structure of the vessels. He could not find the microscopic connections between the arteries and veins, but they were discovered not many years later by Malpighi.[1] In the membranous lungs of frogs and turtles, Malpighi found a *rete* or network of vessels connecting the artery and vein, so that the blood was not poured out into spaces, but was driven through tubules. He concluded that if in one case the ends of the vessels are brought together in a rete, similar conditions exist elsewhere, and he observed the circulation taking place in the diaphanous anastomosing vessels of the distended bladder of frogs. Leeuwenhoek (1698) clearly figured the minute vessels which pass from the arteries to the veins in the caudal fin of eels, and noted that the line of separation between the artery and vein is arbitrary.

The vessels which connect the arteries with the veins, because of their hair-like minuteness, were later called *capillaries*. Physiologically they form the most important part of the vascular system, because through their thin walls osmotic and gaseous exchange can take place most readily, and because in their narrow tubes the blood stream is finely divided and thus presents the greatest relative surface. Anatomically they are most fundamental and consist essentially of endothelial tubes. All larger vessels, not only the arteries and veins, but also the heart, are derived from endothelial tubes and retain their endothelial lining. The endothelium, however, becomes surrounded by layers of smooth muscle fibers and connective tissue, which form the substance of the vessel walls. The arteries in general have thicker and more elastic walls than the veins, and tend to remain open after death; the thinner walls of the veins are prone to collapse. In sections, therefore, the arteries appear round, the veins flattened.

Origin of Blood Vessels. In the last century His advanced the theory that in certain regions and for a limited period of time special cells develop which by elongation and vacuolization or special arrangement form blood vessels, and that all future blood vessels arise by the proliferation and extension of these original cells. As a group these cells were called the *angioblast*. In the chick the angioblast develops in the area opaca, at some distance from the body of the embryo, between the entoderm and the splanchnic mesoderm, in connection with the blood islands. Groups of cells are seen in embryos of the second day adhering to or forming a part of, the overlying mesoderm, while below a space occurs bounded partly by the entoderm as a single layer of cells. Some of the

[1] Malpighi, 1661.

cells become detached from the groups forming free primitive blood cells while others spread around the space and over the entoderm. Thus a cavity is made lined with flattened cells as an endothelial layer. The interpretation of the successive steps is, however, frequently difficult, and some authors maintain that the entoderm plays a large part in the formation of the islands.

In man, according to Hertig,[1] the origin of the angioblast arises first from the trophoderm of the chorion (see p. 541), in the early presomite stage. The trophoderm is the outer, ectodermal layer of the blastocyst, and from its inner surface cells are delaminated, some of which become

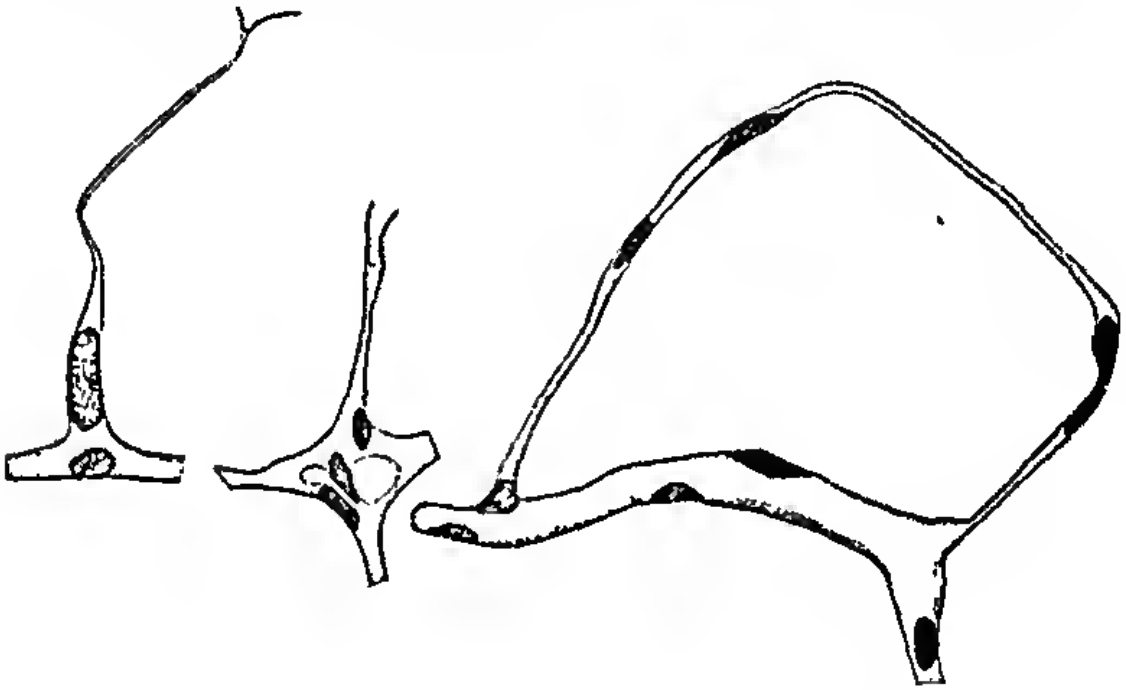


Fig. 188.—Capillary Sprouts. (Benninghoff.)

primitive mesoderm, others angioblasts. The latter multiply, form elongated, syncytial masses, and send out sprouts to join other similar masses to form nets. They develop vacuoles, either inter- or intracellular, the hollow portions being called *angiocysts;* later these coalesce to form vascular nets. Other investigators have thought that the angioblasts were derived from the mesodermal covering of the body stalk. All three germ layers have thus been cited as giving rise to the angioblast, which in turn was thought to produce all future blood vessels.

Another group of anatomists rejected this latter idea and maintained that blood vessels develop in situ in many regions of the embryo, by the vacuolization of mesenchymal cells, or by the flattening of these cells to form endothelial walls around former intercellular spaces, and that these isolated spaces later anastomose to make many of the intraembryonic

[1] Hertig, 1935.

vascular trunks. Such spaces have been found in regions beyond the limits of careful injections of the existing vascular net. Some authors have been able to reconstruct a network of solid strands of angioblast connecting with these isolated spaces, but others have found vessels in areas from which such strands have apparently been experimentally blocked.

The extension of the vascular net, whether it originates from one or many foci, is accomplished by the outgrowth of new sprouts through the

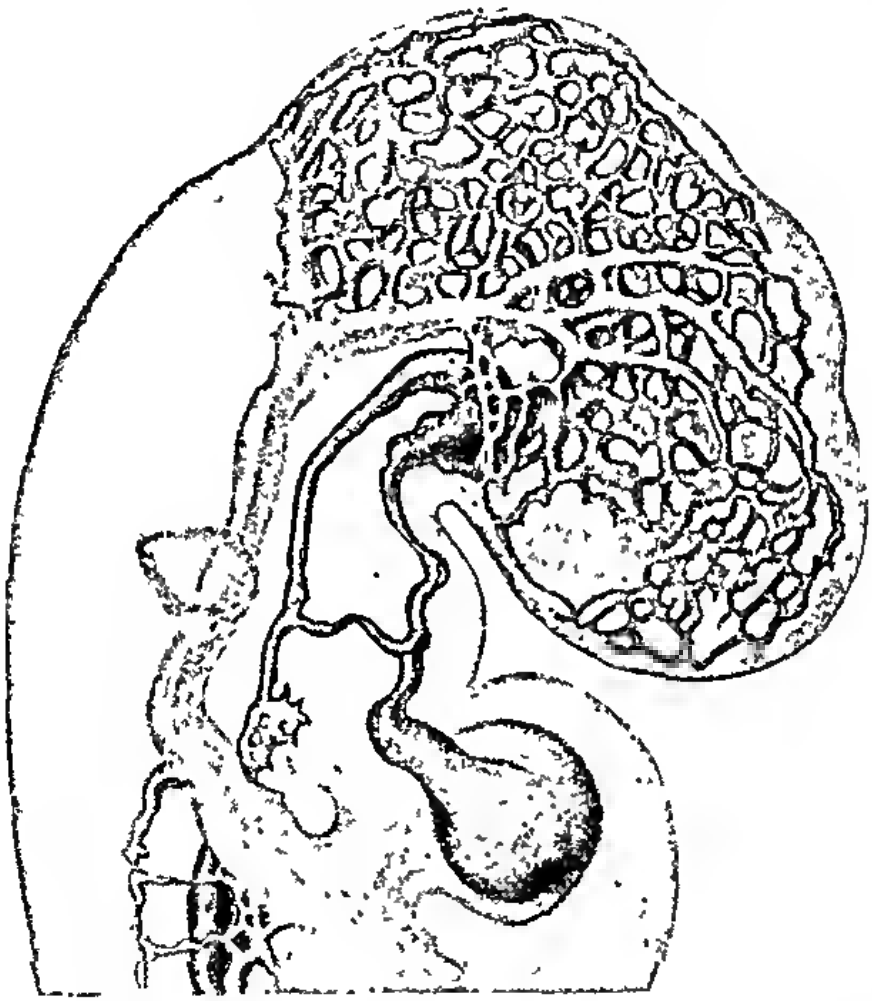


FIG 189.—BLOOD VESSELS OF A VERTEBRATE EMBRYO FORMATION OF VESSELS FROM A CAPILLARY NETWORK. INJECTED CHICK EMBRYO OF 25 SOMITES (EVANS)

mesenchyma, with which they often seem to be intimately connected. The sprouts are at first solid, but soon become hollow, either by the extension of the parent cavity or by the formation of isolated cysts. They may fuse with similar offshoots from the same or other vessels. The process has been followed in tissue cultures[1] which also show that portions of the sprouts may become isolated during growth, a fact which may serve to harmonize the two views as to the origin of the intraembryonic blood vessels.

[1] LEWIS, W. H., 1931.

The formation of a definite system of arteries and veins out of a general network may be partly explained on mechanical principles. The vascular outgrowths must take certain courses marked out by the epithelial structures. Thus in early stages they may grow between the somites, but not into them, producing a series of segmental vessels; they pass around the front of the fore-gut and up and down between its lateral outpocketings, so that the regular system of aortic arches appears to depend upon these epithelial obstructions; and they are guided along the under surface of the developing brain in a very characteristic manner. Epithelial obstructions, therefore, determine the position of the capillary plexuses. In each plexus the favorable channels enlarge and become the main arteries and veins, sending forth new branches and acquiring thick walls; whereas the vessels in which the current is slow remain small or disappear. These factors are further considered by Thoma.[1]

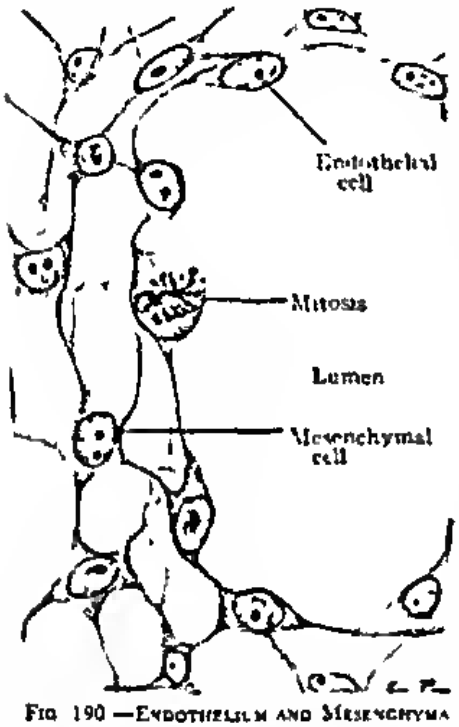


FIG. 190.—ENDOTHELIUM AND MESENCHYMA
A portion of a cross section of a small blood vessel with surrounding mesenchyma. Heart of a 96 hr chick embryo. Bouin fixation, hæmatoxylin and eosin.

The way in which main trunks develop from indifferent networks has been described by Evans on the basis of extraordinarily perfect injections; thin fluid introduced into the vessels of a living chick embryo is distributed throughout the vascular system by the action of the heart.[2] The process of the growth and extension of the capillary net into new areas and of the development of arteries and veins is even more strikingly displayed in numerous papers by the Clarks.[3] They insert a transparent chamber into the ear of a rabbit and watch from day to day the growth of vessels into the chamber.

The formation of anomalous vessels readily takes place by the persistence and enlargement of channels usually unfavorable. This is discussed by Bremer in explanation of various anomalies of the renal arteries.[4] Innumerable forms of human vascular anomalies may thus be explained embryologically; some of them represent persistent vessels which are normally important at a certain stage of development, and others represent connections which are as abnormal in the embryo as in the adult.[5]

A very characteristic form of circulation occurs in certain organs, in which the endothelium of the vessel walls is closely applied to the epithelium of the secreting tubules, or other parenchymal structures (Fig. 191). The walls of the vessels are as thin as those of capillaries, but their diameter is much greater, so that they have been described as lacunar

[1] THOMA, 1893. [2] EVANS, 1909. [3] CLARK AND CLARK, 1932.
[4] BREMER, 1915. [5] LEWIS, F. T., 1909*a*.

vessels or '*sinusoids*,'[1] the term *sinus* being generally applied to the large thin-walled veins in the dura mater about the brain and to other large thin-walled vessels (see below). Since they occupy the space between rounded cords or tubules, the sinusoids are of irregular shape, not definitely tubular. Apparently the close apposition of the endothelium, on all sides, to the cells of the parenchyma is the most essential characteristic of these vessels and must be of considerable physiological significance. There are few or no connective tissue cells between the thin lining of the vessel and the epithelial tissue which it nourishes. Capillaries, on the contrary, have a different history and are embedded in connective tissue, even though occasionally they approach close to an epithelium, sometimes appearing to enter it.

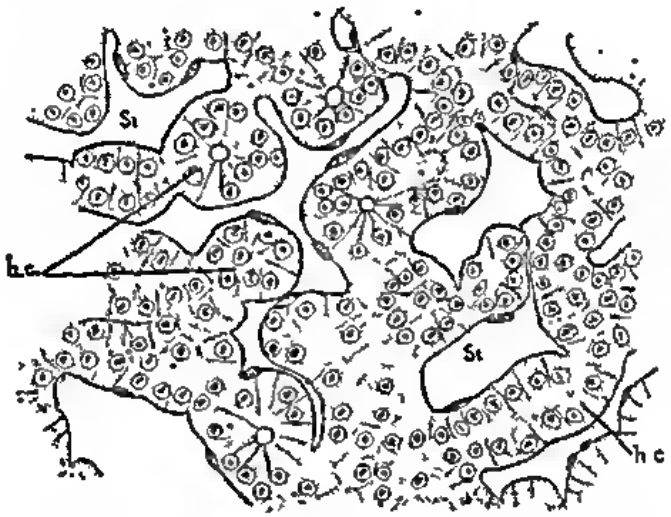


Fig. 191.—Sinusoids (Si) in the Liver of a Chick Embryo of Eleven Days. × 300. (Minot)
h c., cords and tubules of hepatic cells

Where sinusoids are most highly developed, as in the liver and Wolffian body of embryos, they possess another very significant characteristic. They are not connections between an artery and a vein, like the capillaries, but are subdivisions of veins. Thus in the liver, as shown in the diagram, Fig. 192, the portal vein enters the organ and is subdivided by cords of hepatic cells into sinusoids, such as are shown in section in Fig. 191. These reunite to empty into the vena cava inferior. The sinusoids of the liver have therefore been described as formed by the *intercrescence* of vascular endothelium and hepatic parenchyma. This arrangement of veins constitutes the hepatic portal circulation, taking its name from the entering vessel. The same type of venous circulation occurs in the Wolffian bodies, where it constitutes the 'renal portal circulation,' although it has no connection with the portal vein. It is probable that this form of circulation, which is generally lacunar or sinusoidal, represents a primitive type of vascularization, since a single vessel passing by or through an organ provides it with both afferent and efferent vessels. The arterio-venous circulation requires the presence of two vessels with currents flowing in opposite directions. There are indications that various organs in the human embryo have a transient 'portal circulation' before the arteries connect with the network and become the main afferent channels.

[1] Minot, 1900.

Where cords of epithelial cells grow into an area already supplied by an arterio-venous capillary bed, as in the parathyroid gland, or where the capillary net is merely formed of especially large vessels connecting artery and vein, as in the bone marrow, the term sinus is preferable, even though it is also used for such dissimilar structures as the sinuses of the lymph glands.

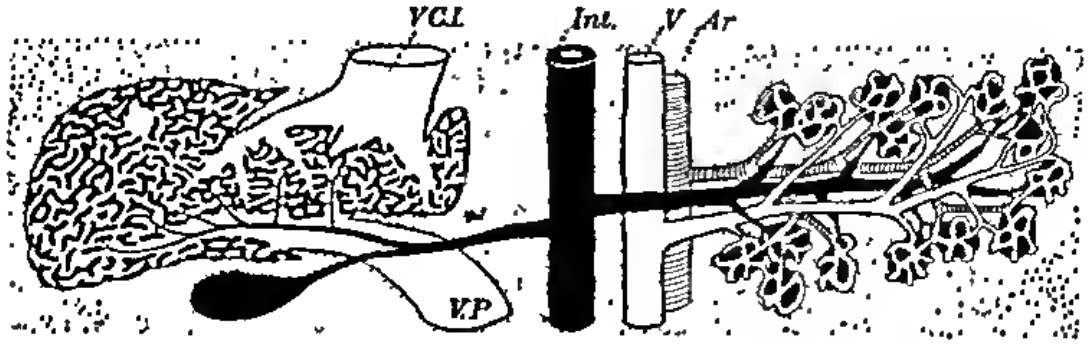


Fig. 192.—Diagram Showing on the Left the Liver and Its Sinusoids, on the Right the Pancreas and Its Capillaries

The connective tissue is represented by dots. Ar., Artery; Int., intestine; V., vein; C. V. I., vena cava inferior; V. P., portal vein.

Capillaries. The capillaries are essentially endothelial tubes of varying diameter, the smallest being so narrow that the blood corpuscles must pass through them in single file. Their walls are composed of very flat cells, some elongated with relatively smooth borders, others shorter with irregularly wavy outlines which are clearly demonstrated in silver nitrate preparations. Kölliker[1] compared the shapes of endothelial cells to 'double-pointed steel pens': they would be then hexagonal. The arrangement of cells around the circumference of a capillary is not a simple rolling up of hexagonal cells, but more complicated leading to cells with few and many sides. The basic question of the shapes and the arrangement of cells in capillary endothelium is discussed by F. T. Lewis.[2] Each endothelial cell contains an oval nucleus which is frequently seen bulging into the lumen in preserved specimens. This appearance is probably caused by a post-mortem contraction; in life the lining is very smooth. The cytoplasm is finely granular, especially around the nucleus otherwise it usually appears quite structureless. Pigment granules, principally hæmatogenous, have been observed in the capillary endothelium of the uterus and granules reducing osmium tetroxide in those of the brain. Blood corpuscles often pass out of a capillary between the

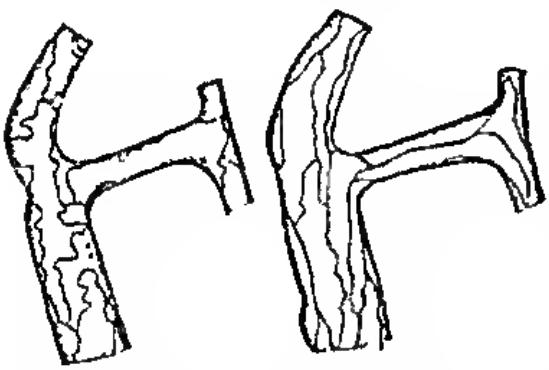


Fig. 193.—Capillaries Showing Cell Outlines, silver-nitrate preparation (Zimmermann).

[1] Kölliker, 1867. [2] Lewis, F. T., 1933a.

cells, particularly where the pointed ends meet. Since there are no preformed openings for this purpose the endothelial cells come together after the corpuscles have passed out. Clark and Clark[1] describe changes in the blood vascular endothelium in the living animal. They find under normal conditions blood cells float along without sticking to the wall but under mild stimulation leucocytes stick momentarily. Injured endothelium becomes more sticky and either recovers or breaks up into solid hyalin globules which are ingested by histiocytes (macrophages). Capillaries are surrounded by a delicate network of interlacing reticular fibers, arranged mainly longitudinally, and it is not likely that they form a complete coat anywhere. Between this network and the endothelial wall there is found a thin peri-vascular space. Along capillaries are observed cells of various sorts—adventitial cells or histiocytes, undifferentiated mesenchymal cells, occasional nerve cells and widely scat-

FIG. 194.—ROUGET CELLS CLASPING A CAPILLARY. THE ENDOTHELIAL NUCLEI ARE FAINTLY SHOWN. (Vimtrup)

tered, elongated branched cells called *Rouget*[2] *cells* or pericytes. The Rouget cells occur on many, if not on all capillaries and discussions as to their nature have led to much disagreement. Vimtrup,[3] and Bensley and Vimtrup[4] consider them as muscle cells, the contraction of which reduces the size of the capillary lumen. Other histologists regard them as non-muscular and in no way concerned with capillary contraction. Clark and Clark[5] observe in the living animal, that growing capillaries contract independent of and before the appearance of any Rouget cells. When they are stimulated mechanically with a microdissecting needle they round-up and retract their processes without producing any effect upon the capillary wall, unless the needle touches the endothelium, which then contracts locally (Zweifach).[6]

Although capillaries vary in diameter (4.5–12 μ), those in a given territory are found quite uniform, both as to caliber of individual vessels and the size and pattern of the meshes in the network. The closest meshes and largest capillaries occur in secretory organs and in the lungs, which are therefore abundantly supplied with blood. The muscles are well supplied by slender capillaries in a rectangular meshwork. Serous membranes and dense connective tissue have a scanty blood supply,

[1] CLARK AND CLARK, 1935. [2] ROUGET, 1873. [3] VIMTRUP, 1922.
[4] BENSLEY AND VIMTRUP, 1928. [5] CLARK AND CLARK, 1925 and 1940b.
[6] ZWEIFACH, 1934 and 1937.

from narrow capillaries in a coarse net. A peculiarity of capillary circulation is that some vessels may remain closed during the ordinary use of a part—the inactive phase—but convey blood under other circumstances —the active phase. Observations on living capillaries in a transparent chamber inserted in a rabbit's ear, reveal that even at rest the vessels are constantly changing, some contracting while others suddenly expand after protracted closure.

For a modern conception of the capillaries the student is referred to A. Krogh, 'The Anatomy and Physiology of Capillaries,' 1929 and to the numerous papers by E. R. Clark and E. L. Clark.

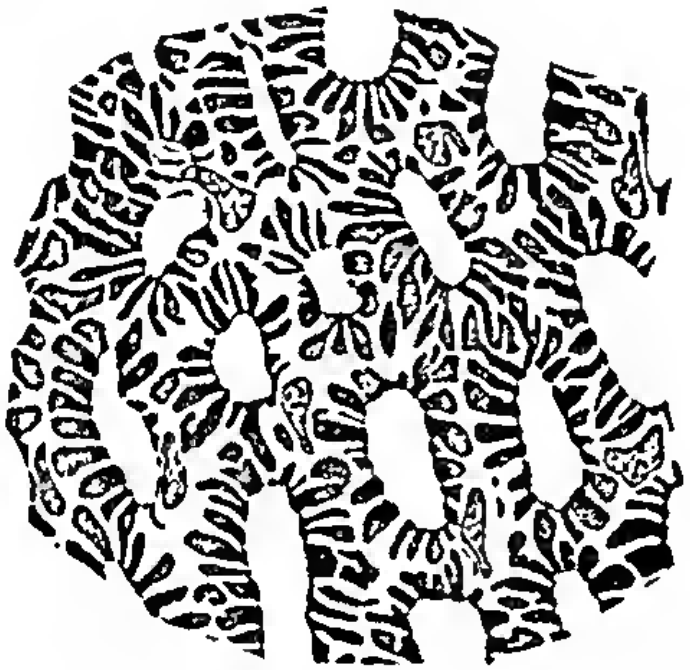


FIG 195.—NEGATIVE PICTURE OF PERICYTES. CAPILLARY NET IN THE TURTLE LUNG. DEMONSTRATED SILVER METHOD. × 830 (*Zimmermann*)

ARTERIES. The walls of the arteries are composed of three layers—the *tunica intima*, *tunica media* and *tunica externa* (often called the tunica adventitia or simply the adventitia). The intima usually the weakest layer, includes the endothelium and generally an underlying inner elastic membrane, separated from the endothelium by a small amount of cellular and fibrous tissue. This subendothelial tissue or striated layer of Kölliker shows a varying development in the different arteries; it forms a relatively thick layer in some vessels and is wanting in others. Although the general direction of the argyrophil, collagenous and elastic fibers is longitudinal, they often appear to be arranged spirally because of the torsion of a vessel. In the intima of some arteries fibrocytes are said to form a syncytium with the fibers of different sorts passing in the intercellular spaces. Large branched fibrocytes—'Langhans cells'—and histiocytes have been described in the subendothelium of larger arteries. The inner elastic membrane (*membrana elastica interna*) is composed of a

network of fine elastic fibers which may be so close as to form a fenestrated membrane in the aorta and larger arteries near the heart. It has been described as formed of three lamellæ—an inner and outer one of coarse fibers separated by a thin intermediate stratum. Dees-Mattingly[1] observes only two layers, a very thin feltwork of fine elastic fibers, giving the appearance of a solid sheet with fenestra or openings in it and a more open-meshed plexus of coarser fibers adhering to its intimal side. She could see no coarse fibers on both sides of the thin membrane.

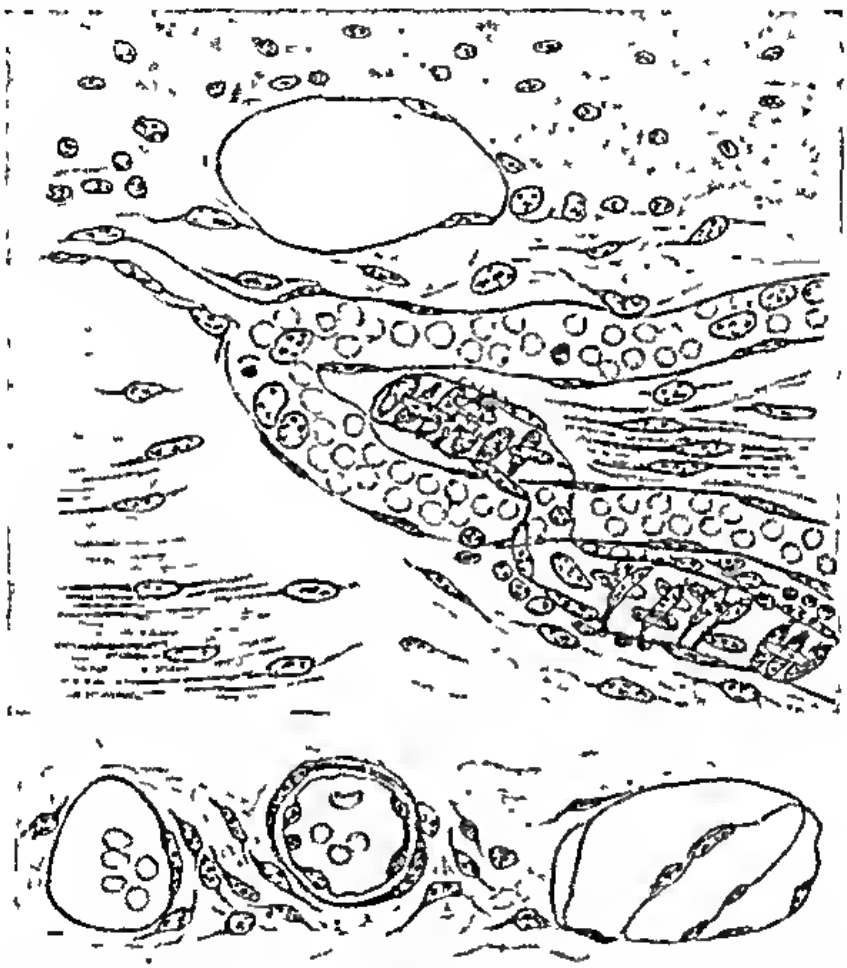


FIG 196.—SMALL VESSELS BETWEEN THE MUSCULAR LAYERS OF THE SMALL INTESTINE

Above, a lymphatic vessel in cross section, at the left a capillary entering a looped venule, crossing the venule is an arteriole with a few circular muscle fibers, seen in section at the sides and cut longitudinally either above or below the endothelial nuclei In the lower part of the figure a venule, an arteriole (not contracted) and a lymphatic vessel with valves

The media is made up mostly of smooth muscle and elastic tissue, the amount of each determining in a measure the type of artery. Near the heart the larger vessels are called '*elastic arteries*' and contain a predominance of elastic tissue and little smooth muscle. The medium-size and smaller arteries conveying the blood to different parts of the body are known as '*muscular arteries*' in them the media is composed of layers of circularly or spirally arranged smooth muscle fibers intermingled with

[1] DEES-Mattingly, 1923.

thin nets of elastic tissue and reticular fibers. Often in lengths of macerated artery, the smooth muscle can be unraveled in a continuous spiral. At the border of the media and the externa there is found in many arteries a condensation of longitudinal elastic fibers in the form of an outer elastic membrane (*membrana elastica externa*).

The tunica externa varies greatly in thickness in arteries of different sizes. It may be very thin in some vessels and have widths up to or even

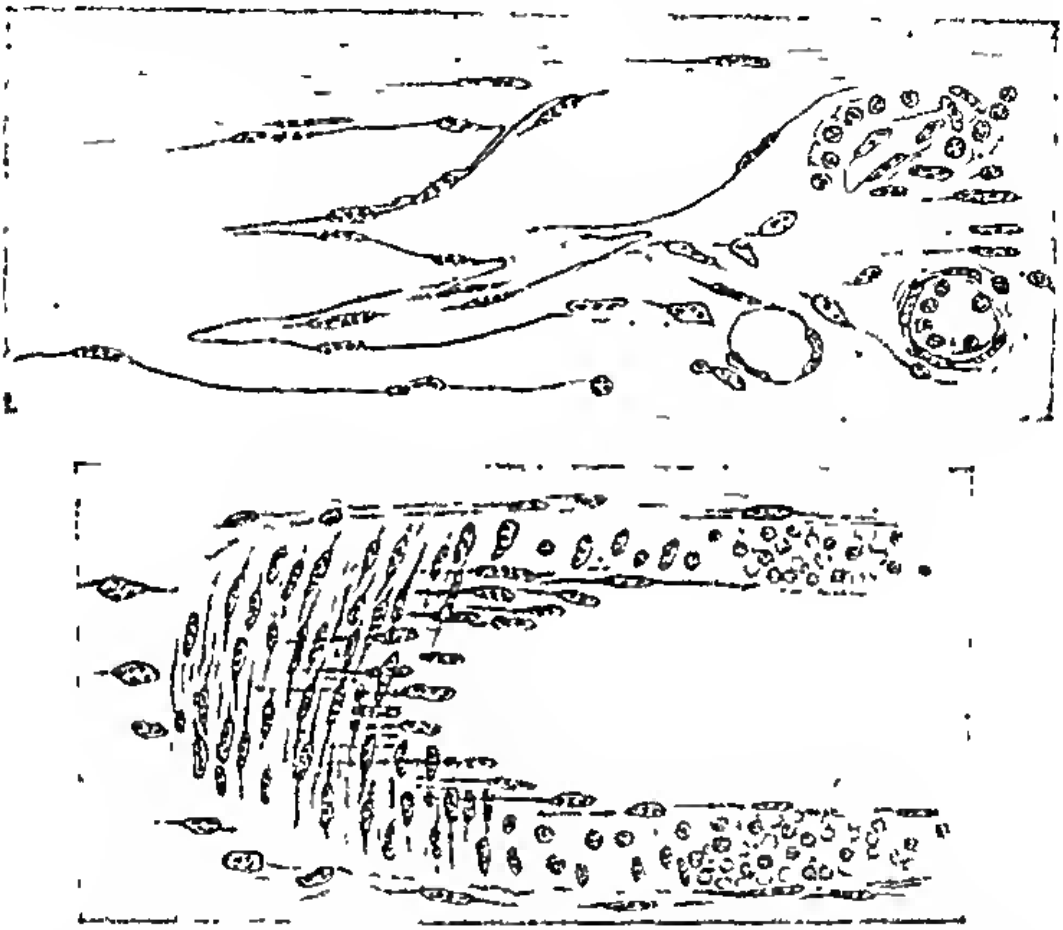


FIG. 197.—FROM THE SAME REGION AS FIG. 196

A lymphatic vessel is shown cut lengthwise at a valve, just distal to an entering branch. At the lower right corner an arteriole, the endothelium thrown into ridges by the contracted muscles. The lower figure is a small artery cut obliquely; note the endothelial ridges, containing nuclei, of the top wall of the vessel, and the transversely running muscle, changing to transversely cut muscle fibers as the sides of the vessel come into the section

exceeding the thickness of the media in others. Collagenous fibers prevail in the externa intermixed with elastic fibers and in some vessels a few smooth muscle fibers. It is in general a loose layer and blends peripherally with the surrounding connective tissue.

The small terminal arteries are called *arterioles*. They are endothelial tubes encircled by scattered smooth muscle fibers (Fig. 196). The oval nuclei of the endothelium are seen to be elongated parallel with the course of the vessel. As is usually the case, the walls of the endothelial cells are not visible. The rod-shaped nuclei of the muscle fibers are at right angles with the axis of the vessel. In a somewhat larger artery,

the muscle fibers form a single but continuous layer, the media, outside of which the connective tissue is compressed to make the externa. Its fibers tend to be parallel with the vessel. The walls of such an artery are so thick that it is possible to focus on the layers separately.

The larger arteries are lined with endothelium similar to that of the capillaries, as shown in silver nitrate preparations. This endothelium rests on a delicate layer of connective tissue containing a few flattened cells and a network of fine elastic fibers. The meshes of the fibrous and elastic tissue are elongated lengthwise of the vessel, and on surface view they present a longitudinally striped appearance. In addition to this *subendothelial tissue* and the endothelium, the intima includes the *inner elastic membrane.* This is usually a conspicuous layer thrown into wavy folds by the post-mortem contraction of the vessel. It is easily seen with ordinary stains, appearing as a refractive layer, and is deeply colored by

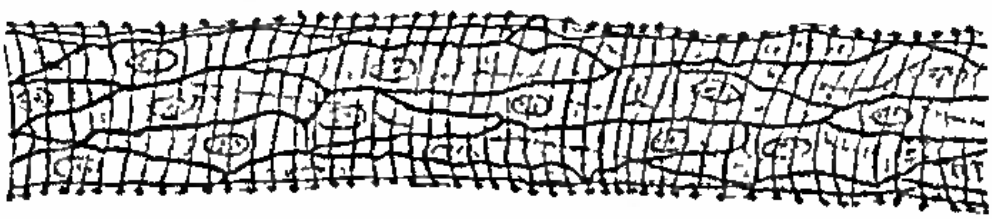


Fig. 198.—A Small Artery in the Mesentery of a Cat Showing Outlines of the Endothelial Cells and Surrounding Smooth Muscle.
Silver nitrate injection. Hæmatoxylin.

resorcin-fuchsin and other elastic tissue stains. In smaller arteries the endothelium appears to rest directly upon the elastic network which replaces this membrane; and in such large ones as the external iliacs, the principal branches of the abdominal aorta, and the uterine arteries in young persons, the subendothelial tissue is said to be lacking. The inner elastic membrane is not a continuous sheet of tissue, since it is perforated by elongated apertures; it forms a *fenestrated membrane* and the development of such membranes from elastic networks has already been described. The membrane is particularly thick in the larger arteries of the brain, and it is sometimes double.

The media, which consists of but a single circular layer of muscle fibers in the smallest arterioles, becomes many-layered in larger arteries. Generally the fibers are all circular or perhaps oblique, but in the loose musculature of the umbilical arteries longitudinal fibers are numerous. Longitudinal fibers are said to occur in certain other vessels near the intima, being especially well developed in the subclavian artery. The post-mortem contraction of the circular fibers, which throws the intima into folds, causes a spiral crumpling of certain muscle nuclei, the significance of which has already been discussed. Between the muscle fibers there are circular elastic fibers, or plates in the larger vessels, which are

thrown into wavy folds. Radial fibers, which connect these in a general network, are slender and require special staining. White fibers are present, apparently formed in considerable part by the muscle fibers which they bind together, since no connective tissue cells are present. The proportion between the muscular and elastic tissue in the media varies in different arteries. In the smaller vessels, the muscular tissue predominates, and this is true also of the cœliac, femoral and radial arteries. But in the common iliac, axillary and carotid arteries the elastic

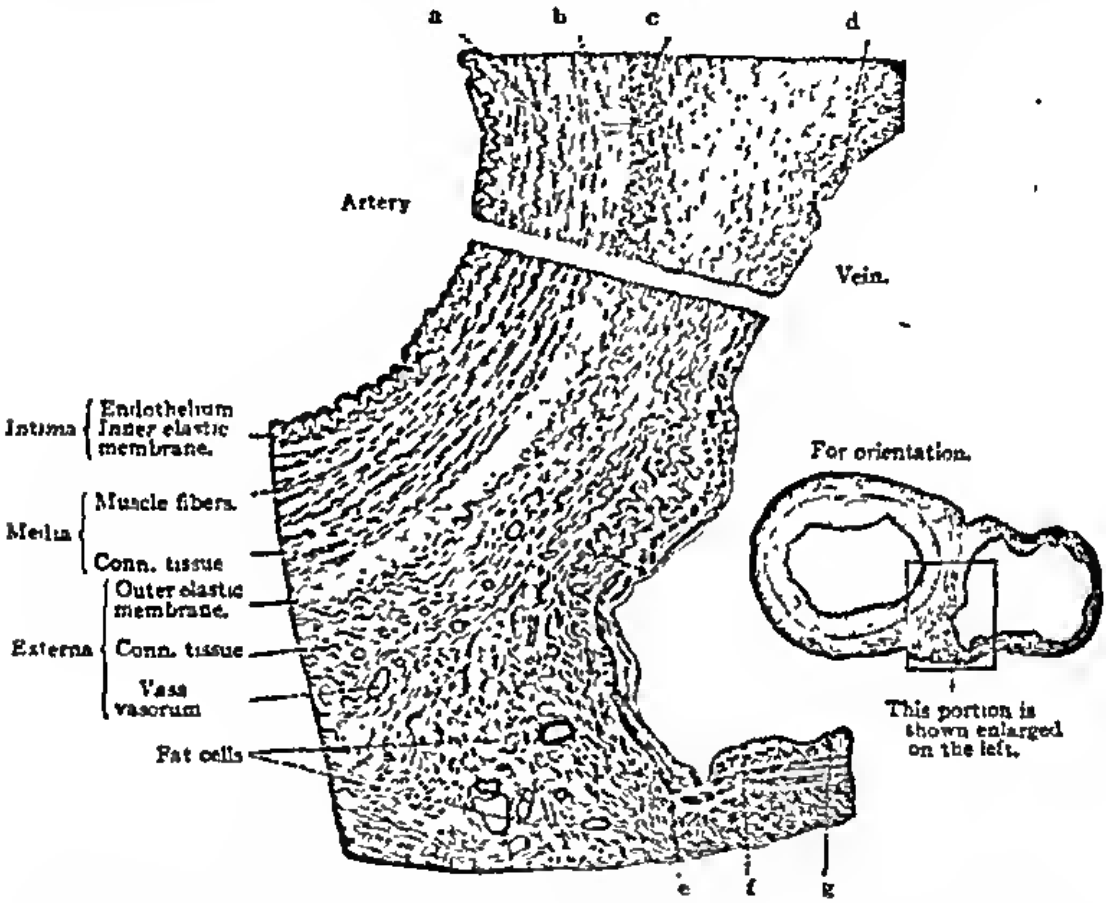


FIG. 199.—A SECTION THROUGH A HUMAN ULNAR ARTERY AND VEIN, SHOWING THE WALL OF THE ARTERY ON THE LEFT AND OF THE VEIN ON THE RIGHT. THE UPPER PART OF THE FIGURE (a–d) IS FROM A SECTION OF THE SAME VESSELS STAINED WITH RESORCIN-FUCHSIN, AN ELASTIC TISSUE STAIN. × 550.

a, Circular, and b, radial elastic fibers of the media of the artery, c, external elastic membrane, d, elastic fibers in the media of the vein, e, circular, and g, longitudinal muscle fibers of the media, f, endothelium.

tissue prevails, and in this respect they resemble the largest arteries—the aorta and pulmonary artery. A detailed description of the relations of muscle and elastic fibers in the different arteries is given by Benninghoff.[1]

The externa is a connective tissue layer which sometimes contains scattered bundles of longitudinal muscle fibers. It has many longitudinal elastic fibers, which are particularly numerous toward the media, where they are often grouped as the *external elastic membrane*. This is not a fenestrated membrane, but is merely a dense zone of longitudinal fibers.

[1] BENNINGHOFF, 1927.

It is said to be well developed in the carotid, brachial, femoral, cœliac and mesenteric arteries, and to be absent from the basilar and other cerebral arteries.

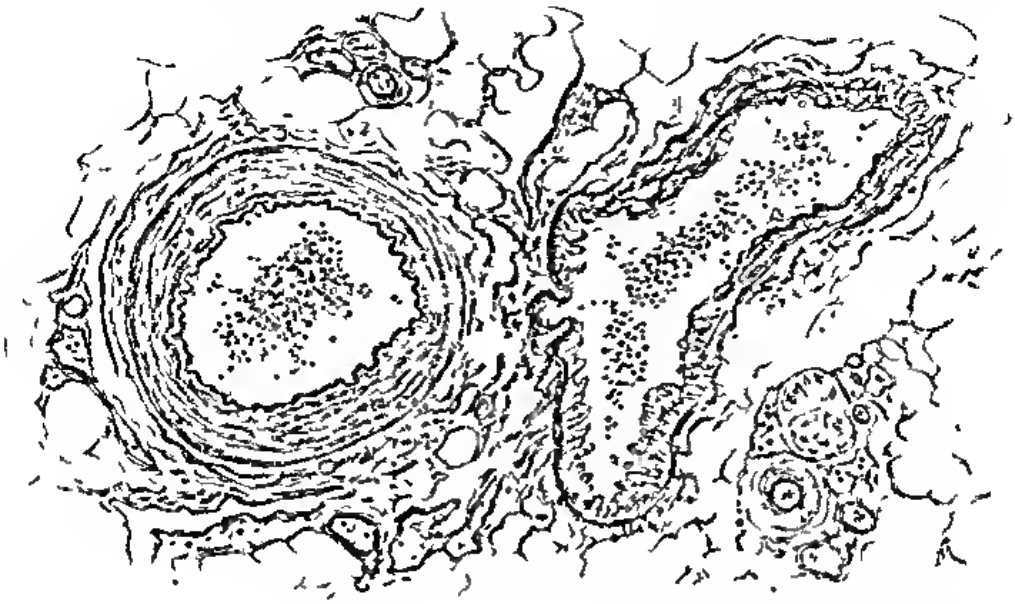


Fig. 200.—A Cross-section of a Small Artery and Vein in the Connective Tissue Surrounding the Human Ureter

Artery on the left, vein on the right. Zenker fixation, hæmatoxylin and eosin.

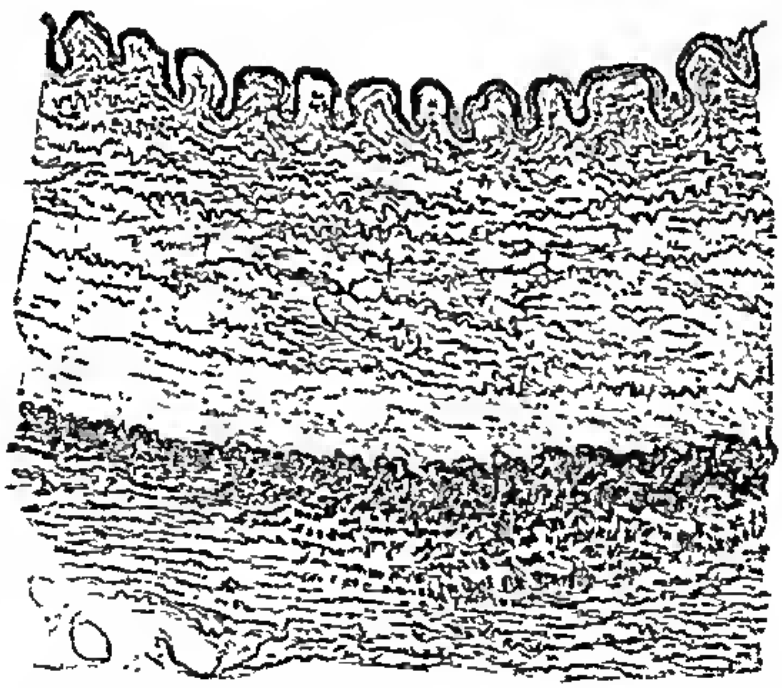


Fig. 201.—A Cross-section through the Radial Artery of a 30 Yr. Old Woman

Sublimate-acetic acid fixation, orcein. × 110. Elastic tissue dark, muscles unstained. (von Ebner.)

Nerves and vessels ramify in the externa.[1] The walls of the larger arteries are supplied with small blood vessels, the *vasa vasorum*, derived from adjacent arteries. These are distributed chiefly to the externa: they

[1] Clark, Clark and Williams, 1934.

may penetrate the outer part of the media but do not reach the intima. Lymphatic vessels form perivascular plexuses which have been described as penetrating the externa. There is some doubt whether all these vessels in the arterial wall are true lymphatic vessels lined with endothelium or unlined tissue spaces. True lymphatics have been observed in the walls of some of the larger blood vessels, as the aorta, and seen to form in the pig a definite plexsus. The subject is discussed by Iwanow, who calls these intra-vascular lymphatic vessels the *Vasa lymphatica vasorum sanguinorum.*[1] The nerves are medullated and non-medullated. They include vasomotor fibers which innervate the smooth muscle cells, and sensory or afferent nerves which have terminal arborizations in the intima and in the externa. Other nerve fibers end in lamellar corpuscles in the externa of the aorta and other large vessels.

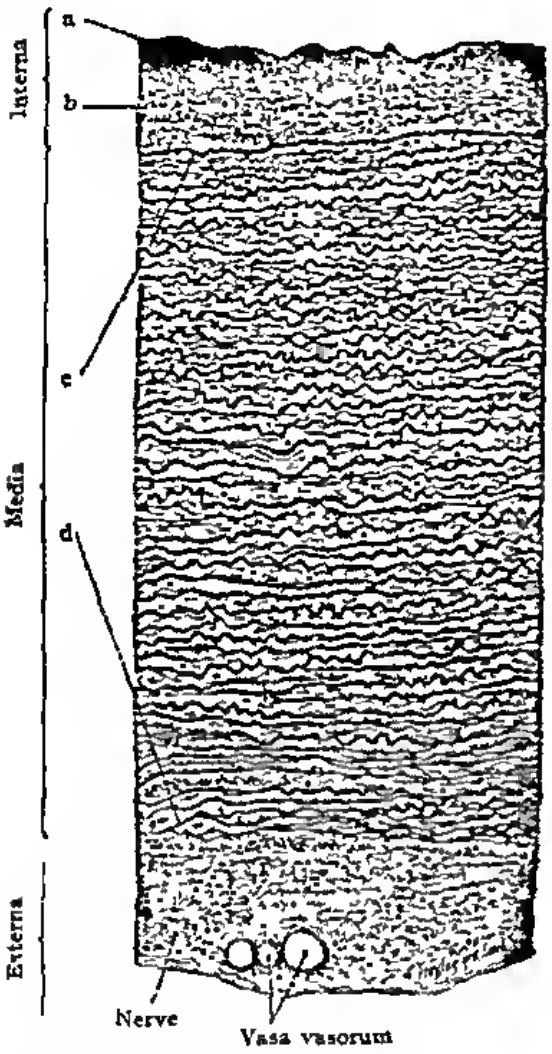


FIG. 202.—FROM A TRANSVERSE SECTION OF THE HUMAN THORACIC AORTA, STAINED WITH RESORCIN-FUCHSIN × 80

a, Endothelium, b, subendothelial fibrous tissue, c, d, elastic membranes of the media

Ganglia are not seen in the walls of the vessels, and the sympathetic fibers to the muscles therefore travel considerable distances to their terminations. In this respect the nerves to the smooth muscles of the vessels differ from those to the musculature of the digestive tube.

In the largest arteries (the aorta and pulmonary arteries) the intima is very broad (Fig. 202), and it increases in thickness with age. Its endothelial cells are less elongated than those of smaller arteries. They rest on a fibrous subendothelial tissue, containing flattened, stellate or rounded cells, and networks of elastic tissue. The elastic fibers are thicker toward the media, finally producing a fenestrated membrane which corresponds with the inner elastic membrane of smaller vessels, but which is scarcely thicker than adjacent elastic lamellæ. The broad media consists of elastic membranes and muscle fibers, but the elastic tissue greatly predominates. On section the wall of the fresh aorta consequently

[1] IWANOW, 1933.

appears yellow, and not reddish like the more muscular walls of smaller arteries. The elastic tissue is arranged in a succession of circular fenestrated membranes connected with one another by oblique fibers. Between them are the muscle cells. In the inner layers of the media, the muscle cells form an anastomosing syncytium of short, broad and flattened elements, somewhat resembling cardiac muscle, but in the outer layers the fibers are of the ordinary type. The externa contains no outer elastic layer and is relatively thin; its inner elastic portion may have been taken over into the media.

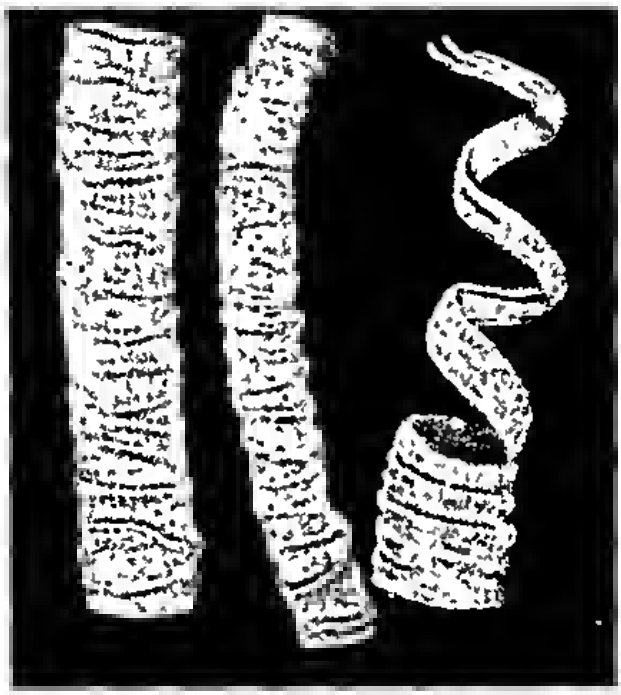


FIG. 203.—CAMERA LUCIDA DRAWING OF MACERATED BRANCHES OF ADULT HUMAN CEREBRAL ARTERIES FROM WHICH THE ADVENTITIA HAS BEEN STRIPPED

Scanty muscle replaced by transverted and longitudinal folding of the lamina elastica interna. × 16. (R. C. Strong—*courtesy of Wistar Institute.*)

SPECIAL ARTERIES. These include arteries in several parts of the body which have a structure dissimilar to that found in other vessels of the same size elsewhere. The cerebral arteries are characterized by having exceedingly thin walls for their size; in the fresh they look more like veins than arteries. The thinness of the arterial wall becomes significant when the question of rupture of vessels is taken into consideration. Triepel[1] suggested that the cerebral arteries differed from similar sized arteries elsewhere in the body in three features: in the specially well developed membrana elastica interna, in the slight development of elastic tissue among the circular smooth muscles of the media, and the striking decrease of elastic fibers in the externa. The internal elastic membrane decreases as the size of the vessel decreases and finally disappears in the precapillaries. In the average small cerebral artery there

[1] TRIEPEL, 1897.

is a predominance of collagenous fibers over both elastic and smooth muscle fibers. The smallest arteries seem to have only an endothelium surrounded with collagenous tissue as with age the media undergoes a fibrosis, a frequent hyalinization and more rarely a calcification.[1] The renal arteries show an extraordinary development in elastic tissue elements which terminate in the afferent artery of the glomerulus. Such an arrangement probably guards the kidney against any sudden rise in blood pressure and favors the discharge of blood into the efferent artery. The umbilical arteries are surrounded by longitudinal smooth muscle fibers outside the endothelium and circular muscle around them. The elastic tissue fibers are very few in number and lie mostly between the muscles.

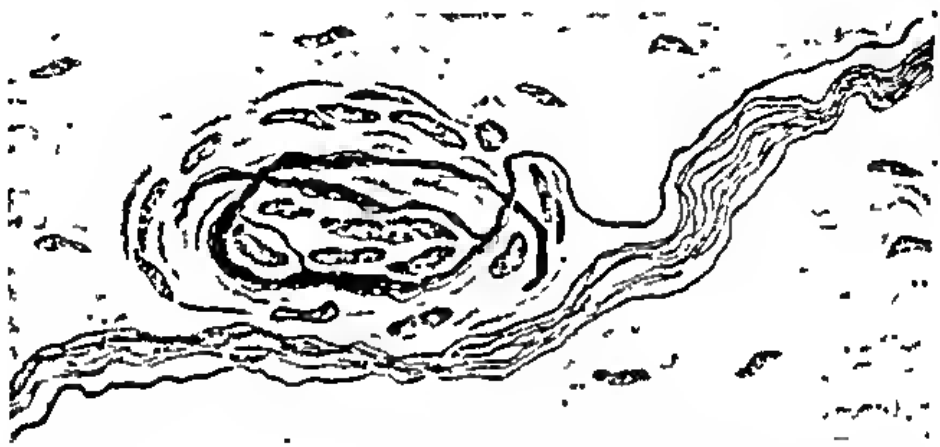


FIG. 204.—NERVE END-APPARATUS IN THE ADVENTITIA OF THE HUMAN FEMORAL ARTERY. A TYPE OF DOUBLE CORPUSCLE (Hirsch).

VEINS. Since the artery to any structure and the returning vein are often side by side, they are frequently included in a single section and may readily be compared. In embryos the veins are of much larger diameter than the corresponding arteries, and they have thinner walls. Although the difference in diameter is less marked in the adult, it generally remains a distinctive feature, and the difference in the thickness of the walls becomes accentuated. In comparing the diameters of the ulnar vein and artery in Fig. 199, it should be remembered that the ulnar artery is usually accompanied by two returning veins, only one of which is shown in the figure. Because of their thinner walls, which contain relatively little elastic tissue, the veins are generally partly collapsed; the lumen is therefore irregular, whereas that of the arteries tends to be round. Small veins full of blood may be round, however, and the arteries are sometimes irregularly contracted.

The walls of the veins, like those of arteries, are composed of three layers, the intima, media, and externa. The intima includes the primary

[1] BAKER, 1937.

endothelium, which is composed of polygonal cells, generally shorter and broader than those of arteries. The endothelium rests on a thin layer of subendothelial fibrous tissue. The inner elastic membrane of arteries is represented in the smaller veins by a thin homogeneous membrane, but in larger veins it is replaced by a network of elastic fibers. In

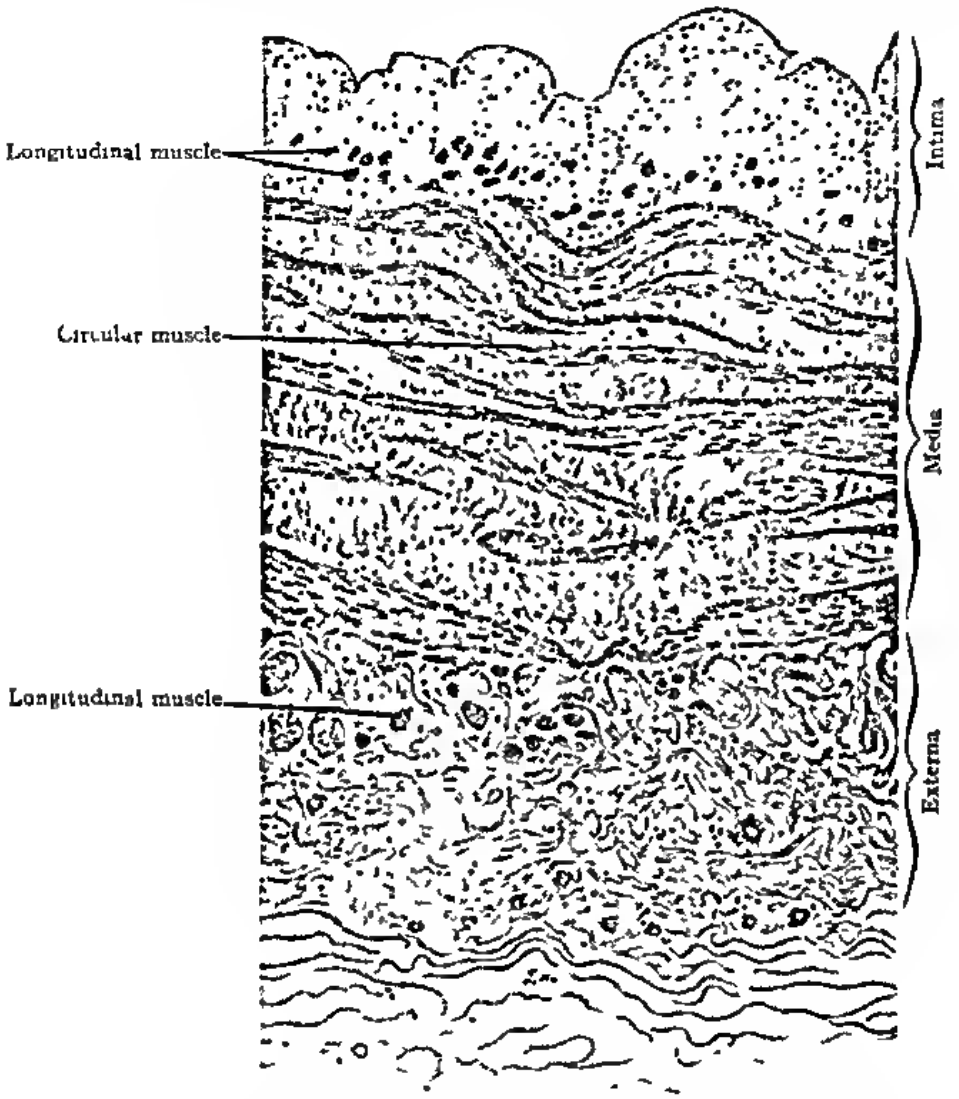


Fig. 205.—A Portion of a Cross Section of the Human Brachial Vein. × 100. Absolute alcohol fixation. Hansen's hæmatoxylin. (von Möllendorff)

addition to these structures the intima of certain veins contains scattered oblique and longitudinal muscle fibers; they are said to occur in the iliac, femoral, saphenous and intestinal veins, the intramuscular part of the uterine veins, and especially in the dorsal vein of the penis near the suspensory ligament.

The media shows great variation. It is generally a thin layer consisting of circular muscle fibers, elastic networks and relatively abundant connective tissue, and is best developed in the veins of the lower extremity (especially the popliteal). In those of the upper extremity it is

not so well marked, and it is still thinner in most of the larger veins of the abdominal cavity; it is reduced to fibrous tissue and is essentially absent from the vena cava superior, the veins of the retina, of the pia and dura mater, and of the bones.[1] In the portal vein, on the other hand, the muscular wall is very powerful, since it must collect blood from capillaries and force it through the liver sinusoids.

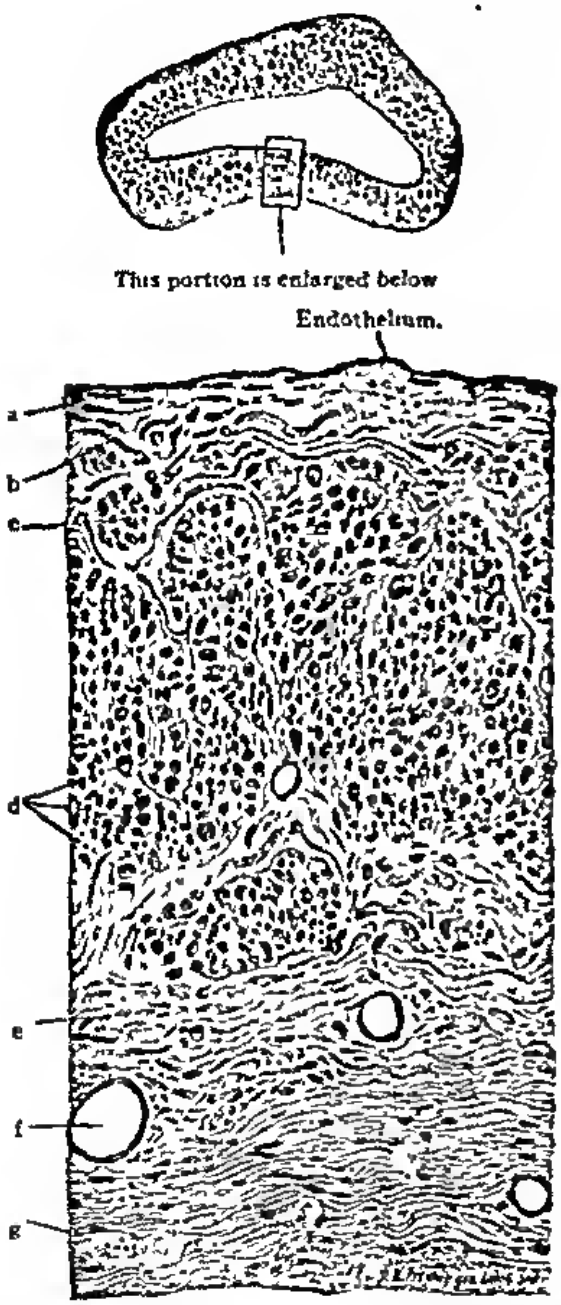


FIG. 206.—FROM A CROSS SECTION OF A HUMAN SUPRARENAL VEIN, STAINED WITH HÆMATOXYLIN. X 240

a, Circular muscle fibers of the media, b, connective tissue, c, d, longitudinal muscle fibers of the externa; e, connective tissue, f, small vein, g, fat cell

The externa is the most highly developed layer of the veins. It consists of interwoven bundles of connective tissue, elastic fibers, and longitudinal bundles of smooth muscles which are more abundant than in the arteries. In certain veins (*e.g.*, the main trunk of the portal, the renal and suprarenal veins)[2] the longitudinal muscle forms an almost complete layer of considerable thickness (Fig. 206).

The valves of veins originate as paired more or less transverse ridges of endothelium which become invaded by mesenchyma. Fig. 207 depicts five phases in the formation of a venous valve. The first valves arise in the human embryo at about 3.5 months, those in the veins of the upper extremity appearing a little earlier than in the lower.[3] Each valvular cusp is shaped like the half of a cup attached to the wall of the vein so that its convex surface is toward the lumen. The valves are generally found just distal to the point where a tributary empties into the vein and they prevent its blood from flowing away from the

[1] BOUIN, *1929*.

[2] See BARGMANN, W., 1933 Zeitschr. f. Zellf. u. mikr. Anat. Bd. 17, for a discussion of the structure of the suprarenal vein in man and different species of mammals.

[3] KAMPMEIER AND BIRCH, 1927.

heart. They are most numerous in the veins subject to external pressure and muscular movement as in the veins of the extremities, but appear also in the intercostal, azygos, spermatic and certain other veins; none are found in the vertical trunks of the superior and inferior venæ cavæ, or in the cerebral veins and in veins of the bone marrow. Peculiar funnel-shaped valves are described by Kiss in the veins of the penis.[1] According to Edwards, in the veins of the extremities, the vein at the site of a valve is elliptical in cross-section, the major axis being parallel to the skin or

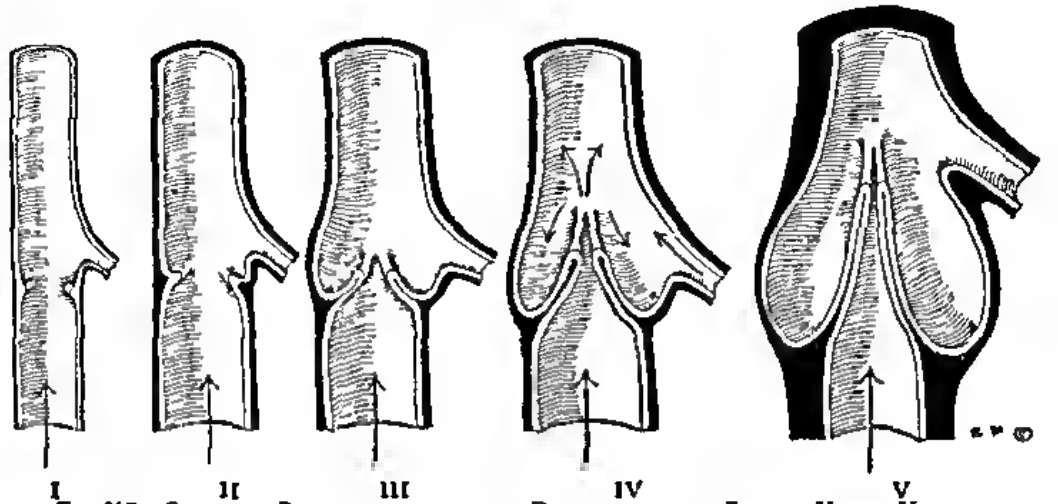


FIG 207.—SCHEMATIC REPRESENTATIONS OF THE DEVELOPMENT OF A BICUSPID VENOUS VALVE.
Stages I to IV, from 3 5 to 5 months old human embryo Stage V, at term Endothelial layer, white, mesenchyma layer, black (*Redrawn, slightly modified after Kampmeier and Birch—courtesy of the Wistar Institute*)

its tangent. The two cusps arise from the long curves of the ellipse so the aperture between their free margins is parallel to the overlying skin. The advantage of this arrangement is that the compression transmitted to the veins by the overlying structures produce secure apposition of the cusps to each other and thereby ensure the competency of the valve. He thinks that a similar relationship presumably exists between the venous valves and adjacent surface planes in the interior of the body.[2] The endothelial cells on the surface of the valve toward the lumen of the vein are elongated parallel with the blood current and beneath them there is a thick network of elastic tissue. On the side of the valve toward the wall of the vein, the long axis of the cells is transverse and there the cells rest upon fibrous connective tissue. Smooth muscle cells are found in the valves of the larger veins, the axis of the fibers being parallel to the axis of the cusp. Under normal conditions the valves contain no blood vessels, but sprouts from vessels in the walls of an inflamed vein may invade them.

Veins are well supplied with nutrient vessels or vasa vasorum and are easier to see than in arteries, because of the looseness of the venous

[1] KISS, 1921. [2] EDWARDS, E. A. 1936.

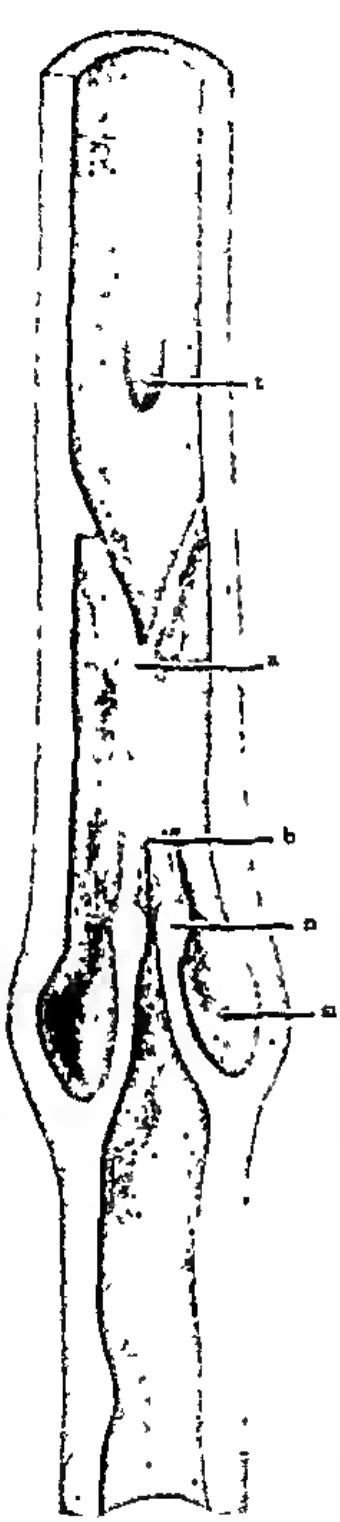


FIG. 208.—RECONSTRUCTION OF SEGMENT OF THE VENA SAPHENA MAGNA OF A 186 MM (5 5 MONTHS) FETUS × 100

Shows below a perfect bicuspid valve, b; immediately above (interval, 150μ) a vestigial one, a, tributary, t, sinus, si, nodular thickening of cusps, n (Redrawn, slightly modified *after Kampmeier and Birch*—courtesy of the Wistar Institute)

wall. These nutrient vessels may even reach the intima. Lymphatic vessels are found in the adventitia of the larger veins and in some vessels may form extensive plexuses. For a more detailed account on veins see—K. J. Franklin's 'A monograph on veins,' 1937.

The prevalence of elastic tissue in the arteries, especially those nearest the heart, provides a mechanism for guarding the thin-walled capillaries from the force of the heart beat. Water pumped through a thin rubber tube will emerge in a constant stream, but through a rigid pipe it will come out in separate jets. Thus when the arteries are hardened by disease the pulse can be seen beneath the finger nails. The musculature of the arteries serves to regulate the caliber, and govern the amount of blood to the different parts of the body.

The longitudinal muscles of certain veins may act as efficaciously as circular muscles to force the blood toward the heart, if valves are present. The volume between valves is reduced by the contraction of either set of muscles, or by pressure from surrounding structures. The latter applies especially to the thin-walled lymphatic vessels, and indicates the advantage of exercise or massage.

ARTERIO-VENOUS ANASTOMOSES. In different parts of the body many arterioles connect directly with venules instead of through a network of capillaries. These connections are called *arterio-venous anastomoses*. As an arteriole passes to a venule, the subendothelial elastic tissue disappears and the endothelium lies close upon a modified musculature. The smooth muscle cells become shorter and wider and take on an 'epithelioid' appearance, looking more like polyhedral epithelial cells than muscle cells. The nucleus of these cells is round in section and does not stain very well; the cytoplasm is pale and rather homogeneous appearing. The cells lie within a delicate connecting tissue network and the whole of the vessel has a thickened adventitia of loose collagenous connective tissue. Arterio-venous anastomoses may be straight or coiled and look more like an arteriole with a thickened wall than a venule. They were observed originally on the more exposed parts of the body as the fingers, toes, nail-

bed, lips, eyelids, tip of the ear and tongue, but since have been found in the deeper tissues. They were first considered a 'warming mechanism' ensuring an adequate supply of blood to those parts exposed to cold. Schumacher[1] regarded the coccygeal body in man and the glomeruli

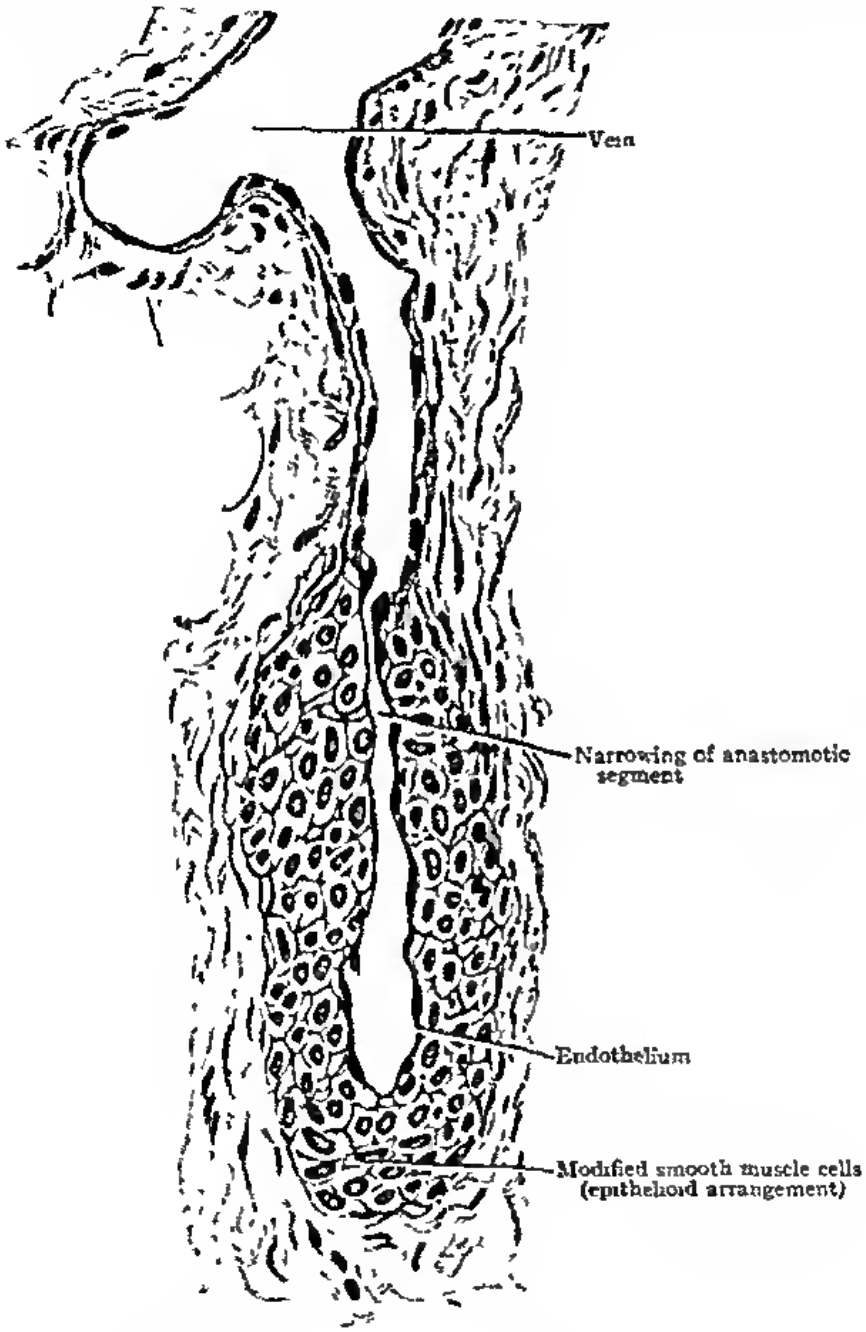


FIG. 209.—ARTERIO-VENOUS ANASTOMOSIS. EXTERNAL EAR OF A RABBIT. × 400. *Zenker fixation, haematoxylin and orcein.* (Tischendorf.)

caudales in animals, as arterio-venous anastomoses. He held that these anastomoses were blood pressure regulators and that temperature regulation was secondary. Benninghoff[2] has suggested that in capillary areas, in which there is little need at the time for blood, the vessels may collapse

[1] SCHUMACHER, 1907. [2] BENNINGHOFF, 1930.

in part and an arterio-venous anastomosis would then protect the capillaries by increasing the pressure and preventing stasis. The formation and functioning of these anastomoses have been observed by Clark and Clark[1] in the living animal. Through transparent chambers inserted into the ear of a rabbit, they watched for months the growth of vessels and anastomoses. They found them numerous (25–55 in 1.6 sq. cm.) and the most contractile vessels in the ear.

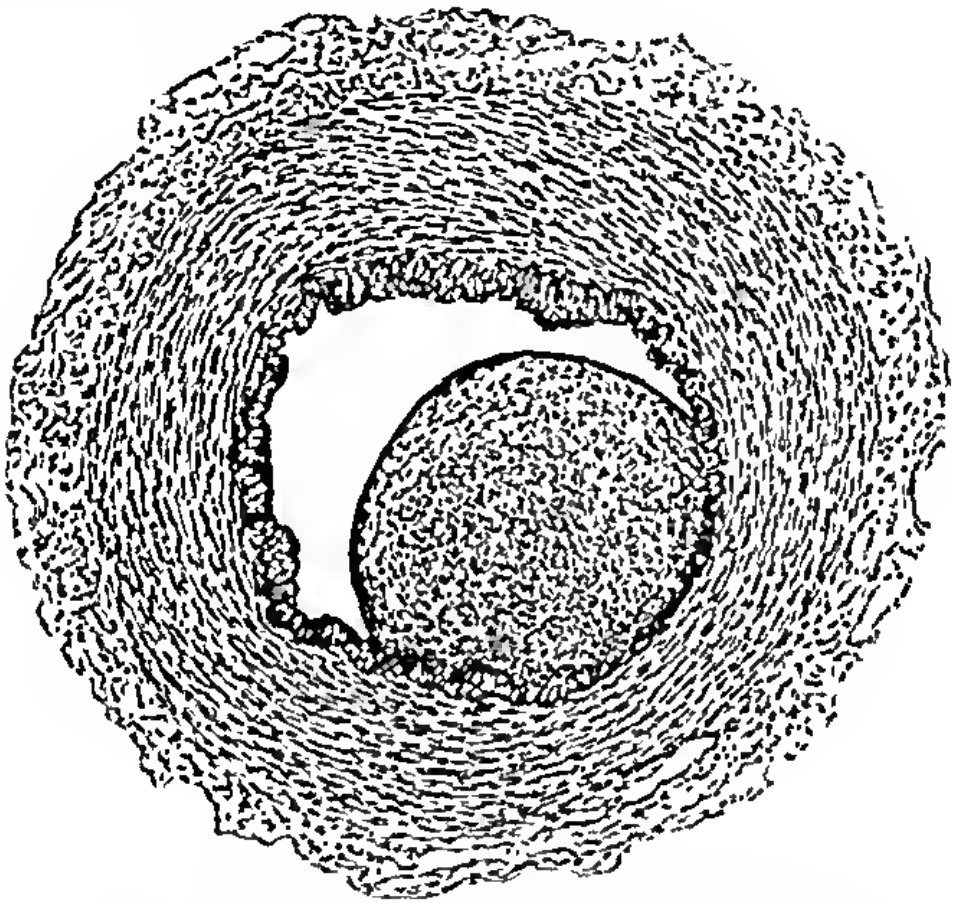


Fig. 210.—Intima Cushion in a Cross Section of the Deep Artery of the Penis. Human. Sublimate formaldehyde, acetic acid fixation, Held's molybdenum haematoxylin. × 80. (Stieve.)

Besides the arterio-venous anastomoses some arteries, as the umbilical, thyroid, bronchial, renal, prostate and penis arteries contain *longitudinal* thickenings of intima or *intima cushions* which act like a valve on the contraction of the vessels. In cross-section these cushions are seen projecting crescent-like from one side of the vessel into the lumen. Such an arrangement occurs in certain veins also and functions for local control of blood flow and pressure.

Carotid Body. The *carotid body* (Glomus caroticum) is a small reddish structure about 5–7 mm. long, 2.5 mm. broad and 1.5 mm. thick, situated close to the bifurcation of the common corotid artery. It is composed of irregular masses of pale-staining, polygonal, epithelioid

[1] Clark and Clark, 1934 and 1938.

cells, resting against the endothelium of lacunar blood vessels. Numerous medullated and non-medullated nerve fibers, derived mainly from the glossopharyngeal nerves and from the superior cervical ganglion of the sympathetic enter the body and after branching end on the epithelioid cells. A few ganglion cells may be present. It was formerly considered to be one of the paraganglia, but the cells do not show the chromaffin reaction, nor are there any evidences of secretory granules in them. Extracts of the gland do not give the reaction for epinephrin. Smith[1] states that 'there is no evidence to warrant the inclusion of the carotid

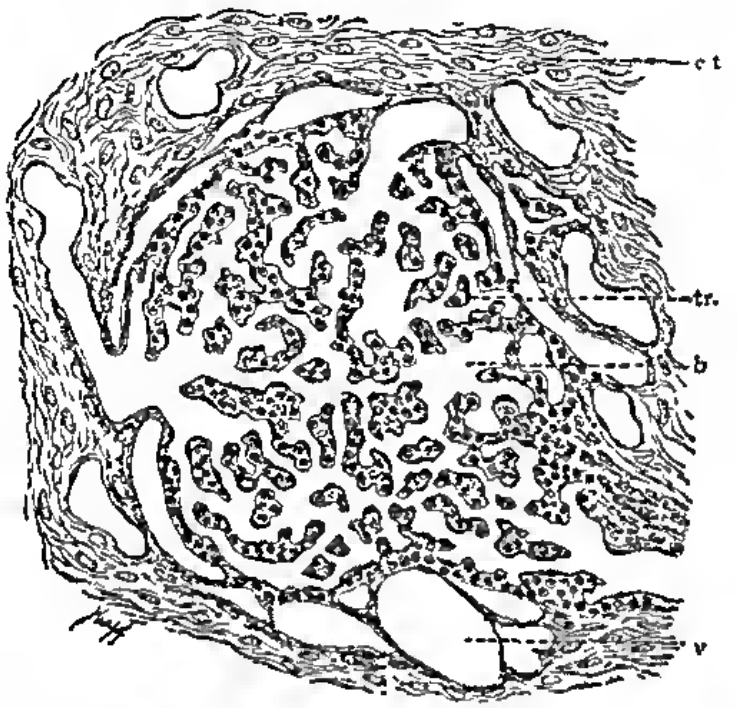


FIG. 211.—SECTION OF A PART OF THE GLOMUS CAROTICUM OF MAN (Schaper)
b, Sinusoid, v, efferent vein, tr., trabecula, c.t., connective tissue septum

body in the endocrine system.' It is now regarded as a special sensory organ or 'chemoreceptor' for the internal carotid artery. At the commencement of the artery there is a bulbar swelling called the *carotid sinus*. DeCastro[2] describes the nerve endings in the walls of the sinus as of two types—one diffuse arborizations, the other more circumscribed and terminating in reticular, plate-like endings or menisci. The region of maximal innervation is at the greatest bulge.

AORTIC BODY. The *aortic body* (Glomus aorticum) is less circumscribed and subject to more variations in different species than the carotid body. It consists of irregular masses of epithelioid cells extending from the arch of the aorta to the base of the heart, in the interval between the ascending aorta and the pulmonary artery. The nerves are derived from the vagus and sympathetic, the fibers after branching repeatedly end on the surface of the epithelioid cells in reticulated swellings and clubs,

[1] SMITH, C., 1924. [2] DECASTRO, 1928.

and minute rings. Nonidez[1] says there are no essential differences in the structure and innervation of the carotid and aortic bodies and suggests that the aortic bodies are chemoreceptors for the pulmonary artery.

The Coccygeal Body. The *coccygeal body* (Glomus coccygeum) is situated in man ventral to the tip of the coccyx and is homologous to the glomeruli caudales of the tailed mammals. It is a small rounded mass

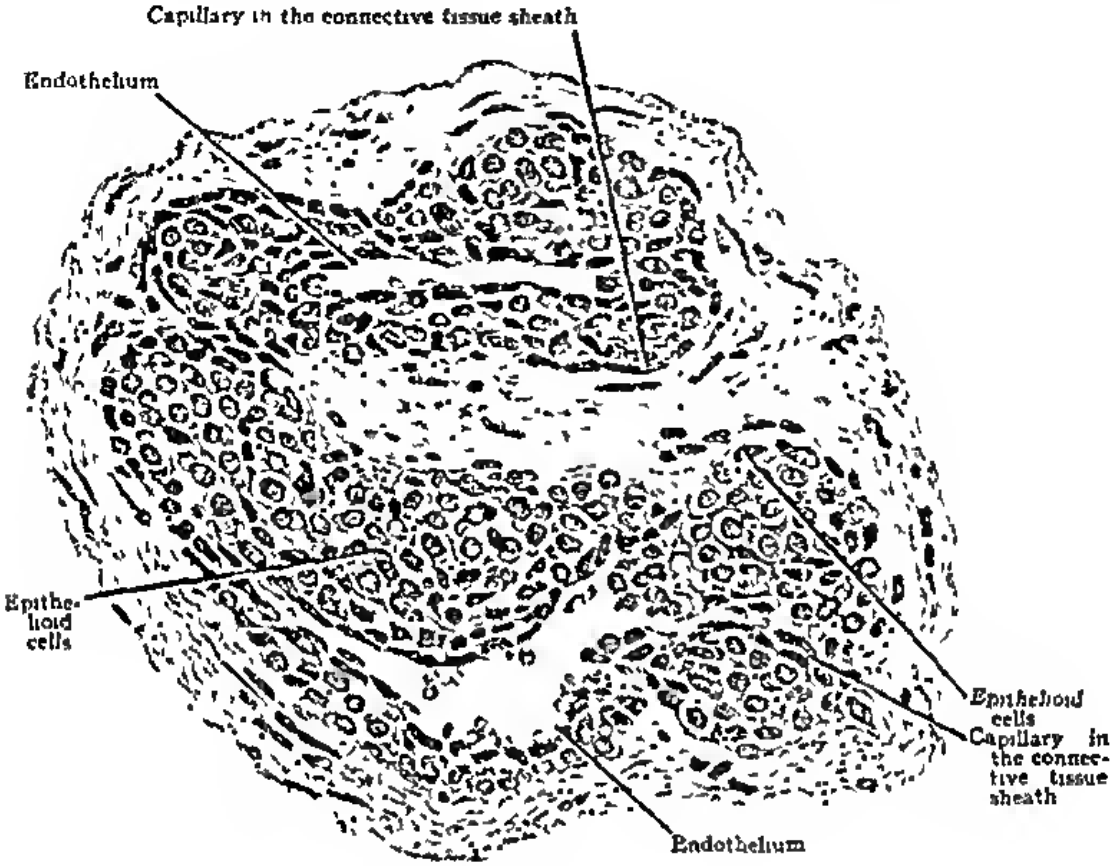


Fig. 212.—Section of a Coccygeal Body (Glomus Coccygeum). Human (Walker)

about 2.5 mm. in diameter and consists of tortuous blood vessels which can be injected from the middle sacral artery. The vessels lack a membrana elastica interna and the endothelium lies close to a thick epithelioid layer of polygonal cells. Between the vessels, which are sometimes open and at other times closed there is a connective tissue stroma which is said to thicken with age.[2] The epithelioid cells are clear, pale-staining and do not show the chromaffin reaction.[3] Schumacher[4] considers them as modified smooth muscle cells.

THE HEART

Development (based on the chick embryo). The heart, like most of the other early blood vessels, develops by the coalescence of endothelial-

[1] Nonidez, 1936. [2] Walker, 1904. [3] Stoerk, 1906.
[4] Schumacher, 1938.

lined cavities situated between entoderm and mesoderm. The area occupied by vessels is thus limited at first by the extent of the mesoderm, which spreads from the primitive knot in all directions (p. 58). Toward the head it grows forward on either side of the axial structures (forming the somites), and thence spreads laterally, splitting into two sheets to enclose the cœlom. The mesoderm enters the head process while the latter is still flat, grows forward on each side, and spreads across the median line in front of the medullary groove. In this position also it splits into somatopleure and splanchnopleure, enclosing a median portion of the cœlom, connecting the two lateral halves of this cavity. This connection is limited anteriorly by the proamnion, a median area not invaded by the mesoderm. Blood vessels may occur, therefore, between the entoderm and the somites, and in the splanchnopleure laterally and in front of the medullary groove (Fig. 213, A).

With the further growth of the axial structures the head process projects forward above the general surface, and the layers immediately in front of the medullary groove are rolled under to form the floor of the fore-gut and the ventral part of the head. Diagrams B and C show this result. The entoderm and the median cœlom are rolled forward as though attached at the points 'a' and 'b.' The median somatopleure remains relatively small, while the splanchnopleure is stretched and the part nearest the head is inverted. Throughout the lateral splanchnopleure the vessels rest on the entoderm and are covered by bulging mesoderm, seeming to push up into the cœlom. In this inverted splanchnopleure they seem to hang down from the floor of the fore-gut into the cœlom beneath. Here, where the right and left vascular systems can fuse, the heart develops. It connects at its present caudal end with the right and left vitelline veins, and is continued forward by the two aortæ. The aortæ, in their position next the entoderm, run forward below the fore-gut (ventral aortæ), turn up around the tip (first aortic arches), and continue caudally dorsal to the fore-gut (dorsal aortæ), as is shown in D. The heart constitutes the fused portion of this right and left system which lies within the median cœlom.

The diagrams (Fig. 213) are purposely exaggerated in that they show a well-developed vascular net in the embryo before the projection of the head process, whereas actually the splanchnopleure undergoes its folding and inversion while vasculogenesis is still going on. Also, in the chick, on which the diagrams are based, the forward projection of the head process plays the greater part in the inversion; in mammals, however, this process is accompanied by a rolling in of the lateral splanchnopleure, as is suggested by F and G. In some mammals two lateral hearts are temporarily present, though they soon fuse to form the definitive heart; in all forms it is common to find transitory median septa indicating the fusion of lateral halves.

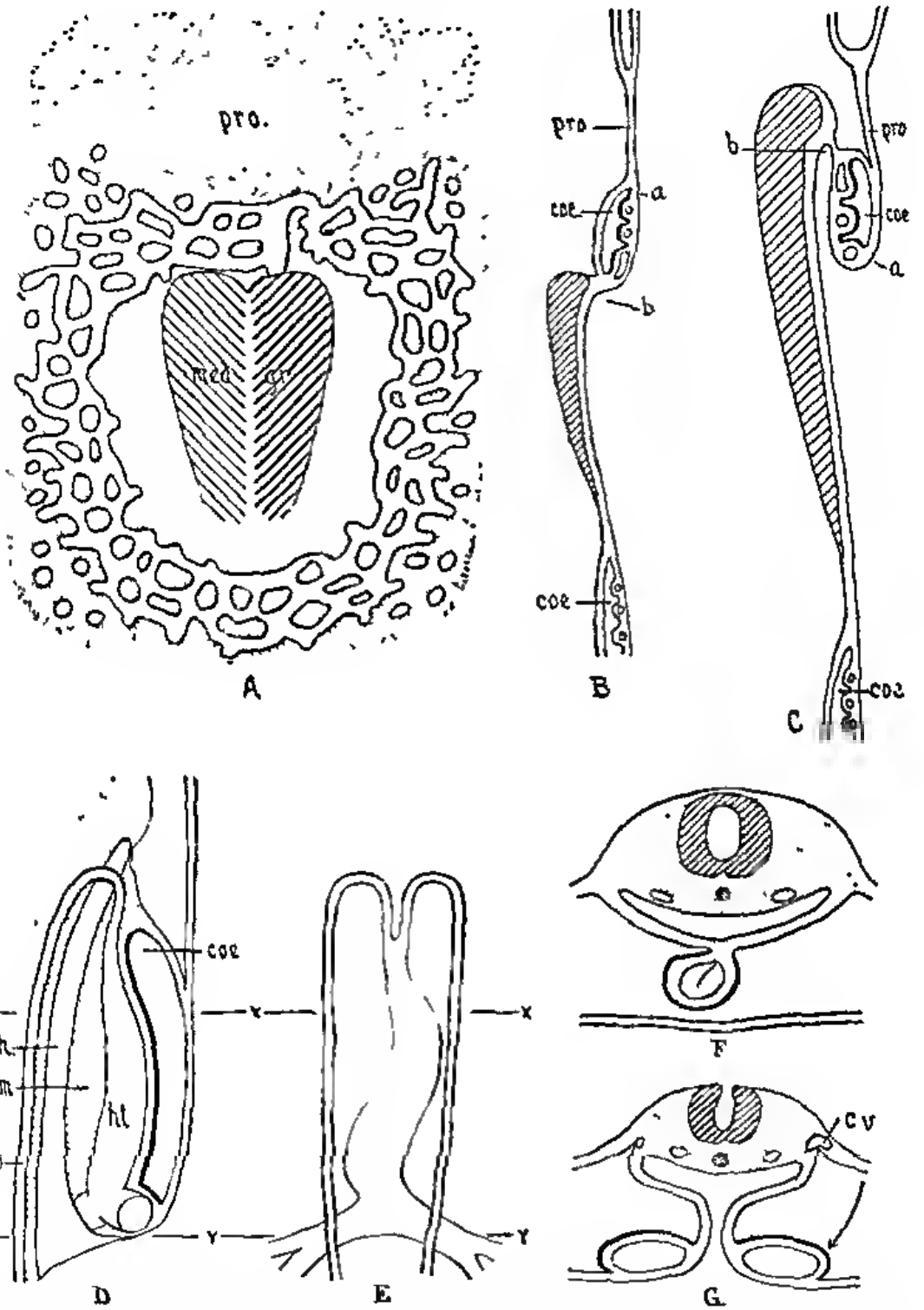


FIG 213.—DIAGRAMS OF THE DEVELOPMENT OF THE HEART

A *dorsal view of hypothetical embryo before the head is protruded forward* The *mesoderm (stippled) extends* all around the medullary groove, **med gr**, except at the proathmion, **pro** The blood islands and blood vessels lie in the splanchnopleure, and can thus extend across the median line in front of the medullary groove B sagittal section of same embryo, showing vessels between entoderm and splanchnic mesoderm C, older embryo, showing how the growth of the medullary tube forms the fore-gut and inverts the splanchnic layer of the anterior median cœlom, **coe**, with its vessels **a** and **b** are comparable points in the two drawings D sagittal view of older embryo (chick of 10 segments) showing tubular heart **ht**, aorta, **ao**, and dorsal mesocardium, **d m** E, dorsal view of endothelial heart and aortæ of same embryo **F and G**, transverse sections at levels **x-x** and **y-y** respectively The endothelial heart is covered by thickened splanchnic mesoderm, the myoepicardium **c.v**, anterior cardinal vein

With the further growth of the embryo a second system of veins develops in the body and body-walls or somatopleure. These veins, the paired *cardinal system*, drain the body branches of the aortæ, and consist of the anterior and posterior cardinal veins on each side (from the head and tail regions respectively) which unite in the common cardinal veins or ducts of Cuvier. These latter lie in the somatopleure, and in order to enter the venous end of the heart which lies in the splanchnopleure they

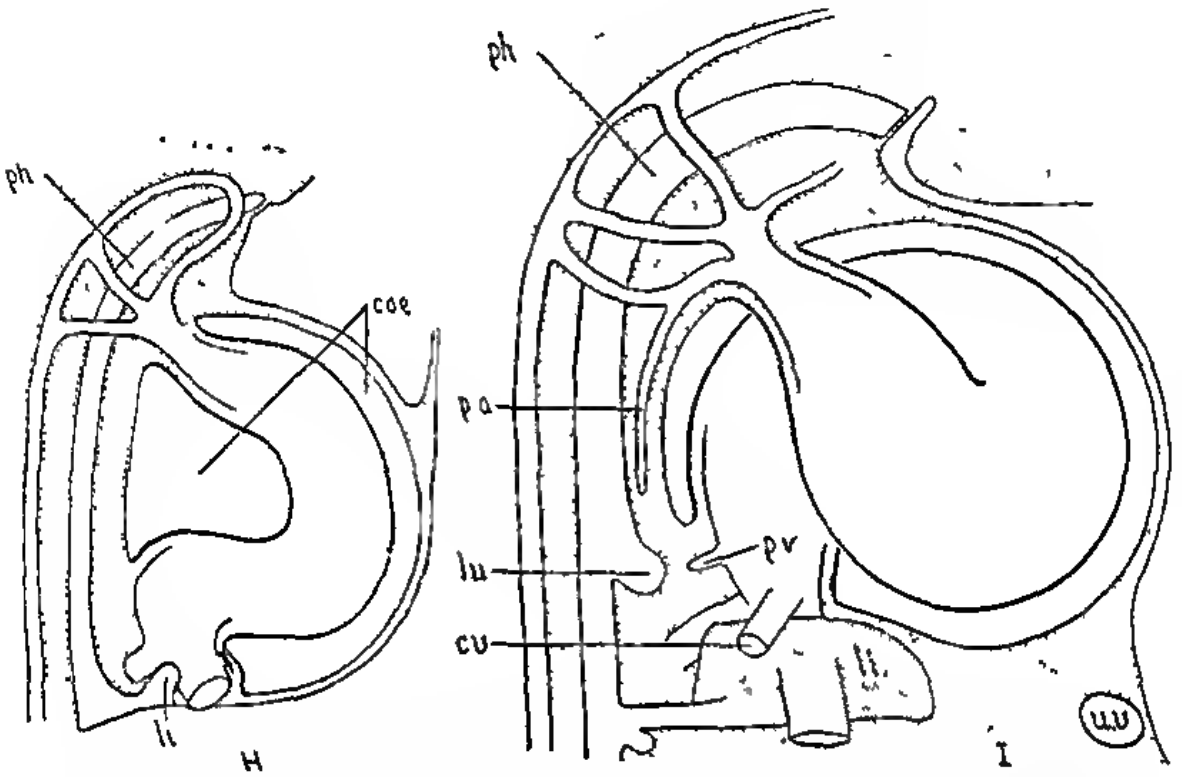


Fig. 214

II, sagittal view of embryo with three aortic arches and heart bent in sagittal plane. The dorsal mesocardium is ruptured. li., liver diverticulum. I, embryo with III, IV (aortic) and pulmonary arches. The heart has assumed a loop form. lu, lung bud, toward which grow the pulmonary artery and vein, p. a and p v, the latter from the endothelial wall of the atrium through the remains of the dorsal mesocardium. c v, common cardinal vein, u v, umbilical vein.

must cross the cœlom. This is accomplished by the rolling in of the body-walls in the direction of the arrow in *Fig. 213, G*, and the increase in size of the vitelline veins, until the somatic mesoderm and that covering the veins meet and fuse. Across this bridge, which forms part of the adult diaphragm, the cardinal veins open into the caudal end of the heart.

A third system of veins, the *allantoic* or *umbilical*, runs from the placenta in the somatopleure of the umbilical cord and joins the common cardinal veins. In human embryos they develop before the vitelline veins.

The heart now consists of an endothelial tube almost completely covered by a reflexion of the splanchnic mesoderm, by which it is attached to the floor of the fore-gut. This attachment is the dorsal meso-

cardium. The heart grows rapidly, chiefly in length, within the pericardial cavity, as this median portion of the cœlom may now be called, and the cavity does not share in this growth. The heart therefore bends, at first sinuously, to the left caudally, to the right cranially (E); later it is forced into a deep sagittal curve (Fig. 214, H). This stretches and finally ruptures the mesocardium, and the heart becomes a double-walled tube (endothelium covered by splanchnic mesoderm), attached at its two ends, but otherwise free in the pericardial cavity. The outer heart layer is called the myoepicardium, since from it are formed the musculature (myocardium) and covering layer (epicardium) of the heart.

FIG. 215.—COMPOSITE DRAWING FROM SEVEN FRAMES OF MOTION PICTURE FILM OF LIVING CHICK'S HEART, TO SHOW SUCCESSIVE POSITIONS OF TWO BLOOD STREAMS.

Continued lengthening of the heart tube causes the deep curve to change to a loop-form (I). Similar changes can be followed by bringing the two ends of a flexible tube closer together, thus imitating a relative growth of the tube within a confined space. In the loop form the heart tube is forced to twist spirally; if the aortic limb passes to the right of the venous limb, as in I, the result is a dextral spiral. This position is normal, but certain anomalies of the heart show that the loop may occasionally be formed on the other side, with a sinistral torsion.

So far the heart is a single tube, without valves, which forces the blood toward the head by peristaltic action, though there are as yet no definite muscle fibers and no connection with the nerves. For convenience it has been divided into six areas: the sinus venosus, at the entrance of the veins; next, the atrial region, where the first bend occurs;

the atrio-ventricular canal; the ventricular region at the bottom of the loop; the bulbus, and the conus, continuing in order to the ventral aorta.

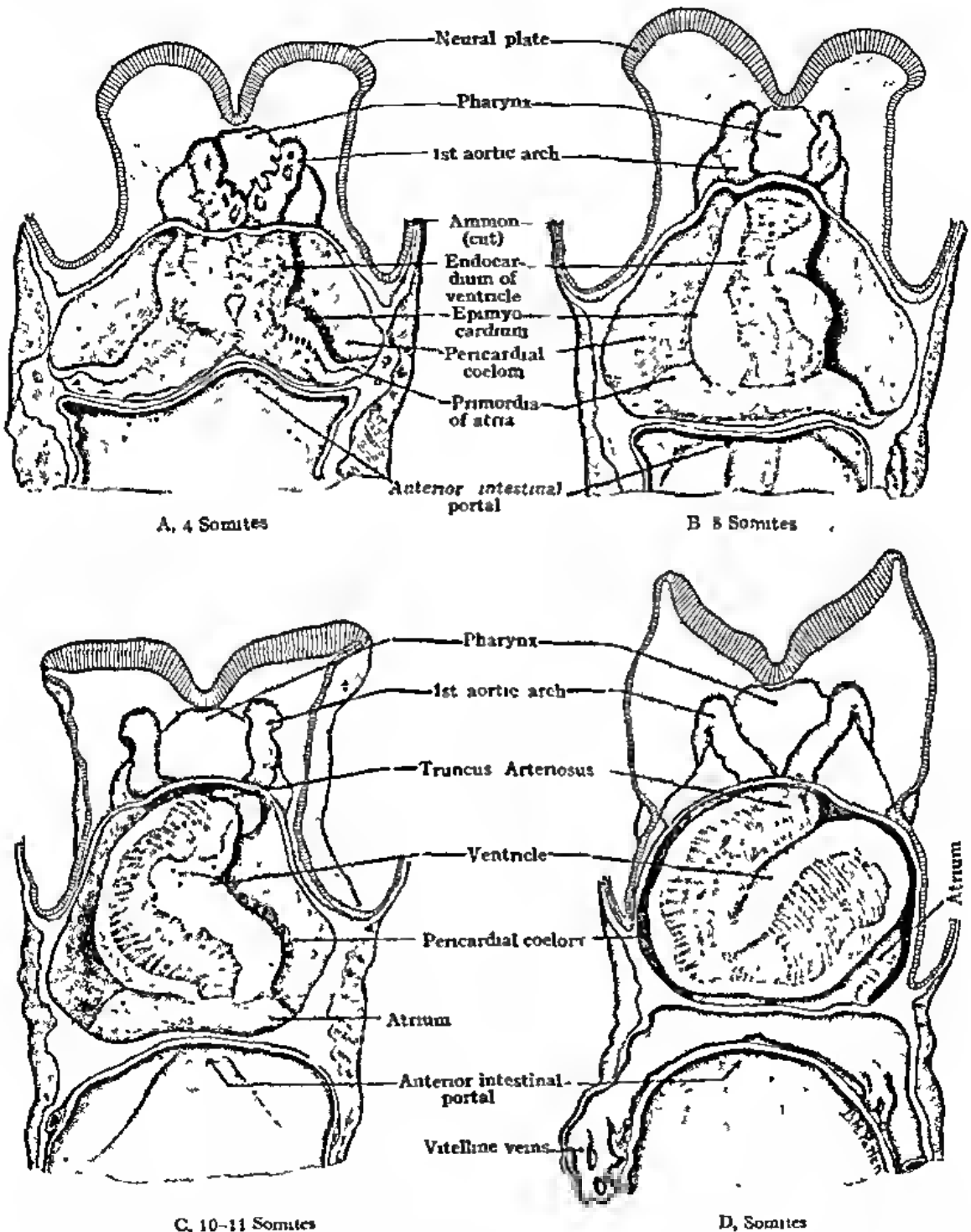


Fig. 216.—Four Stages in the Formation of the Heart. The Pericardial Chamber Has Been Opened by Ventral Dissection and the Myocardium Is Represented as Semitransparent so the Endocardium Shows Through. (Morris "Human Anatomy," from Patten's redrawing of reconstructions by Davis.)

Valves later develop[1] between the sinus and the atrial region (temporarily), in the atrio-ventricular canal, and in the bulbus or conus. Such a single heart is found in the adult fish, where the blood receives oxygen

[1] Mall, 1912.

from the gills on its way to the body; but with the development of the lungs in air-breathing vertebrates a separation of the pulmonary from the systemic circulation becomes necessary. This is accomplished in part by the development of paired longitudinal ridges bulging into the lumen of the tube and finally meeting, thus dividing the single endothelial tube into two channels. The endothelium initiates this ridge growth by an increased multiplication of cells, and only much later does the mesoderm participate to form muscular walls for the new channels within the heart or coats for fully separated vessels. For this reason the earlier stages of the process can best be studied from casts of the cavities of embryonic

FIG. 217.—SECTION THROUGH THE HEART OF A 6.7 MM. HUMAN EMBRYO. × 75. (BREMER.)

hearts.[1] The process is complicated, the result of the action of hydrodynamic forces, but can be briefly stated as follows.

Blood enters the heart by the two vitelline veins; at first these run in the same plane from right and left, but as the heart coils tightly the dorsal wall of the ventricle lifts the bulging right side of the atrium so that the right vein entering it points slightly upward or dorsally, while the left vein mouth points correspondingly downward. The two streams thus become separated and can take individual courses through the heart.[2] Since the atrio-ventricular canal has been moved to the left by the coiling of the heart, the right venous stream is directed toward it through the middle of the atrial cavity. The left stream, flowing across the floor of the atrium, meets the rounded right wall of this cavity and is deflected back along the roof to the left wall and thence down the

[1] BREMER, 1928. [2] BREMER, 1932a.

left wall of the atrio-ventricular canal. The *left stream* thus forms a spiral about the right (Fig. 215), and the two enter the ventricular portion of the heart side by side. The force requisite to cause this spiral course comes from the vigorous beats of the right and left walls of the atrium, which act successively because morphologically the right wall is the more caudal (Fig. 213, E) and therefore feels the peristaltic wave first.

The ridges which divide the heart longitudinally develop apparently between the two streams. In the atrio-ventricular canal are formed two broad ridges, the endocardial cushions, which divide the tube into two lateral halves, one for each stream. In the bulbar region two linear bulbar ridges, commencing as lateral indentations in the notch between the fourth aortic arches and the pulmonary arches (Fig. 214, I), extend caudally and split the common aorta into a ventral tube connecting with the third and fourth aortic arches (systemic) and a dorsal tube continuing into the pulmonary arches and pulmonary arteries. As they grow caudally along the heart tube, the ridges follow the dextral spiral, as is shown by the adult relations of the aorta and pulmonary artery. When they reach the atrio-ventricular canal they are thus rotated to a dorso-ventral position on the tube, and hence meet accurately the similarly oriented endocardial cushions, with which they fuse. The right stream is thereafter conducted spirally to the pulmonary arches, and the left to the systemic aorta. The two ventricles are irregular enlargements of the two channels, and, though morphologically dorsal and ventral, appear as right and left cavities because of the torsion of the ridges.

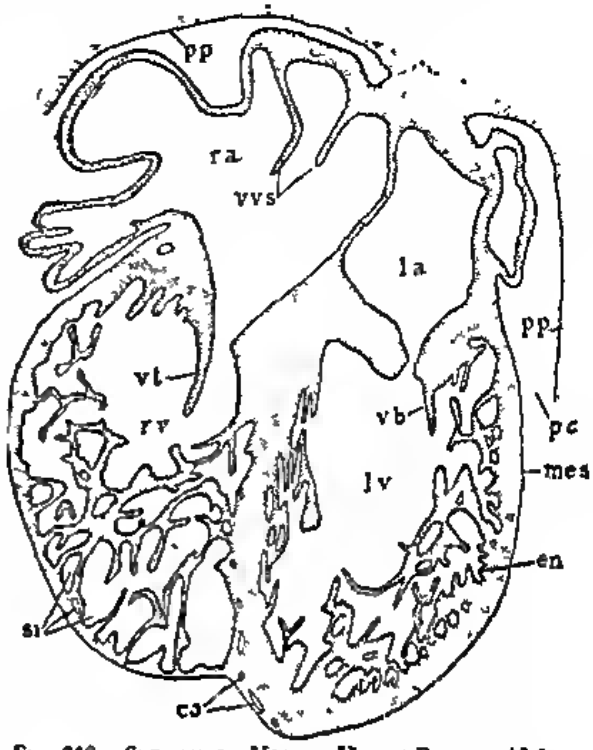


FIG. 218.—SECTION OF HEART, HUMAN EMBRYO, 13.6 MM. ca, Capillaries, en, endothelium; l. a, left atrium, l. v, left ventricle, mes, mesothelium (of the epicardium, or visceral pericardium), p. c. pericardinal cavity, p. p, parietal pericardium, r. a, right atrium, r. v, right ventricle, si, sinusoids, v. b, bicuspid valve, v. t, tricuspid valve, v. v. s, valves of the venous sinus.

The division of the common atrial cavity into the right and left atria is accomplished by the growth of the curtain-like interatrial septum, which forms in the sagittal plane to the left of the entrance of both the vitelline veins. With the lengthening of the fore-gut and the growth of the liver between the caudal end of the heart and the anterior intestinal portal, these veins are carried backward and are directed cranially as

they approach the heart, instead of mesially from left and right, as formerly. The conditions causing the atrial spiral of their streams no longer exist, but the initiation of the ridge formation in the atrio-ventricular canal has already been accomplished. Both veins, and their tributaries the common cardinal and umbilical veins, now enter the right atrium, whence the blood is carried to the pulmonary arches. From the left atrium the pulmonary vein grows through the caudal remains of the dorsal mesocardium toward the lung bud, and blood from this is directed through the heart toward the systemic arches.

During embryonic and fetal life the interatrial septum remains incomplete, being pierced by holes, one of which remains as the foramen ovale. In mammals the closure of this foramen at birth is aided by the growth of a second interatrial septum, parallel with the first, which fuses with it.

Layers of the Heart. Early in the development of the heart a third layer, consisting of mesenchyma, forms between the endothelium and mesothelium. It gives rise to the cardiac musculature, and toward the mesothelium it also produces connective tissue. The wall of the heart in the adult is divided into three layers, the *endocardium*, *myocardium* and *epicardium* respectively. The endocardium consists of the endothelium, which is continuous with that of the blood vessels, and of subendothelial reticular tissue. According to Mall, this tissue is derived from the endothelium. The myocardium is the muscle layer, which is thin in the atria, but very thick in the ventricles; in the left ventricle it is much thicker than in the right. The epicardium consists of the pericardial epithelium together with underlying connective tissue. This layer is also called the *visceral pericardium*, and with the parietal pericardium it bounds the pericardial cavity, forming a closed sac containing the pericardial fluid. The general relations of these layers in an embryonic heart are shown in Fig. 218. The epicardium is a smooth layer. The musculature of the ventricles is arranged in trabeculæ covered with endothelium, between which there are blood spaces classed as sinusoids. In the adult the musculature is more compact, but internally it is indented by many clefts and irregular spaces, extending among the *trabeculæ carneæ* and the conical papillary muscles.

Endocardium. The endocardium[1] is a smooth layer covering the inner surface of the heart, the papillary muscles, the chordæ tendineæ and the

[1] The name *endocardium* was given to the internal membrane of the heart, and to its inflammation the term *endocarditis* by J. B. Bouillaud, *Traité clinique des maladies du cœur, précédé de recherches nouvelles sur l'anatomie et la physiologie de cet organe*, Paris, 1835. In his *Anatomie descriptive* (1803), Bichat described the lining of the heart as the 'membrane interne.' He stated that this covering is subject to 'ossifications' especially over the aortic and mitral valves and that only in rare cases can they be considered the cause of death.

cardiac valves. Its thickness varies, it being thicker in the atria than in the ventricles and the thickness is said to increase about fourfold from birth to adulthood. The endocardium consists of an endothelium, the single layer of flat cells being shorter and wider than those lining the blood vessels entering and leaving the heart. Beneath the endothelium lies a layer of loose connective tissue which unites it to the underlying *lamina propria*. This layer is made up of denser connective tissue and many elastic fibers. Elastic fibers are more highly developed in the atria than in the ventricles; they occur either as networks of thick fibers or fuse to form fenestrated membranes. Smooth muscle is found also in the lamina

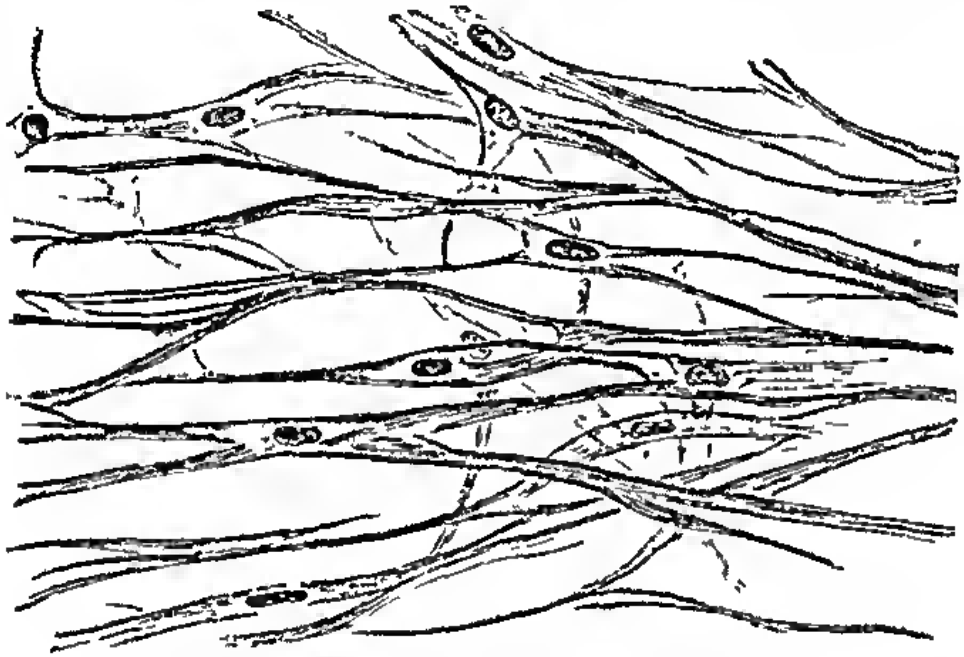


Fig 219.—Syncytium of Smooth Muscle in the Endocardium of the Right Chamber of the Heart of an Executed Man. ×350 (Benninghoff)

propria; the fibers are more numerous where the wall of the heart is smooth and they are most abundant in front of the root of the aorta. Shaner[1] observed a large amount of smooth muscle in the reptilian heart. A subendocardial layer, the *tela subendocardiaca*, binds the lamina propria to the myocardium. It is of looser texture than the lamina propria consisting of collagenous fibers intermingled with coarse elastic fibers and contains in addition Purkinje fibers, masses of adipose cells and networks of blood and lymphatic vessels. Besides the usual smooth muscle fibers, branched cells with longitudinal fibrils, but otherwise bearing a superficial resemblance to fibrocytes are observed in the lamina propria. These branched cells have been described by Benninghoff[2] and are perhaps related to the contractile-fiber cells of Kölliker.

There has been much discussion on the homology of the endocardium and the layers of the peripheral blood vessels. According to one

[1] Shaner, 1923. [2] Benninghoff, 1926.

view, the endocardium corresponds to the tunica intima of the vessel, while others regard it as corresponding to the whole thickness of the vessel wall. A third view has been advanced in which the endocardium corresponds to the tunica intima and tunica media of the vessels and the rest of the cardiac wall to the tunica externa or adventitia. The question cannot be settled until the origin of the connective tissue and muscle have been determined as coming from the endothelium, or from

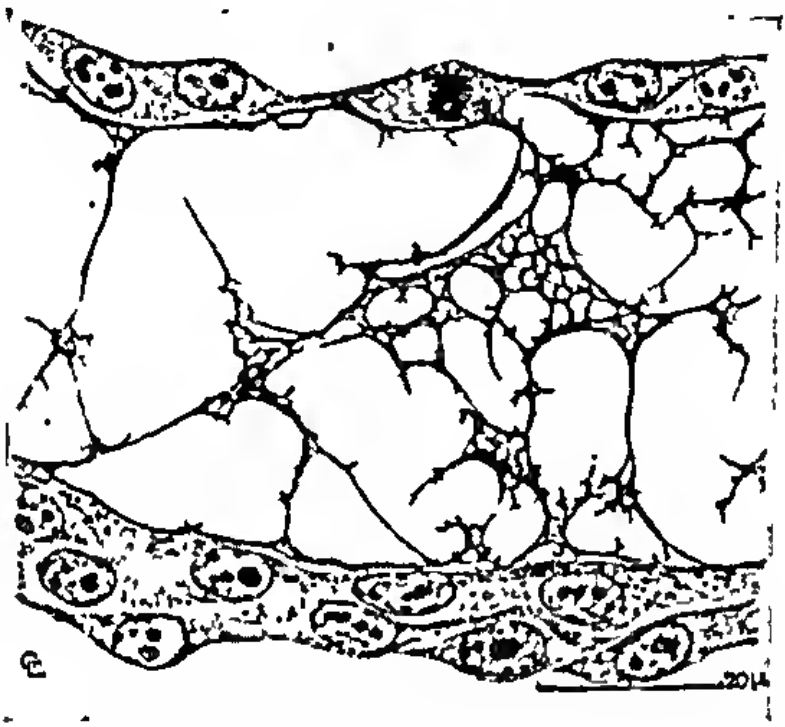


FIG. 220.—CARDIAC JELLY IN CHICK EMBRYO OF 53 HRS. INCUBATION
Above, a row of endothelial cells; below, the myocardium; between the two is a coagulation of mucoid substance. Bouin fixation, Mucicarmine and hæmatoxylin (Garrault).

the myo-epicardial mantle. These two structures are at first separated by a space containing a viscid fluid, the *cardiac jelly*.[1] Garrault[2] observes, in the chick embryo that mesenchymal cells migrate into this jelly from the myo-epicardial mantle toward the end of the third day of incubation (Fig. 221). The elastic tissue of the endocardium, sparse at birth, is not continuous into the aorta and pulmonary artery, the *annuli fibrosi* of the cardiac skeleton interrupts any continuity.

The atrio-ventricular valves are essentially folds of endocardium containing dense fibro-elastic tissue continuous with similar tissue in the annuli fibrosi. The valves contain muscle fibers toward these rings, and elastic fibers which are prolonged into the chordæ tendineæ. On the atrial surface strands of muscle are found continuous with the myocardium of the atria; they run forward in the tela subendocardiaca more or less parallel to the long axis of the cusp. Less constant extensions of

[1] DAVIS, 1924. [2] GARRAULT, 1934.

ventricular musculature on to the cusps were described by Bernays[1] and several later writers. They occur as isolated bundles running a short distance from the base on the cusp. Blood vessels probably do not extend in man normally beyond the musculature on the cusps, but under pathological conditions they may occur throughout the valves. The semilunar valves of the pulmonary artery and aorta contain neither muscle nor blood vessels. Their elastic fibers are found chiefly on the ventricular sides of the valve and in the *noduli* which are thickenings in the middle of the circumference of each segment, to perfect their approxi-

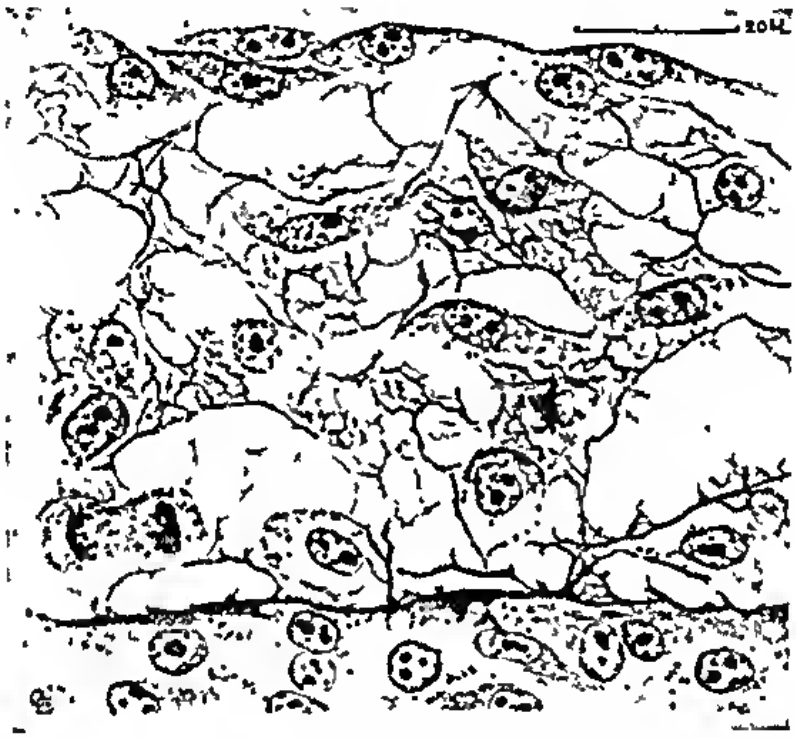


FIG. 221.—CARDIAC JELLY IN CHICK EMBRYO OF 72 HRS. INCUBATION
The mucoid substance between the endothelium and the myocardium is infiltrated with numerous cells which are actively multiplying. (Garrault.)

mation when closed. The yellow color of the noduli is due in part to deposition of fat.

Histiocytes are found abundantly throughout the subendothelium on the atrial side of the cardiac valves, where they are said to be about as numerous as fibrocytes. On the ventricular side, the fibrocytes outnumber the histiocytes. Harper[2] injected rabbits with a solution of trypan blue and observed particles of the dye stored in both histiocytes and undifferentiated mesenchymal cells, but none in fibrocytes. He suggested that the undifferentiated mesenchymal cells on stimulation form in situ additional histiocytes which frequently accumulate in the intermediate portion of a cusp near the surface. Within the lamina fibrosa of the valves and in the central fibrous core of the chordæ tendineæ very few cells segregate particles of the dye.

[1] BERNAYS, 1876. [2] HARPER, 1943.

Myocardium. The myocardium consists of muscle fibers arranged in layers or sheets, which are wound about the ventricles in complex spirals, making a vortex at the apex of each ventricle. If the heart is boiled in dilute acid these layers may be unwound, and the heart has frequently been investigated in this way, as by Mall.[1] The layers are composed of cardiac muscle, which is a syncytium of striated fibers with central nuclei and intercalated discs, as already described (p. 168). Cardiac muscle is shown in longitudinal section in Fig. 136, and in transverse

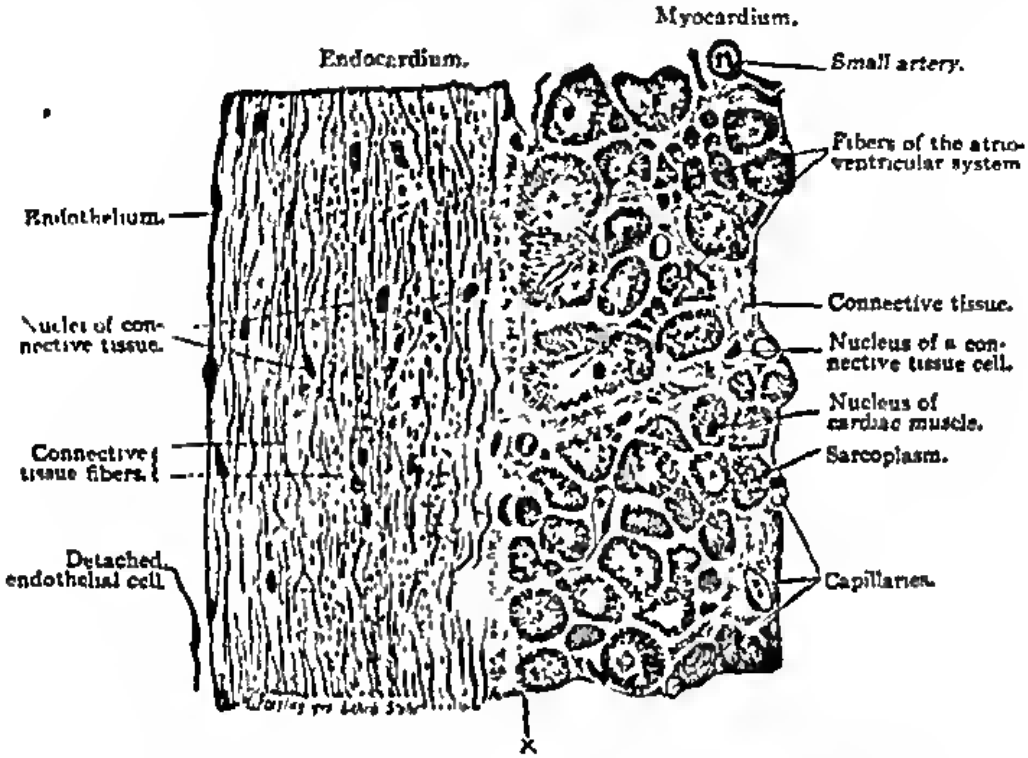


Fig. 222.—From a Cross Section of the Pectinate Muscle of a Human Heart (Right Atrium). × 240. The muscle fibrils in transverse sections appear as points, at x they are radially arranged.

section in Fig. 139. Between the muscle fibers there are capillary branches of the coronary vessels which ramify in the epicardium. The capillaries *come into close relation with the muscle fibers and some of them extend* into the endocardium. Certain vessels, especially in the right atrium, empty into the cavity of the heart as small veins known as the *vena minimæ* (or veins of Thebesius) Minute veins in the papillary muscles have been described as opening into the ventricle at both ends.

A structure of great functional importance is a small band of muscle fibers, associated with nerves, which passes from the septum between the atria into the septum between the ventricles. This *atrio-ventricular bundle* or 'bundle of His' (discovered independently in 1893 by Kent and by His, Jr.) represents the only connection between the musculature of the atria and ventricles; it passes through the fibrous tissue where the *annuli*

[1] Mall, 1911.

fibrosi come together. The position of the bundle is shown in Fig. 223. after Curran.[1] Extensive branches in the atria come from both sides of the heart into the inter-atrial septum, and converge from the fossa ovalis, the roots of the tricuspid valve and the orifice of the coronary sinus to form the *atrio-ventricular node*, 'a small mass of interwoven fibers in the central fibrous body of the heart.' The main bundle, 2–3 mm. wide, runs from this node into the inter-ventricular septum, passes under the *pars membranacea*, and divides into two branches which are distributed to the right and left ventricles respectively. Their extensive ramifications have been modeled by DeWitt[2] and by Johnstone and Wakefield,[3] and

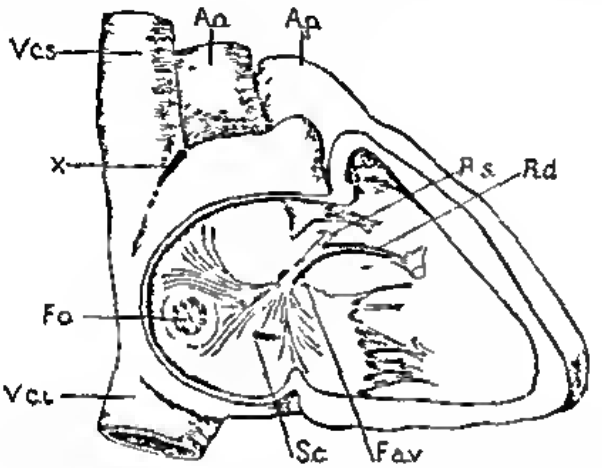


FIG 223.—THE ATRIO-VENTRICULAR BUNDLE (F. a. v), AND THE POSITION OF THE SINO-ATRIAL NODE' (x) IN A HUMAN HEART (*Curran and Aschoff*)

Ao, Aorta, A.p, pulmonary artery, F.o, fossa ovalis, S c, coronary sinus, R d, right branch of the atrioventricular bundle, and R s, its left branch, V c. i, vena cava inferior, V c. s, vena cava superior

the subject is more fully considered by Tawara,[4] whose careful dissections and histological drawings well repay examination.

The atrio-ventricular bundle is composed of muscle fibers which are pale macroscopically. They are larger than those of ordinary cardiac muscle, but contain fewer fibrils, peripherally placed and surrounded by abundant sarcoplasm (Fig. 224). In the ventricle they are specially rich in glycogen. In the node, however, according to DeWitt, the fibers, though varying greatly in size, are much smaller than those found elsewhere in the heart. Several of them unite at a point, producing stellate groups, and the entire node is an intricate network.

The fibers of the atrio-ventricular bundle resemble those described by Purkinje in the sheep, horse, cow and pig, but which he could not find in the rabbit, dog and man.[5] In the walls of the ventricle, immediately beneath the endocardium, he observed 'first with the naked eye, a network of gray, flat gelatinous threads, which in part were prolonged into the papillary muscles, and in part passed like bridges across the separate folds and clefts.' Under the microscope, they appeared very granular, but he decided

[1] CURRAN, 1909. [2] DEWITT, 1909. [3] JOHNSTONE AND WAKEFIELD, 1922.
[4] TAWARA, 1906. [5] PURKINJE, 1845.

that they were probably muscular. Purkinje's fibers are regarded as imperfectly developed muscle fibers. In the human heart they are not so distinct from the other cardiac muscle fibers as in the sheep. It is possible that they are directly continuous with the cardiac syncytium, although, as noted by DeWitt, if the transition is gradual, it will be very difficult to observe in sections.

At the junction of the superior vena cava and the atrium, Keith and Flack have described a peculiar musculature embedded in densely packed connective tissue, composed of striated, fusiform fibers, plexiform

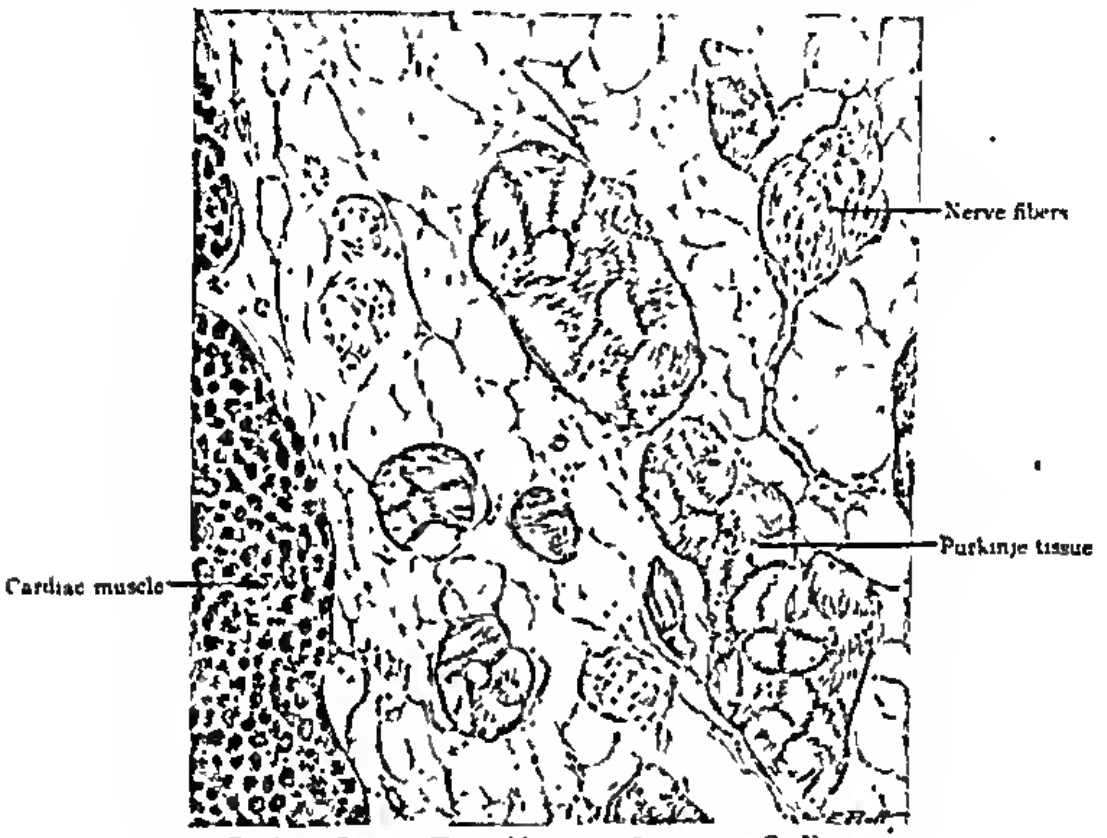


FIG. 224.—PURKINJE TISSUE. MODERATOR BAND OF THE OX HEART. Formaldehyde fixation. Hæmatoxylin and eosin.

in arrangement, with well-marked elongated nuclei, 'in fact, of closely similar structure to the node.'[1] These fibers are said to be in close relation with the vagus and sympathetic nerves; they have a special arterial supply. According to Keith and Flack they are situated at the junction of the sinus venosus and the atrium, and they form the *sino-atrial node* (sino-auricular node). This second node is found immediately beneath the epicardium in the position shown in Fig. 223. In it the impulse for the heart beat is believed to originate, and to be transmitted to the atrio-ventricular node; the latter correlates the contraction of the atrium with that of the ventricle. Interference with this system leads to the condition known as 'heart-block.'

[1] KEITH AND FLACK, 1907.

Developmentally the atrio-ventricular bundle represents the persistent remnant of the muscular connection between atria and ventricles, most of which has been interrupted by the formation of the annuli fibrosi. The embryological origin of its extensions is described by Stiénon[1] and by Benninghoff.[2]

Epicardium. The epicardium is a connective tissue layer, covered with simple flat mesothelium and containing elastic fibers and many fat cells. The latter are distributed along the course of the blood vessels.

Pericardium. The heart is enclosed in a fibro-serous sac called the *pericardium*. Above, the outer fibrous layer—the *tunica fibrosa*—fuses to the walls of the great vessels entering and leaving the heart and below, in man and the anthropoid apes, it adheres to the diaphragm. It is made up

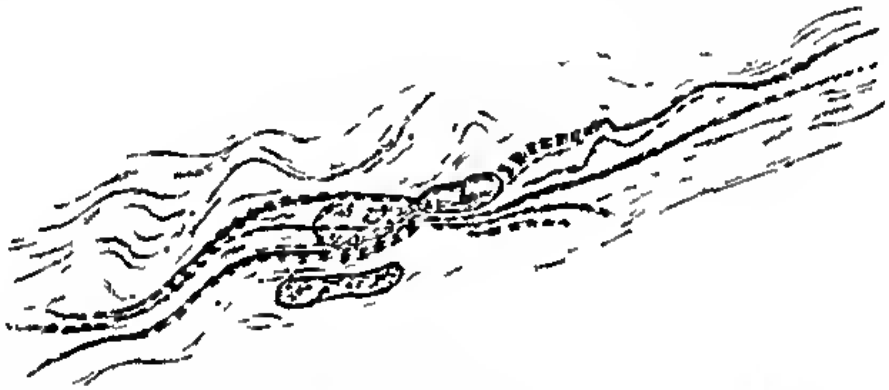


FIG 225.—A SINGLE MUSCLE FIBER IN THE MIDDLE OF RICH CONNECTIVE TISSUE FROM THE SINUS NODE OF THE HUMAN HEART. × 1000. (Burian.)

of interlacing bundles of collagenous fibers and a few elastic fibers and is strengthened by collagenous bands having a distinctive arrangement which Popa and Lucinescu[3] call the 'mechanostructure' of the pericardium. Small arteries from neighboring vessels pass into the fibrous layer and from plexuses in the more superficial parts branches continue into the subserous tissue. The nerves are small and are derived from the phrenic, vagus and sympathetic nerves. The serous layer—the *tunica serosa*—is divisible into two parts, a thicker parietal layer lines the fibrous pericardium and a thinner visceral layer forms the epicardium which invests the heart and those parts of the great vessels within the sac. The space between the parietal and visceral pericardii is called the pericardial cavity. Normally it contains only a small amount of fluid (*liquor pericardii*) but in inflamed conditions the quantity may be increased considerably. The serous layer is smooth and glistening, being covered with flattened irregularly polygonal mesothelial cells attached to the fibrous layer by a delicate connective tissue rich in elastic fibers. When seen on the surface, the cells often contain cytoplasmic vacuoles, and between them are wide intercellular spaces. Some of the cells contain more than one

[1] STIÉNON, 1925. [2] BENNINGHOFF, 1930. [3] POPA AND LUCINESCU, 1932.

nucleus. Poska-Teiss[1] has recorded as many as twenty-three nuclei in a single cell in the human serous pericardium and a similar multinucleate condition has been observed in a number of mammals and birds.[2] This binucleate and multinucleate condition is accompanied by a division of centrioles. Mitoses are not seen.

Vessels and Nerves. The branches of the coronary vessels pass from the epicardium into the myocardium, forming capillaries in intimate relation with the muscle fibers. The heart wall is thus supplied with aerated blood from the root of the aorta, as well as by the blood within its own cavities; on the left side this is aerated, but not on the right.

The question as to whether the coronary vessels in man connect with the Thebesian vessels and thus with the heart cavities is important because of its relation with coronary thrombosis, the blockage of the coronary arteries, which usually causes sudden death, but not invariably. In the heart of adult frogs, the system of intermuscular clefts or lacunar vessels is the only blood supply of the ventricular musculature; the coronary vessels are limited to the epicardium. In turtles the coronary vessels supply an outer layer of the ventricular muscles, but the greater part is still nourished by the central lacunæ or sinusoids. This sinusoidal circulation, which is characteristic of the adult heart in lower vertebrates, occurs also in mammalian embryos, but it becomes vestigial in adult mammals. There are normally, however, some connections between the peripheral coronary and the internal sinusoidal circulation,[3] which may become enlarged if the obliteration of the coronary orifices develops slowly. The heart valves have an abundant circulation in embryos, but gradually lose it in adult life.

The lymphatic vessels, draining toward the base of the heart, are very abundant, and true lymphatic vessels are found in all layers of the heart. The tissue spaces in the myocardium are also extensive.

The nerves to the heart have already been described as forming the cardiac plexus. This plexus receives branches from the vagus and from the sympathetic cardiac nerves proceeding from the cervical sympathetic ganglia. It sends its fibers toward the heart, where they follow the coronary vessels in their ramifications. The *cardiac ganglion* is associated with the superficial part of the cardiac plexus, and is under the arch of the aorta. Other small ganglia occur on the posterior wall of the atria, and scattered ganglion cells are found along the atrio-ventricular bundle. They have been reported along the nerves elsewhere in the heart. The ganglion cells are probably in connection with efferent fibers from the central nervous system, which include two sorts—fibers from the ventral ramus of the accessory nerve, which pass out with the branches of the vagus and inhibit cardiac actioo; and fibers from the spinal nerves, by way of the inferior cervical ganglion, which accelerate it. Histologically nerve endings have been seen both within and around the capsules of cardiac ganglion cells. It is said that the medullated nerve fibers from

[1] Poska-Teiss, 1936. [2] Tonkoff, 1899. [3] Wearn et al., 1933.

the central system end within the capsules; and that non-medullated branches from adjacent sympathetic ganglia end outside of them. Motor endings in contact with cardiac muscle have also been found. Sensory endings have been described both in the epicardium and endocardium. They consist of terminal ramifications forming 'end-plates.' Some of these fibers presumably connect with sympathetic cells near at hand; others are terminations of afferent medullated fibers which are said to pass to the medulla, along the vagus trunk, as the 'depressor nerve.'

LYMPHATIC VESSELS

General Features. The lymphatic vessels are far less conspicuous than the blood vessels, but they are no less important and are widely distributed throughout the body. Those which occur in the mesentery and are filled with a milky fluid after intestinal digestion has been going on are the most conspicuous. These 'arteries containing milk' were observed by Erasistratus, an anatomist of Alexandria who died in 280 B. C., but the observation was discredited by Galen. When Aselli in 1622 found the white vessels in a living dog which he had opened, and had shown by cutting into them that they were not nerves, it was essentially a new and great discovery. Aselli observed that the vessels were filled only after digestion, at other times being scarcely visible. He traced them to a mass of lymph glands which he mistook for the pancreas, and believed that they passed on into the liver (De lactibus sive lacteis venis, 1627). Years before the physiological observations of Aselli, Eustachius (who died in 1574) had described the main trunk of the lymphatic system in his treatise on the azygos vein.[1] He states that from the posterior side of the root of the left jugular vein 'a certain large branch is given off, which has a semicircular valve at its origin, and moreover is white and full of aqueous humor.'

'Not far from its source, it splits into two parts which come together a little further on. Giving off no branches, and lying against the left side of the vertebræ, having penetrated the diaphragm, it is borne along to the middle of the loins. There, having become larger and folded around the great artery, it has an obscure ending, not clearly made out by me up to the present time.'

The vessel so well described by Eustachius is now known as the thoracic duct (*ductus thoracicus*). It extends in man from the level of the second lumbar vertebra in front of the bodies of the vertebræ and empties its contents into the blood at the junction of the left internal jugular and left subclavian veins. It receives all the lymph from below the diaphragm and above from the trunk of the body, the left side of the head and the left arm. On the right side a number of trunks drain the

[1] Eustachius, 1707.

right side of the head, the right arm and adjacent territory. These vessels may empty separately at the junction of the right internal jugular and right subclavian veins or they unite in part to form a right lymphatic duct (*ductus lymphaticus dexter*). Since the right duct has no connections with the lymphatics below the diaphragm, except possibly from the upper surface of the liver, it is much smaller than the thoracic duct.

FIG. 226.—A PORTION OF THE WALL FROM THE UPPER PART OF THE THORACIC DUCT HUMAN ADULT. WEIGERT'S RESORCIN-FUCHSIN AND PICRO-INDIGO CARMINE (KAJAVA)

The connection between the lacteal vessels in the mesentery, seen by Aselli and the thoracic duct observed by Eustachius was demonstrated physiologically by Pecquet (Experimenta nova anatomica, 1651). He found a whitish fluid coming from the vena cava superior of a dog from which the heart had been excised and observed that its flow was increased by pressure on the mesenteries. Moreover, he described the receptaculum chyli, or enlargement of the thoracic duct dorsal to the aorta, which receives the chylous fluid. This is now called the *cisterna chyli*. The distribution of the lymphatic vessels, which are ramifications of these main trunks, was followed out by skillful injections and the results of such studies were presented in great folios by Mascagni (1787) and Sappey (1874). Wide variations are seen in the course of the thoracic duct, which is found not infrequently double and connected by anastomoses. These variations are very numerous caudally, where the cisterna chyli is often absent and replaced by several lumbar trunks.

In structure the thoracic duct resembles a vein. Its wall is made up of three layers—a tunica intima, a tunica media and a tunica adventitia. Kajava[1] calls the media the 'connective tissue-muscular zone.' The intima which has a variable thickness (13–22 μ) consists of an endothelium lining the duct, collagenous and elastic fibers, smooth muscle and in places an elastica interna. The endothelial cells when seen on surface have serrated outlines and they dovetail one into the other.

[1] KAJAVA, 1921.

Collagenous tissue is sparse in some parts and more abundant in others. Both the elastic tissue and the bundles of smooth muscle fibers are disposed chiefly longitudinally. The muscle bundles are better developed in the lower than in the upper part of the duct and in places form local pillow-like thickenings which cause the intima to bulge into the lumen. These pillow-like ('Intimakissen') are thought by Kajava to be compensatory against dilation of the duct. The elastica interna which thins out or disappears toward the venous valve is composed of networks of

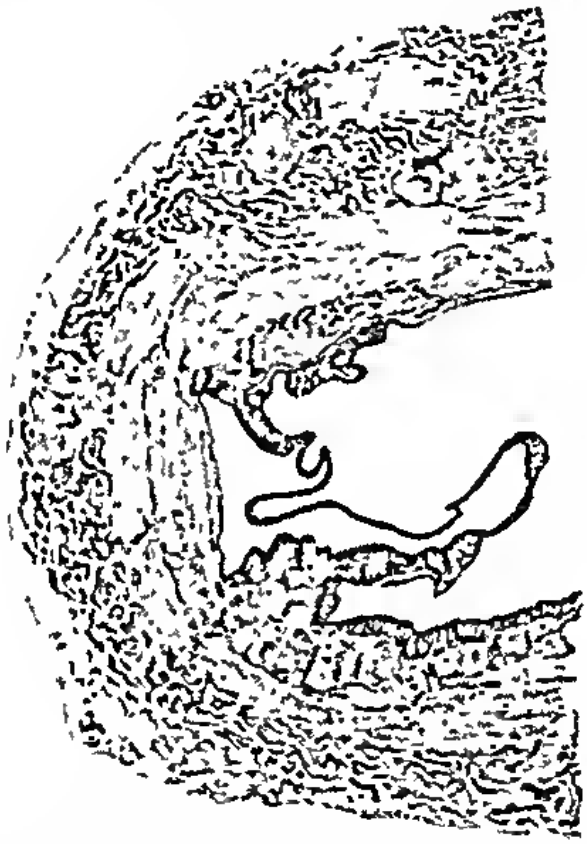


FIG. 227.—A PORTION OF THE WALL OF THE LUMBAR LYMPHATIC TRUNK WITH A VALVE. HUMAN ADULT. WEIGERT'S RESORCIN-FUCHSIN AND PICRO-INDIGO CARMINE (Kajava.)

fine longitudinally disposed fibers. The tunica media or connective tissue-muscular zone of Kajava is the best developed part of the duct and may reach a thickness of 56 μ. Where thickest it consists of an inner and outer layer of longitudinal smooth muscle and a middle layer of circular fibers. In reality the muscle fibers in all three layers are arranged spirally. The outer layer which may be absent in places is generally the least developed, while the middle layer makes up about two-thirds of the musculature. Between the bundles of smooth muscle there are found numerous bundles of collagenous fibers and a few fine elastic elements. The converse occurs in the horse where the media possesses rich nets of elastic tissue and little or no muscle. The tunica adventitia is a small border zone attaching the duct to surrounding structures. It is composed principally of longitudinal collagenous fibers, some fine elastic fibers

and here and there single smooth muscle fibers. The adventitia is provided with vasa vasorum, besides numerous fine medullated nerve fibers of which some may be traced into the intima. Adipose cells occur in variable numbers.

Fig. 228.—Nerve Fibers in the Adventitia of the Thoracic Duct of a Dog. Methylene Blue. (Kytmanoff.)

The wall of the cisterna chyli is similar but thicker than in the thoracic duct. It contains numerous so-called 'Intimakissen.' In the right lymphatic duct, the borders between the three zones—intima, media and adventitia are less distinct than in the thoracic duct, otherwise the general microscopic structure is found similar. The thoracic duct as all larger lymphatic vessels possesses valves. These are endothelial duplications with thickened bases and contain besides collagenous fibers longitudinal smooth muscle. Kampmeier[1] studied the development of the valves and found their number inconstant. The venous valve of the thoracic and right lymphatic duct is not always functionally secure and not infrequently blood backs from the vein into them.

Origin. Lymphatic vessels originate in embryos of 8 to 10 *mm.* after the main blood vessels have become well established. Their exact mode of origin is still in doubt. At first they were considered as the result of the coalescence of isolated tissue spaces, which had dilated and received an endothelial lining by the flattening of the surrounding mesenchymal cells, later acquiring an opening into the jugular veins. Sabin[2] then found that the peripheral lymphatics were derived by the growth of sprouts from four original sacs, the jugular and iliac pairs, which could be injected directly or through the veins, the connection being guarded by valves. The sacs were filled at a certain period with stagnant blood, and

[1] Kampmeier, 1928*a*. [2] Sabin, 1902.

were considered to have budded off from the veins. Lewis,[1] following the method of reconstruction from sectioned embryos, recognized multiple outgrowths from many veins, not always in communication with the parent veins. He noted especially that the jugular sacs are formed by the

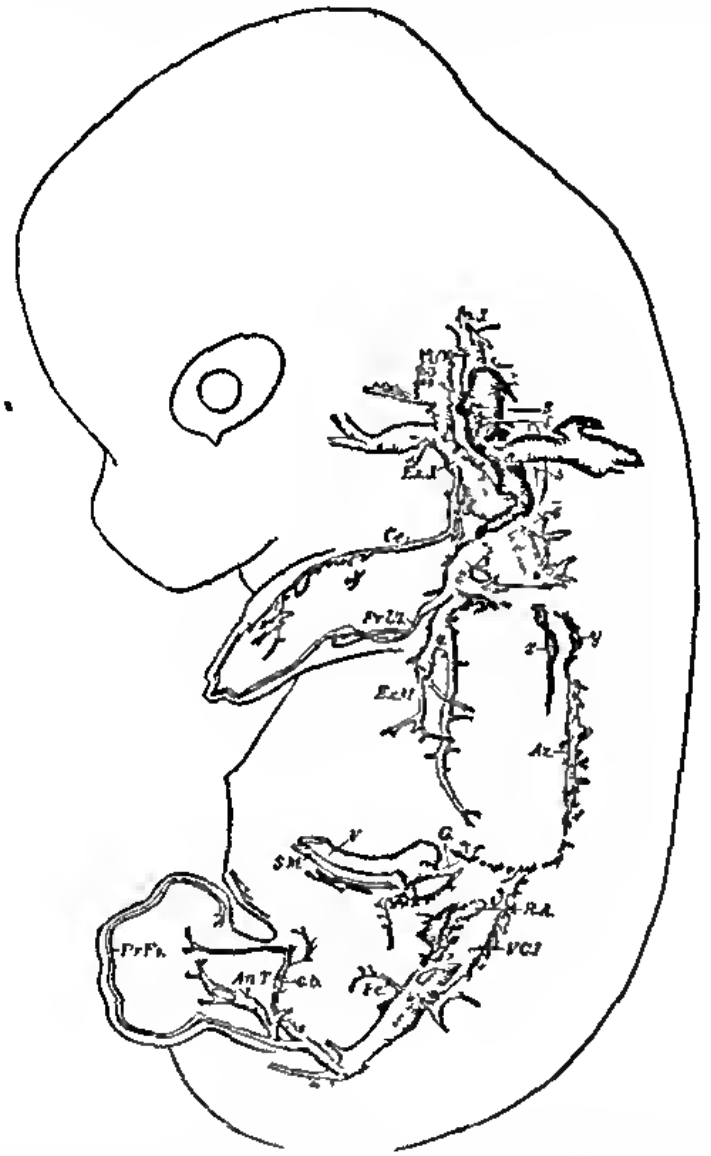


FIG. 229.—LYMPHATIC VESSELS AND VEINS IN A RABBIT OF FOURTEEN DAYS, EIGHTEEN HOURS, 14.5 MM. × 11.5 (LEWIS)

The lymphatics are heavily shaded, x being a vessel along the left vagus nerve and y along the aorta. The large jugular lymph sac is in contact with the internal jugular vein, In. J.; it passes to the junction of the external jugular (Ex. J.) and subclavian veins, the latter being formed by the union of the primitive ulnar, Pr. Ul., and external mammary veins, Ex. M. The mesenteric sac is in front of the vena cava inferior (V. C. I.) and below the renal anastomosis (R. A.). Other veins include Az., azygos, V., vitelline, G., gastric, S. M., superior mesenteric, etc. The figures indicate the position of the corresponding cervical nerves.

coalescence of several venous outgrowths, and that smaller sacs arise from the subcardinal and mesenteric veins at a slightly later date (rabbit), and that still later other lymphatics appear along the azygos and cutaneous veins, apparently from independent, detached outgrowths (*Fig.* 229). *Huntington* and McClure[2] considered that the *primitive* lymphatic vessels, called by them 'veno-lymphatics,' are derived from

[1] LEWIS, F. T., 1905. [2] HUNTINGTON AND MCCLURE, 1910.

these special veins by the process of fenestration or partial linear subdivision, resulting in parallel channels, one venous, the other lymphatic. Later McClure[1] revised the theory of isolated spaces, in that he considered that the main lymphatic trunks appear along the course of disused venous channels as isolated, extraintimal spaces which become confluent as the venous endothelium shrinks and disappears. These are not to be confused, however, with mesenchymal tissue spaces. The connection with the veins comes later, and the lack of it causes the temporary swelling of the various sacs. This view is also held by Kampmeier.[2] The blood in the sacs is thought due to local hæmatopoiesis. Later the sacs subdivide and become the site of chains of lymph glands.

It is probable that the diversity of opinion here recorded is due partly, as in the study of the origin of the blood vessels, to different methods of investigation, whether by injections, serial sections, or embryonic experimentation. Injections must fail to reveal any isolated spaces, while in sections the tissues have been subjected to agents which might cause shrinkage and perhaps segmentation of continuous slender channels.

The later development or growth of the lymphatic vessels, once the main foci are formed, is by the budding of endothelial sprouts, similar to those of the blood vessels. They have been studied[3] in the living tadpole (Fig. 230), and are always solid at the tip, not opening out into the tissue spaces, though the tips may ingest foreign particles which later appear in the lumen. The endothelium is paler and more irregular than that in the blood vessel sprouts, and the two never anastomose, though each type freely forms loops with its own kind. From the capillary net thus formed main channels develop. The mesenteric sac thus becomes connected with the left jugular sac (symmetrical connections with both jugular sacs occur in some animals) and the connecting vessels constitute the thoracic duct. The cisterna chyli is a secondary enlargement dorsal to the aorta. In the adult the sacs are replaced by plexuses of smaller vessels.

Lymphatic Vessels in the Adult. In sections of the intestine from an animal in which intestinal digestion was in progress, lymphatic vessels may readily be found between the muscle layers (Figs. 196 and 197). Their walls are decidedly thinner than those of blood vessels of the same caliber, and their contents are typically a granular or fibrinous coagulum free from red corpuscles, but containing an occasional lymphocyte. It must be remembered, however, that blood vessels seen in sections are not infrequently empty, and that blood corpuscles may be taken into the lymphatic vessels. Having learned to recognize the lymphatics in such favorable situations as the intermuscular tissue, one may readily

[1] McClure, 1910. [2] Kampmeier, 1931. [3] Clark, E. R., 1909.

identify them in the connective tissue layer internal to the circular muscle of the intestine, and in the connective tissue around the bronchioles in the lung; in the embryonic lung they are very conspicuous. They may then be sought for in various organs, but a sharp distinction must be drawn between the endothelium-lined lymphatic vessels and the interfibrillar tissue spaces.

When prepared with silver nitrate, the outlines of the endothelial cells are seen to resemble those of blood vessels, and in the larger lymphatic vessels the endothelium with the underlying connective tissue forms a *tunica intima*. These lymphatics (0.2–0.8 mm. in diameter) are

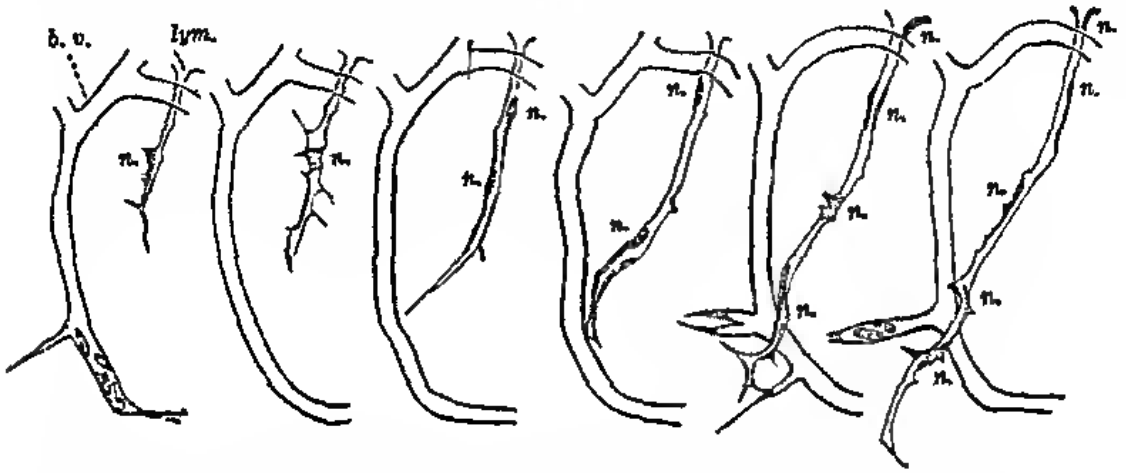


FIG. 230.—SUCCESSIVE STAGES IN THE GROWTH OF A LYMPHATIC VESSEL (lym.) IN THE TAIL OF A TADPOLE (*Rana palustris*) × 135 (Clark)

b. v., Blood vessel, n., nucleus of the lymphatic vessel

often composed of three coats, though loose in texture. The media contains circular smooth muscle fibers and a small amount of elastic tissue; and the externa is composed of longitudinal connective tissue and scattered bundles of longitudinal muscle. Thus they resemble the veins more closely than the arteries. Valves are very numerous in lymphatic vessels. In the small vessels the valves are described as folds of endothelium, such as would be produced if the distal part of the vessel were pushed forward into the proximal part.[1] The vessels are often distended on the proximal side of the valve, producing bulbous enlargements. Owing to the presence of these valves, compression of tissue containing lymphatic vessels, or the contraction of the muscles of the media, causes an onward flow of the lymph. The nerves to lymphatic vessels are like those of the blood vessels. Lymphatics are provided with vasa vasorum. Even small lymphatic vessels are accompanied by blood capillaries,[2] and the larger lymphatics are surrounded by a wide-meshed capillary network resting on the outer side of the lymphatic media.

[1] KAMPMEIER, *1928a*. [2] EVANS, *1907*.

BLOOD

Blood consists of round cells entirely separate from one another, floating in an intercellular fluid, the *plasma*. The plasma in mammals also contains as a regular and apparently important functional constituent, the *blood plates* (or platelets), together with smaller granular bodies. Blood cells or *corpuscles* are of two sorts, (1) *red corpuscles* or *erythrocytes*, which become charged with the chemical compound, *hæmoglobin*, and which lose their nuclei as they become mature; and (2) *white corpuscles* or *leucocytes*, which are of several kinds, all of them retaining their nuclei and containing no hæmoglobin. The redness of blood is not due to the plasma, but is an optical effect produced by superimposed layers of the hæmoglobin-filled red corpuscles. Thin films of blood, like the individual red corpuscles seen fresh under the microscope, are yellowish-green. Blood has a characteristic odor which has been ascribed to volatile fatty acids; it has an oily feeling associated with its viscosity, an alkaline reaction, and a specific gravity said to average in the adult from 1.050 to 1.060.

Red Corpuscles. The non-nucleated red corpuscle (also called erythrocyte, plastid or erythroplastid) is the most important and most numerous element in the blood, and apparently a simple structure to describe, yet even the normal shape of red corpuscles is in doubt. When a drop of freshly drawn mammalian blood is spread in a thin film on a glass slide, beneath a cover glass, it is seen to consist chiefly of biconcave discs, and of those in the form of shallow saucers. They have a remarkable tendency to pile up in *rouleaux*, like rolls of coins. It is said that discs of cork, weighted so that they will float beneath the surface of water, will come together in a similar way if their surfaces have been coated with an oily substance. It is evident that the thin film of blood, though very fresh, is examined under extremely artificial conditions; and from such preparations, conclusions as to the normal shape of the corpuscles should not be hastily drawn.

In well-preserved sections the erythrocytes within the vessels are sometimes seen as biconcave discs, often as cup-shaped corpuscles. Which of these two forms is typical or whether both may be considered normal is still a matter of controversy. The corpuscles are extremely delicate and easily moulded, and pressure from contact with the walls of narrow

Fig. 231.—Cells from Smear Preparation of Normal Human Blood, Wright's Stain.

In the center adult red blood corpuscles, blood platelets and a polymorphonuclear neutrophile. At left above two polymorphonuclear basophiles and two polymorphonuclear eosinophiles. At right above three large and four small lymphocytes, some with granules in the protoplasm. At left below polymorphonuclear neutrophiles, two of these cells, the uppermost and the lowermost of the group, are young, with merely crooked nuclei, sometimes known as band, stab or non-filamentous forms, the mature cells have multilobed nuclei. At right below: six monocytes, some containing more protoplasmic granules than others, in the younger cells the nuclei tend to be rounded, in the adult cells they are horseshoe-shaped, indented or lobed.

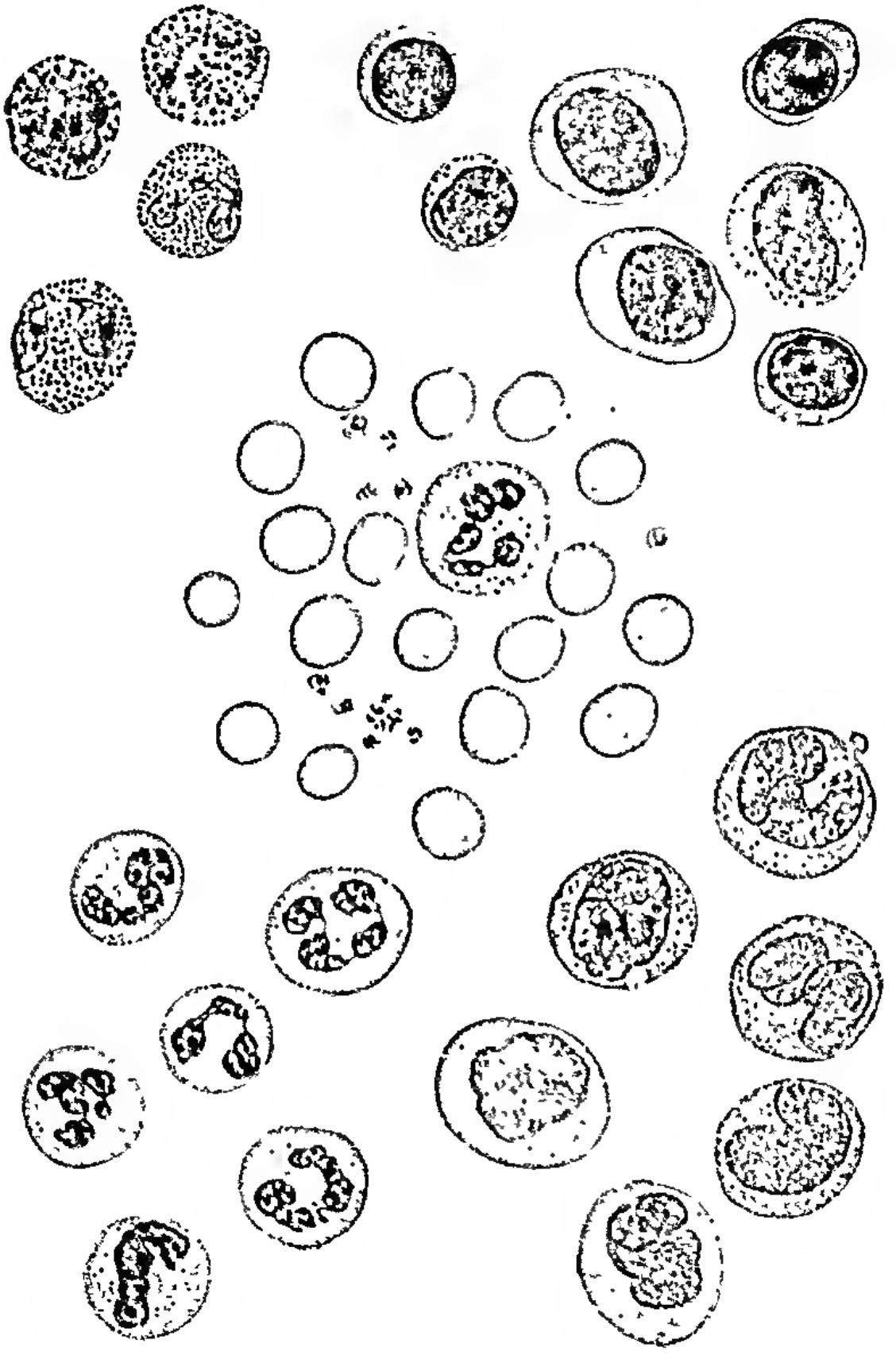


FIG. 231.—(See page 262 for description.)

capillaries or with each other may change their shape. When they are crowded in fixed tissues, square or angular forms are frequent.

Rindfleisch[1] found that the corpuscles in Guinea-pig embryos, after losing their nuclei by extrusion, are at first bell-shaped; but he considered that afterward they become biconcave discs from impact with others in the circulating blood. Commenting upon this statement, Howell[2] writes:

'I feel convinced that the bell shape which Rindfleisch ascribes to the corpuscles which have just lost their nuclei is a mistake. The red corpuscles even of the circulation, as is well known, frequently take this shape when treated with reagents of any kind, or even when examined without the addition of any liquid. It seems very natural to suppose that the biconcavity of the mammalian corpuscle is directly caused by the loss of the nucleus from its interior. Certainly as long as the corpuscles retain their nuclei, they are more or less spherical, and after they lose their nuclei they become biconcave.'

Dekhuyzen discussed *cup-shaped* corpuscles[3] which he found as a transient stage in mammals, and which his assistant saw in blood drawn from his finger. Dujardin[4] found many corpuscles shaped 'like cups, or cupules (acorn cups) with thick borders' in blood altered by the action of phosphate of soda. The first reference to such forms is by Leeuwenhoek (1717) who put a drop of blood in a concoction of pareira brava, and found that most of the globules which make the blood red, have 'a certain bend or sinus receding within, as if we had a vesicle full of water and by pressure of the finger should hollow out the middle of the vesicle as a pit or depression.' Von Ebner, in Kölliker's Handbuch (1902), writes of bell or cap-shaped corpuscles produced in warmed blood by the thickening of the border on one surface of the disc. Weidenreich,[5] after thorough study of blood variously preserved, and also examined while circulating in the mesentery of a rabbit, concluded that 'the red corpuscles of mammals have the form of bells (Glocken).'

Arey[6] reviews the previous work on the shape of red blood corpuscles, and adds some new experiments. He concludes 'that the biconcave disc represents the normal shape of the erythroplastid—the concavo-convex cup being merely an occasional modification,' which may be brought about through irregular shrinkage of the disc. According to this observer, biconcave forms predominate in solutions corresponding to 0.9% sodium chloride concentration (normal saline), but cup shapes appear in less concentrated (hypotonic) solutions. Thus blood obtained by pricking through a hypotonic fixative placed on the finger would give cups; and the same would be true of tissues immediately fixed in such fluids. Many of the usual fixatives are hypotonic. He finds that anæsthetization also may induce cup forms, and thus throws doubt on the validity of many of the results of direct examination of flowing blood in the capillaries. For diagnostic purposes the cups and biconcave forms are both considered normal, as opposed to irregular shapes or variations in size

In examining films of fresh blood, the biconcave discs will be seen to change their appearance as the objective is lowered. When sharply in focus the thin central portion appears light (Fig. 232 A); but in high focus the center is dark, perhaps owing to the dispersal of light by the lenticular corpuscles. The biconcave shape is apparent when the cor-

[1] Rindfleisch, 1880. [2] Howell, 1890. [3] Dekhuyzen, 1899.
[4] Dujardin, 1842. [5] Weidenreich, 1903. [6] Arey, 1917.

puscle is seen on edge (Fig. 232, B). The cup-shaped forms are shown in D; and E represents one of the innumerable shapes due to shrinkage. The cups may be irregularly infolded, presenting shapes which can be imitated by indenting a soft hat. If the corpuscles are placed in water or a dilute solution, their hæmoglobin passes out and water enters, so that they are reduced to transparent membranes or shadows (F). Such forms are often seen in clinical examinations of urine. In dense solutions, and in fresh preparations as the plasma becomes thicker from evaporation, water leaves the corpuscles. They then shrink, producing spiny or nodular round masses of hæmoglobin, known as *crenated corpuscles* (G). The production of all these forms can be watched by adding appropriate solutions at one side of the cover-glass of fresh smear preparations and following the dispersion. In life the corpuscles doubtless change their shape, responding to the variations in their hæmoglobin content and

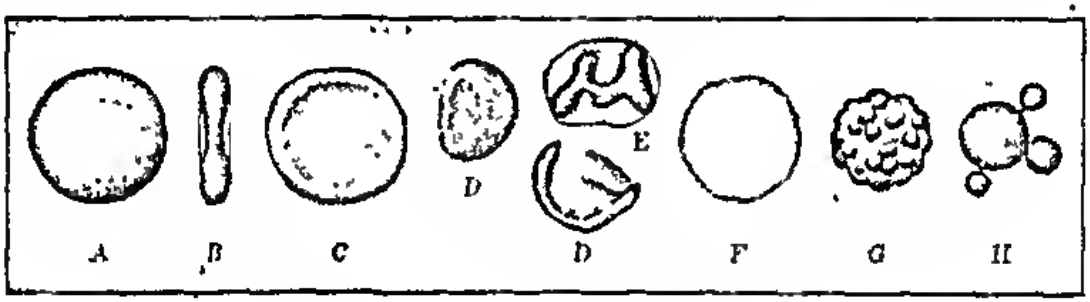


FIG 232.—RED CORPUSCLES IN VARIOUS CONDITIONS

in the surrounding plasma Occasionally they are spherical (according to Schultze and others), and deviations from the primary form are to be expected. In these changes the corpuscles act like membranes filled with fluid. The plastic nature of the membrane is shown by heating the blood film. The corpuscles then become globular and send out slender varicose processes, or round knobs attached by pedicles (Fig. 232, *H*). These small spheres become detached in great numbers. Normally the corpuscles do not present pseudopodia, and are not actively motile, though they may pass out of the vessels by 'diapedesis.'

The dimensions of red corpuscles are quite constant. Those in human blood average 7.5 μ in diameter and ordinarily vary from 7.2 to 7.8 μ. They sometimes surpass these limits. In biconcave form they are about 1.6 μ thick. The cups average 7 μ in diameter and are 4 μ in depth. Spherical corpuscles are said to be 5 μ in diameter. The blood of mammals other than man also contains cups which become discs. The latter are oval in the camel group but round in all others. Their average diameters are less than in man (7.3 μ in the dog, 7.48 μ in the Guinea-pig), but the species of animal cannot satisfactorily be determined from the diameter of the corpuscles. In a given section, as already noted, the

red corpuscles furnish a useful gauge for estimating the size of other structures.

The number of red corpuscles in a cubic millimeter of human blood averages five million for men and four million five hundred thousand for women. By diluting a small measured quantity of blood and spreading it over a specially ruled slide, the corpuscles may be counted, and the

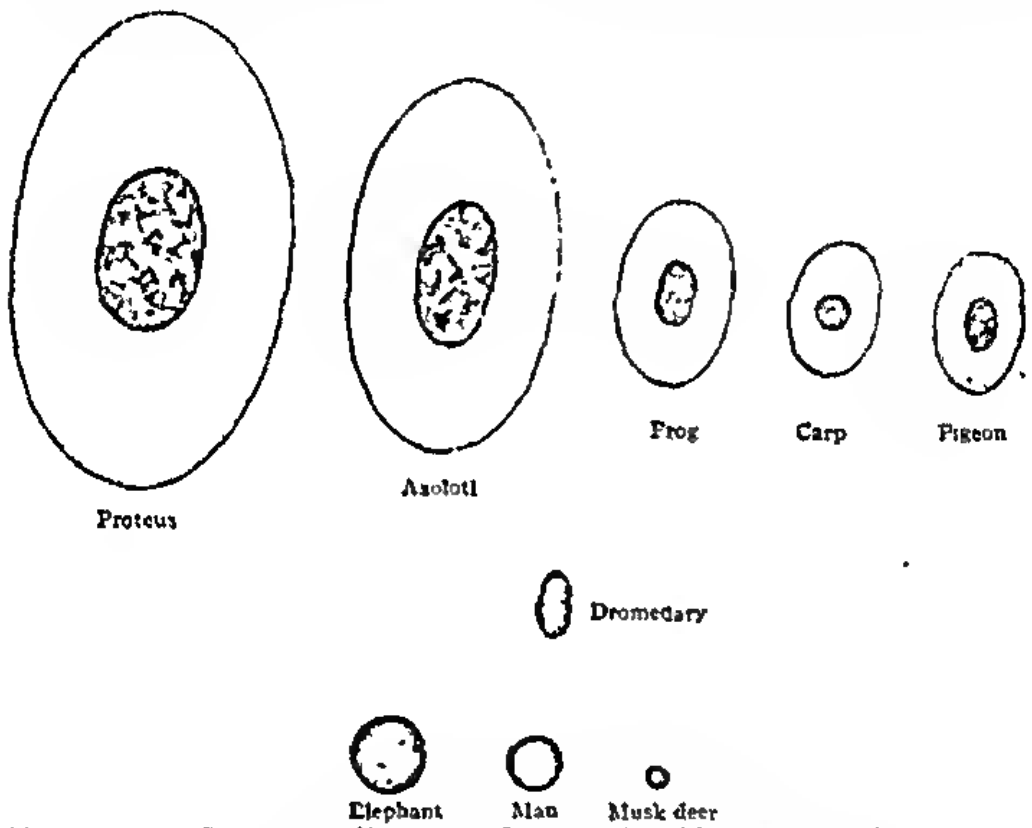


Fig. 233.—Red Blood Corpuscles of Vertebrates Drawn at Same Magnification to Show Comparative Sizes. (Szymonowicz.)

number per cubic millimeter calculated. A diminished number is of clinical importance.

In histological sections the red corpuscles usually appear as homogeneous bodies, refractive and clear, and of a color usually quite unlike that of any other portion of the section. The hæmoglobin stains brilliantly with eosin, orange G, or other acid stains; with iron hæmatoxylin it appears black or gray. The conception gained from their action in fresh blood films that the erythrocytes are membranes filled with fluid is not substantiated in fixed material, for no definite cell membrane can be made out, nor can the protoplasmic reticulum which might hold the hæmoglobin be detected in normal adult red corpuscles. The membrane must be very thin and the contents probably in the form of a colloidal gel.

Hæmoglobin is an exceedingly complex chemical substance which combines readily with oxygen to form *oxyhæmoglobin.* To the latter the bright color of arterial blood is due.

Venous blood becomes similarly red on exposure to air. Through the oxyhæmoglobin, oxygen is transferred from the lungs to the tissues. Hæmoglobin may be dissolved from the corpuscles by mixing blood with ether, and upon evaporation it crystallizes in rhombic shapes which vary with different animals. Hæmoglobin is readily decomposed into a variety of substances, some of which retain the iron which is a part of the hæmoglobin molecule, others lose it. *Hæmatoidin*, considered identical with a pigment (bilirubin) of the bile, is an iron-free substance occurring either as yellow or brown granules, or as rhombic crystals. The crystals may be found in old blood extravasations within the body, as in the corpus luteum of the ovary. *Hæmosiderin*, which contains iron, appears as

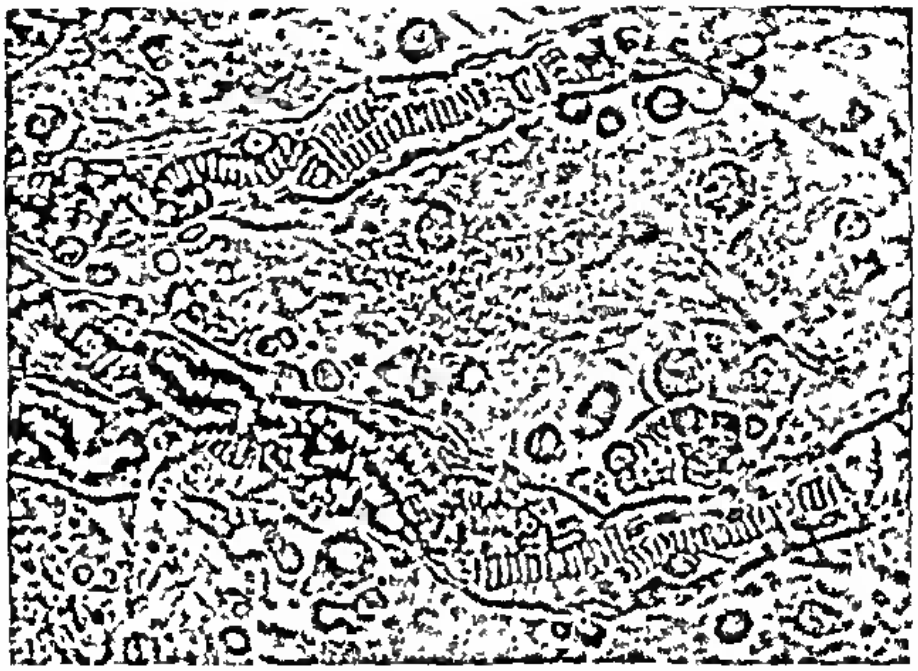


Fig. 234.—Photograph of Living Vessels in Rabbit's Ear, Showing Rouleaux of Red Blood Corpuscles and a Few Extravascular Reds. (Courtesy of E. R. Clark.)

yellowish or brown granules sometimes extremely fine, either within or between cells. The iron may be recognized by the ferrocyanide test which makes these minute granules bright blue. If dry blood from a stain is placed on a slide with a crystal of common salt the size of a pin-head, and both are dissolved in a large drop of glacial acetic acid which is then heated to the boiling point, a product of hæmoglobin is formed, called *hæmin*. It crystallizes in rhombic plates or prisms of mahogany brown color. Such crystals would show that a suspected stain was a blood stain, but they afford no indication of the species of animal from which it was derived. A small amount of hæmoglobin is in solution in the plasma.

The duration of the life of mature red corpuscles is unknown, but is supposed to be brief. They may be devoured intact by phagocytes, but generally they first break into numerous small granules. These may be ingested by certain leucocytes, or by the reticulo-endothelial cells of the liver and other organs. Their products are thought to be eliminated in part as bile pigment. The destruction of red corpuscles occurs especially in the spleen and hæmal glands; to a less extent in the lymph glands and red bone marrow. Pigmented cells in some of these structures derive their pigment from destroyed corpuscles.

Development of Red Corpuscles. Development of the erythrocytes may be studied in the embryo, where all the cells of the blood are passing through stages leading to the mature form, or in the fetus or adult, where the loss of the mature erythrocytes of the circulating blood is being constantly replaced by new cells originating in the so-called blood forming organs.

The first cells in the embryonic blood are apparently all of one sort, derived from the blood islands. They are large, round cells with a delicate membrane and a pale granular protoplasmic reticulum; their relatively large nuclei contain a fine chromatin network with several coarse chromatin masses. Hæmoglobin later develops in their protoplasm, giving it a refractive, homogeneous appearance. Often the hæmoglobin has been more or less dissolved from the corpuscles, which then appear granular or reticular. The developing red blood corpuscles are known as *erythroblasts*, especially in their younger stages when the nuclei are reticular. In later stages the nuclei become densely shrunken or pycnotic, and stain intensely with hæmatoxylin. The entire cells become smaller, and are then called *normoblasts*. During the transition from an erythroblast to a normoblast the cells divide repeatedly by mitosis.

In becoming mature red corpuscles the normoblasts lose their nuclei. Before they disappear, the pycnotic nuclei often assume mulberry, dumbbell, trefoil or other irregular shapes. According to older observations they then fragment, and are dissolved within the normoblasts; but it is now generally believed that they are extruded from the cells, either in one mass or in detached portions, and that the extruded nuclei are devoured by phagocytes. Emmel[1] watched the formation of non-nucleated red corpuscles, or plastids, in living blood from pig embryos. He found that the apparent extrusion of the nucleus is caused by the constriction of the cytoplasm of a nucleated cell, the nucleus and part of the protoplasm finally separating from a non-nucleated portion, the plastid, or red corpuscle. In the adult, however, cells with fragmented nuclei are much more commonly seen than those showing constriction. The loss of the nuclei begins in human embryos of the second month. In embryos of the seventh month, nucleated corpuscles in the circulating blood have become infrequent, and after birth it is rare to find one, except under pathological conditions.

In withdrawing from the circulating blood the nucleated red corpuscles do not disappear from the body. Since 1868 it has been known that the red marrow, found within certain bones in the adult, contains an abundance of erythroblasts, which multiply by mitosis. They are the source of the new corpuscles constantly entering the circulation. Before

[1] Emmel, 1914.

the marrow assumes the blood-forming function, the liver is the chief hæmatopoietic organ. Beginning in embryos of about 7.5 mm., and continuing until birth, erythroblasts are found between the hepatic cells and the endothelial cells of the sinusoids, and in certain stages they occur in vast numbers. Toward birth, however, the erythroblasts in the liver are no longer abundant, and in a few weeks after birth they are said to disappear entirely. Red blood corpuscles are formed also in the embryonic spleen, though to a less extent than in the liver, and in some mammals the spleen normally contains erythroblasts in the adult.

In the bone marrow the types of young cells do not seem exactly comparable to those found in the embryo. The youngest forms correspond to the erythroblasts of the embryo. They are large cells, without hæmoglobin; the clear protoplasm stains with basic dyes and is reticular. The chromatin is in large blocks, and occasionally tends to have a 'cart-wheel' arrangement. But whereas in the embryo the normoblast remains large (megaloblast) and produces a larger erythrocyte (megalocyte) by the loss of the nucleus, in the adult bone marrow the erythroblast constantly shrinks in size as it passes through a more finely graded series of changes, recognizable by the employment of special techniques. With stains combining an acid and a base, the protoplasm of the erythroblast first shows a definite basophilia, then as hæmoglobin increases it becomes polychromatic. The cells are designated basophilic erythroblasts and polychromatic erythroblasts, which are younger than normoblasts. The loss of the nucleus may lead to cells with 'Cabot rings' or with 'Howell-Jolly bodies' (*i.e.*, minute or larger basophilic bodies supposed to represent fragments of degenerating nuclei). The reticulocyte is now recognized as a nearly mature erythrocyte,[1] found normally in small percentage in the circulating blood. It is not recognizable by Wright's technique, but by the use of supravital stains such as cresyl-blue a fine reticulum can be brought out in certain of the red cells, both in the blood and in the bone marrow. The fragmented basophilic material, not in the form of a net, seen sometimes in the erythrocytes in ordinary smears, known as punctate basophilia, is thought to be due to drying or heat, and hence of no significance. Mitochondria are present in the erythroblasts, but decrease in number as the cells mature and are absent in the erythrocyte. In the blood of certain individuals the erythrocytes on standing for some time develop long filaments, which may be motile and may become beaded and then fragment.[2] The significance is unknown.

It will be noticed that the terms applied to developing corpuscles are compounded of words which describe the formative cells, instead of indicating what they produce. Thus *erythroblast* signifies a red formative cell. *Normoblast* (Lat. *norma*, model or type, and

[1] KEY, 1921. [2] AUER, 1933.

Gr. βλαστός, bud) is an objectionable term to designate a nucleated red corpuscle of the usual size and form, in contrast with the large *megaloblasts*. The immature stages of red corpuscles, not normally found in the circulating adult blood, are present in certain of the so-called blood diseases, which are more properly diseases of the blood-forming or blood-destroying organs. Their presence in the blood stream indicates either an over-production of young cells or a too rapid loss of mature erythrocytes, the developmental forms being called on to maintain the normal number.

In regard to the source of the erythroblasts in the spleen, liver and red marrow, two opinions are held. It is well known that in the embryo erythroblasts may wander out of the vessels into connective tissue. Accordingly it is often stated that the circulating erythroblasts, which at first multiply in the blood vessels, later withdraw to the reticular tissue of the liver, spleen, and marrow and there proliferate. Others consider that the erythroblasts are formed *in situ* in these various places from the endothelial or reticular tissue cells. The mature erythrocytes, after a short life in the blood stream, probably fragment and are destroyed either by the phagocytic macrophages and reticulo-endothelial cells of the spleen and liver, or by some chemical dissolution.

Both phylogenetically and ontogenetically the developmental history of the red blood corpuscle is repeated. In adult fishes nucleated cells comparable to erythroblasts are the normal form; in reptiles and amphibians the normoblast is the adult cell. In the latter class, however, there are a few species in which the blood is characterized by the presence of non-nucleated erythrocytes, which are thus not strictly confined to mammals.[1] In young mammalian embryos, again, the cell with vesicular nucleus appears first, later are found forms with contracted nuclei, and still later the non-nucleated plastids. Typical erythroblasts are shown in sections of a chick embryo of 96 hours (Fig. 44), and normoblasts are present in the blood stream of 10 mm. pig embryos.

White Corpuscles. The white corpuscles or leucocytes are those blood cells which retain their nuclei and do not contain hæmoglobin. The number of white corpuscles in a cubic millimeter of human blood is about eight thousand. If it exceeds ten thousand the condition is called *leucocytosis* and becomes of clinical importance. There exists, therefore, normally but one leucocyte for five or six hundred red corpuscles. In the circulating blood the two sorts are said not to be evenly mixed; the leucocytes are more numerous in the slower peripheral part of the blood stream, near the endothelium.

The leucocytes may be divided, according to their nuclear characteristics, into three classes, namely, into lymphocytes, monocytes, and polymorphonuclear leucocytes. According to the character of their cytoplasm they may be classed as granular and non-granular. The polymorphonuclear leucocytes are the granular (often called granulocytes); the non-granular are the lymphocytes and monocytes.

[1] Emmel, 1924.

In the study of the leucocytes it has been found useful to employ numerous techniques. In fresh smears of blood the nuclear characteristics are not clear and the protoplasmic granules can be distinguished only by their size and refractility; but if the cover be ringed with vaseline to prevent drying and the slide kept warm, the living cells can be studied for motility and phagocytosis. In the ordinary blood smears stained by a combination of acid and basic dyes (usually methylene blue and eosin, Romanowsky stains, Wright's stain) the cytoplasm and cytoplasmic granules are differentiated, but the cells are dead and the nuclei not well preserved. In fixed tissues the blood cell nuclei are shown in a condition comparable to that of other tissue nuclei, but the granules in certain cases have been dissolved. Realizing these difficulties, especially important in the studies of the developmental forms of the leucocytes, which themselves are important in blood disturbances where younger forms are called into the blood stream to replace those lost by disease, Sabin and her associates devised a new combined method. 'By the supravital technique, we mean the study of living blood cells, differentiated by means of their reactions to very small doses of certain dyes.'[1] The blood is spread on a slide already coated with a thin film of some non-toxic dye, the cover ringed with vaseline and the preparation examined in a warm-box. The nuclei are not stained, but the activities of the cell can be studied to much better advantage. In the differential study of the leucocytes all these techniques will be drawn upon.

The *polymorphonuclear granular leucocytes* comprise three distinct classes of cells differing in the type of granules present in their cytoplasm. The granules are specific in that all the granules in any one cell are of the same kind, as shown by their size and staining reactions. They are apparently not secretory like those in gland cells, they do not represent phagocytosed materials, and they are constant throughout the life of the cell. The characteristic nucleus is irregularly bent and constricted, showing nodular subdivisions joined by narrower bands, or by mere threads of nuclear material, leading to an endless variety of shapes. The term 'polynuclear,' indicating as it does a cell with many nuclei, is thus incorrect; whereas 'polymorphonuclear,' signifying that the nuclei are of many forms, is eminently fitting. This deformity of the nuclei has been ascribed to degenerative changes, comparable to those seen in the normoblast nuclei; and it is true that the polymorphous forms are derived from spherical, through crescentic shapes, in the developmental stages; but there is no pycnosis, since the individual subdivisions remain vesicular. On the other hand this shape is by some considered to be of advantage as affording more nuclear surface and thus a greater nucleo-cytoplasmic

[1] Sabin, Cunningham, Doan and Kindwall, 1925.

ratio. The relative age of the cell is judged by the increasing subdivision of the nucleus. In normal blood one finds a certain constant proportion of polymorphonuclear leucocytes with one, two, three or more nuclear lobes (Arneth's scale), and an increase of those with fewer lobes—a 'shift to the left' on the scale—indicates a too rapid loss of the older forms and a replacement by younger.[1] The value of this count has been doubted,[2] and another scale is suggested depending on whether the lobes are connected by broad bands or by slender filaments, the former supposedly indicating the younger cells.[3]

Neutrophil. The commonest form of polymorphonuclear leucocyte, and the one usually referred to by that term, is the neutrophil. This type comprises between 60 and 70% of all the leucocytes of the blood. The granules within the protoplasm are closely packed and so fine that they are often hard to distinguish as separate particles, though the cytoplasm looks granular. These cells are easily recognizable in fresh unstained preparations; in blood smears stained by Wright's or some similar method the granules appear purple or lilac by taking both acid and basic dyes simultaneously, and it is from this characteristic that the cells are called neutrophils. They also contain mitochondria, Golgi apparatus, and centrosome, the last two situated in the bay of the typically polymorphic nucleus. They are extremely motile and phagocytic, and often contain vacuoles stainable with neutral red. They produce a proteolytic enzyme which enables them to destroy bacteria or injured tissues, to which they migrate from the blood vessels in great numbers.

The *eosinophil*, found less commonly in the blood stream (2 to 4%) and more commonly in the tissue spaces, especially of the breast and intestinal mucosa, is distinguished by having fewer and larger granules of uniform size, which stain brilliantly with acid dyes. For this reason these cells are also called oxyphils or acidophils. The staining qualities of the granules led to the theory that they were ingested particles of red blood corpuscles or particles containing hæmoglobin derived from degenerating muscle cells. They are very numerous in the vicinity of the atrophying gills of the salamander.[4] This is of interest in connection with cases of trichiniasis in man, in which the parasite causes degeneration of the muscles, and at the same time the number of eosinophils in the blood becomes greatly increased; but eosinophilia also accompanies many other parasitic invasions with no muscular atrophy, and if the granules are hæmoglobin derivatives their presence in the cells is not yet fully explained. The nucleus is seldom divided into as many lobes as in the neutrophil, and is often round or merely crescentic especially

[1] Cooke and Ponder, 1927. [2] Bunting, 1932.
[3] Farley, St. Clair and Reisinger, 1930. [4] Badertscher, 1913

in the tissue eosinophils. Eosinophils are less motile, less phagocytic than neutrophils.

Basophils contain large granules in the cytoplasm which differ from those of the eosinophils in that they take basic stains and are often fewer and of unequal size. With Wright's stain the granules are therefore dark blue and readily distinguishable. The nuclei are seldom more than crescentic. By appropriate stains one can bring out, as in the other granular leucocytes, mitochondria, Golgi apparatus, centrosome, and neutral red granules. They are still less active than the eosinophils in motility and phagocytosis. They are very few in human circulating blood (1–3%), though much more common in some animals, and it is not known whether they are the same type as the mast cell of the connective tissues. The latter are more phagocytic and the granules more uniform in size. The basophilic granules are soluble in water, so that the cells are hardly to be identified in many fixed tissues.

In the study of the blood of laboratory animals it is well to note that the size and staining of the granular leucocytes may differ from the human standard The neutrophils in the mouse and rat do not stain properly with the usual stains; the neutrophiles in the Guinea pig and rabbit take a distinctly eosinophilic tinge, and the individual granules are larger, though still in the proper proportion to the others. Neutral red, used as a supravital dye, is an indicator, turning yellow with alkalis and brick red with acids, so that eosinophilic granules will be yellow, but basophilic granules red. The staining and treatment of the blood under examination should always be kept in mind.

Lymphocytes have already been briefly described among the constituents of connective tissue. Ordinarily they are small cells, about the size of red corpuscles, but since they are spherical they will appear smaller when floating and larger when flattened on the slide. Moreover, larger ones are found so that it is now usual to designate small, medium and large lymphocytes, the latter being two or three times greater in diameter. In the small lymphocytes the cytoplasm forms a narrow rim, sometimes crescentic or almost imperceptible, about the dense, usually spherical nucleus. In the large ones the cytoplasm is much more abundant, the nucleus larger and oval or kidney-shaped. The cytoplasm stains definitely with basic dyes and is peculiarly smooth and non-granular, and there is a very delicate cell membrane. The chromatin of the nucleus is arranged in a network associated with coarse chromatic masses, giving a characteristic checkered appearance. Some of the masses rest against the nuclear membranes, giving somewhat the appearance of the spokes of a wheel (Radkern), more noticeable in the spherical nuclei. The centrosome lies on the side where the cytoplasm is most abundant. Mitochondria are present in lymphocytes except in a small percentage which are supposed to be moribund. Lymphocytes are

actively motile, travelling with nucleus forward, but the motility does not begin in fresh smears until after several minutes. They are only slightly phagocytic, though by the supravital technique and appropriate stains they are often found to contain a few azurophil granules and vacuoles colored by neutral red. The lymphocytes of the adult are derived from the lymph glands and other lymphoid tissue (see p. 288), and are the same as those found in connective tissue. They are supposed to be eliminated from the body by passing through the intestinal epithelium, or they may be transformed into plasma cells. They form 20–25% of all leucocytes.

The *monocytes*, or large mononuclear leucocytes or endothelial phagocytes, are the largest of all the white cells, yet in certain cases they are practically indistinguishable from large lymphocytes. Typically they show a greater proportion of cytoplasm to nucleus, and the nucleus is smaller and more indented, but at times it may be merely oval. The chromatin is more finely divided, less block-like, the whole nucleus giving a paler appearance. With Wright's stain the cytoplasm of the monocytes shows a peculiar muddy blue,[1] distinctively different from the clear basophilia of the lymphocytes. With eosin-azur it is deeply basophilic, with fine red granules. With neutral red in vital staining it contains numerous neutral red granules or vacuoles, and these often cluster around the centrosome near the notch in the nucleus, forming a 'rosette,' which was thought to be a distinctive mark of the monocyte by Sabin; but this formation is also found in certain other cells, plasma cells, some macrophages, and some of the older lymphocytes. In position it occupies the same region in which a Golgi net may be demonstrated by appropriate technique. Mitochondria are numerous. Monocytes are notably motile and phagocytic, being called out especially by the presence of the bacillus of tuberculosis. Their normal number in the blood stream is from 4 to 8%. The manner in which specific disease agents may cause an increase of a single type of blood cell in the peripheral blood stream is not understood.

The monocyte used to be called a 'transitional cell,' believed to be in transition between the lymphocyte and the granular leucocyte. Though this idea has been superseded, there are still many theories as to its derivation. That it is a desquamated endothelial cell, as another of its names would imply, is denied by the Clarks[2] after long study of living vessels; there is not the 'slightest evidence of the formation of 'endothelial leucocytes." Some claim that it may arise from fixed tissue histiocytes, which have found their way into the blood stream, a theory to which its violent phagocytosis might give support, or, on the other hand, it may

[1] Sabin et al., 1925 [2] Clark and Clark, 1927.

arise from the lymphocyte,[1] from which it is at times practically indistinguishable, and be actually a transitional type leading ultimately to a tissue macrophage by withdrawing from the blood stream. Several authors have shown that macrophages[2] and even fibrocytes[3] can develop from normal monocytes. If this latter theory is true (for there is as yet no consensus of opinion in the matter), the lymphocyte must be considered as a young cell which may divide to produce more lymphocytes, but is capable of continued specialization to form plasma cells or monocytes or even macrophages. Lymphocytes are often noticed in mitosis, monocytes very rarely.

The total number of the leucocytes in the peripheral blood, which is that ordinarily taken for examination, and the percentages of the different types are subject to considerable variation. Intense heat, exercise, even change in position or change in the mental state all give immediate increase of white cells which may last for two or three minutes, as though many cells had been stuck to the blood vessel walls and suddenly were swept into the stream by a swifter current. But in addition to these momentary increases certain more permanent ones have been recognized: a digestive increase, one to three hours after meals, an afternoon increase, and an hourly increase, forming a rhythmic pattern traceable through the others.[4] The hourly 'showers' are due mostly to an increase in the neutrophils, and of these most are non-motile, which are presumed to be old, almost dying forms.[5] The lymphocytes seem to have a rhythmic increase of their own at half hourly periods. These periodic 'showers' have, however, been disputed by Ponder and his co-workers who find that the momentary increases may be so great (60–100%) and from so many unpredictable sources that a true rhythmic change could hardly be verified.[6] They failed to find either a digestive leucocytosis or the hourly 'showers.' The subject deserves further study.

The origin of the different types of blood corpuscles and their relations to each other have been the subject of an immense amount of investigation. Strictly speaking, the youngest stages of erythroblasts are leucocytes, in that they retain their nuclei and do not contain hæmoglobin. Many still believe that all types of blood corpuscle, red and white, come from a common stem cell, the *hæmocytoblast*, derived from either the mesenchyma or the reticular tissue of certain organs. This cell, with large vesicular nucleus and basophilic cytoplasm, granular-appearing, but without specific granules, has also been called the mesamœboid cell, primitive lymphocyte, lymphoidocyte, hæmatoblast,

[1] Bloom, 1926*a*. [2] Lewis, M. R., 1925*a*. [3] Carrel and Ebeling, 1926.
[4] Sabin et al., 1925. [5] Henderson, 1928.
[6] Ponder, Saslow and Schweizer, 1931.

and other terms, leading to considerable confusion. The conception that all blood cells are derived from this source is known as the 'monophyletic theory.' Opposed to this is the 'polyphyletic theory,' supported by those who believe in diverse origins of red and white corpuscles, and of the various forms of white corpuscles, each type being distinct from the beginning. A third, the 'dualistic theory,' would provide two parent cells, one for the red corpuscles and one for the leucocytes.

The question is complicated by the extravascular origin of most, if not all, of these cell types in the adult, and the similarity or identity of some of the free cells of the connective tissue and the blood leucocytes. Leucocytes can leave the blood vessels and wander in the tissue spaces, yet the eosinophils and mast cells of the connective tissue are considered by many as distinct from those in the blood. The same distinction does *not seem to be made in the case of the lymphocytes and neutrophilic* polymorphonuclear leucocytes, and the monocytes of the blood are usually considered the same cells as the macrophage of the tissue spaces.

The attempt to trace the lineage of cells depends on the recognition of transition stages between one type and another. The changes may be evident in the shape and character of the nucleus, in the staining of the cytoplasm, or in the acquisition of characteristic granules in the cytoplasm. Unless under exceptional circumstances the variation is slight, and differences of opinion may arise from differences of interpretation. The continuous observation of individual cells as they make their changes has not been accomplished.

Against the monophyletic interpretation, it has been asserted that the lymphocytes of the adult are a different form of cell from the primitive blood cells, and that they are not found in embryos until the time when lymph glands develop. These arise rather late—in rabbits of 25 mm. and in human embryos of 40 mm.[1] Some authors meet this by recognizing two groups of red cells, the primitive intravascular forms that disappear, and the definitive extravascular (i.e. developing extravascularly, from lymphocytes) group that is permanent.

Some of the probable developmental processes will be seen in the study of the blood-forming organs, the lymph glands and the bone marrow. The question is still a live one.

Blood platelets (Fig. 231) are small granular bodies (*Körnchen, plaques*) which were recognized as a normal constituent of the blood by Schultze in 1865. Previous references to them occur, and Zimmermann[2] described them as 'elementary corpuscles,' believing that they gave rise to red corpuscles. They are 2–4 μ in diameter, and between 245,000 and 778,000 have been estimated to occur in a cubic millimeter of human blood.

[1] Lewis, F. T., 1909*b*. [2] Zimmermann, G., 1860.

They are readily reduced to granular débris in ordinary sections, but when well preserved and properly stained, they are found to consist of a central granular core and a hyaline outer layer. Often they appear stellate, and on a warm stage they exhibit amœboid movements. They are concerned in the clotting of the blood, or thrombus formation, and during coagulation threads of fibrin extend out from them. It is possible, however, that they are only passively involved in this process. In the amphibia certain small spindle-shaped cells appear to be similarly related to fibrin-formation, and they are called *thrombocytes;* the same term is sometimes applied to the blood platelets, but the mammalian platelets are not small cells, since they do not contain nuclear material. Their staining reactions lend no support to the idea that they are frag-

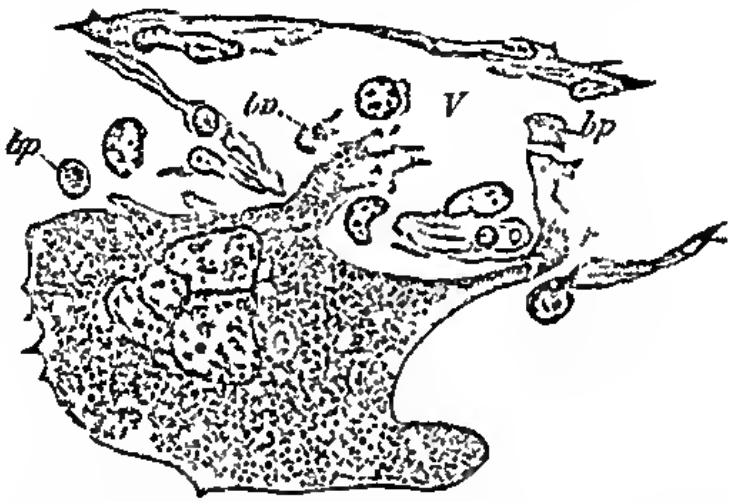


Fig. 235.—Giant Cell from the Bone Marrow of a Kitten, Showing Pseudopodia Extending into a Blood Vessel (V), and Giving Rise to Blood Platelets (bp). (J. H. Wright.)

ments of disintegrating red or white corpuscles. In blood clots several days old, blood platelets are still found, indicating that they have more than a transient existence.

The source of the blood platelets was demonstrated by J. H. Wright[1] in 1906. Fig. 235 represents a giant cell of the bone marrow, sending out two processes or pseudopodia into a blood vessel; the endothelium is interrupted at their place of entrance. By the special stain which Wright perfected, the cytoplasm of the giant cell consists of an endoplasm, containing red or violet granules, and a clear blue exoplasm, identical respectively with the central granules and clear peripheral cytoplasm of the blood platelets. The platelets are clearly detached portions of the pseudopodia. Fig. 235 shows a few such detached platelets, and one which is budding off from a pseudopodium, but the color contrasts which make the original preparations convincing are scarcely indicated in the reproduction. Wright found that only few of the giant cells showed this

[1] Wright, 1910.

condition, and although the process takes place also in the spleen this source seems almost inadequate to supply the enormous number of platelets in the blood. Some authors have disagreed with Wright's findings. Bunting,[1] while accepting the theory in general, calls attention to an 'unusual compensatory or vicarious formation of bodies, analogous, at least, to the normal blood-platelets,' arising from the pseudopodia of

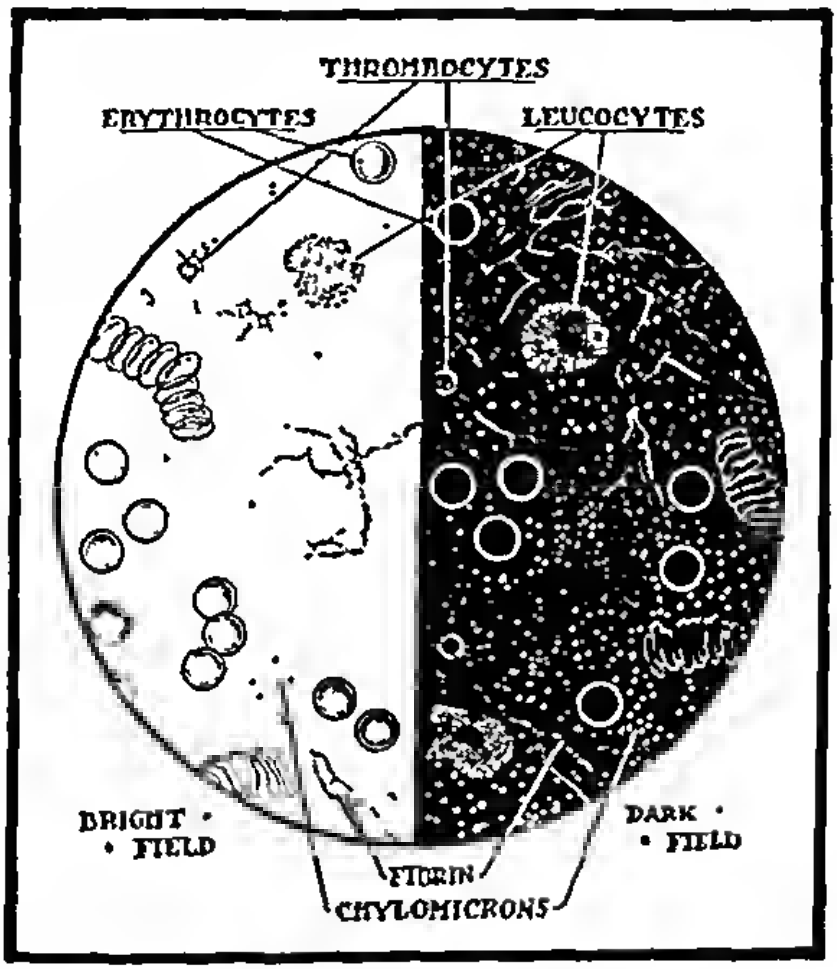


FIG. 236.—FRESH BLOOD WITH CHYLOMICRONS.
Right half of field, under dark-field and left half under light-field (Gage and Fish.—courtesy of the Wistar Institute)

large lymphocytes containing azurophil granules, in a case of influenza. Emmel[2] finds that in some of the lungless amphibia platelets are present in the blood, derived from the nucleated thrombocytes by segmentation of the cytoplasm.

Plasma is the fluid intercellular substance of the blood. It contains various granules, some of which are small fat drops received from the thoracic duct. These increase after the ingestion of a fat meal, and are absent unless fat is eaten. They are carried to the tissues for their nutrition, and thus gradually disappear from the plasma. They are usually ultramicroscopic in size, but revealed by dark-field illumination. Gage[3]

[1] BUNTING, 1920. [2] EMMEL, 1925. [3] GAGE AND FISH, 1924.

has studied them carefully and proposes the name of 'chylomicra.' Other granules occurring in variable quantity are refractive particles, not fatty, either round or elongated; they are known as hæmatoconia (or hæmoconia). In ordinary sections the plasma appears as a granular coagulum. In the process of clotting, fibrin forms from the plasma, and with the entangled corpuscles, it constitutes the blood-clot; the fluid which remains is the serum. The process of fibrin formation is of considerable histological interest, owing to a possible analogy with fibril formation in connective tissue.

For more details the student is referred to H. Downey, 1938, Handbook of Hematology, 4 vols., 1448 illustrations, New York. This is the most comprehensive and modern work on the blood and the blood forming and destroying organs.

LYMPH

The contents of the lymphatic vessels is called lymph. This fluid is not identical with plasma, or with tissue fluid, yet all three are similar. Nutrient material passes from the plasma into the tissue fluid which bathes all the cells of the body; and in return the products of metabolism of the non-epithelial cells enter the tissue fluid from which they may be taken over into either the plasma or lymph, first passing through the endothelial walls of the vessels. Thus in the intestine much of the absorbed fat is transferred across the tissue spaces to the lymphatic vessels (lacteals) within which it forms a milky emulsion known as *chyle*. This form of lymph mingles with other varieties coming from the various parts of the body, and together they are poured into the plasma at the jugulo-subclavian junction. Histologically lymph appears as a fine coagulum, containing lymphocytes and large mononuclear phagocytic cells. The cells are not abundant. Occasionally other forms of blood corpuscles are found in lymphatic vessels, but the lymphocytes greatly predominate.

The fluid within the peritoneal, pericardial, and pleural cavities contains normally a small number of cells, which have been classed by Emmel[1] in two groups: the basophilic and usually phagocytically active cells or 'cœlomic macrophags,' and the eosinophilic, non-phagocytic cellular elements. Emmel derives the former chiefly from the proliferation of the lining mesothelium, the latter from degenerating red blood corpuscles. Lymphocytes are rare or entirely lacking.

The cerebrospinal fluid, varying chemically from both blood plasma and lymph, has extremely few cells, mostly lymphocytes with an occasional large mononuclear leucocyte. Pathologically other cell types may be present in large numbers.

[1] Emmel, 1916.

Special Histology

BLOOD FORMING AND BLOOD DESTROYING ORGANS

BONE MARROW

Bone marrow is the soft tissue found within the central cavities of bones. Its source in the embryo is the vascular mesenchyma invading a cartilage which is being replaced by bone. Early in its development it contains osteoblasts and osteoclasts, and these cells may be found in adult marrow, where it is in contact with the bone. The greater part of the mesenchyma becomes reticular tissue with fat cells intermingled. Where the fat cells predominate, as in the shafts of the long bones, the tissue is called 'yellow marrow,' as distinguished from 'red marrow' where fat cells are infrequent. The meshes of the reticular tissue are occupied by an extraordinary variety of cells, most of which are called *myelocytes* (marrow cells). In ordinary sections the tissue of the marrow appears to be riddled with large round holes. Under high magnification the holes are seen to be fat cells, the nuclei of which are here and there included in the section (Fig. 237). The reticular tissue framework of the marrow consists of flattened cells, generally seen cut across; their nuclei then appear slender and elongated. The abundant meshwork of fibrils associated with these cells is not apparent in ordinary sections, because the great number of cells in the meshes overlap the reticular strands. These floating cells comprise hæmatocytoblasts (or 'stem cells'); promyelocytes (myeloblasts); myelocytes which are neutrophilic, eosinophilic or basophilic; erythroblasts and normoblasts; young lymphocytes; mature corpuscles both red and white; and giant cells.

The hæmocytoblasts (as described by Maximow and Bloom[1]) are large cells, 'of lymphoid nature.' These authors believe that they occur extravascularly, and may produce all types of blood cells, red as well as white. Sabin[2] and her associates regard this same type of cell as already marked as the progenitor of granulocytes by containing mitochondria differing from those of the lymphocyte series, and therefore call them myeloblasts. They think them derived from the reticular tissue cells of the framework. Downey[3] recognizes this cell as the 'primitive lymphocyte,' and distinguishes a second type as the myeloblast. All agree that the primitive cell is characterized by its large, round, vesicular nucleus,

[1] Maximow and Bloom, 1932. [2] Sabin, 1932. [3] Downey, 1932.

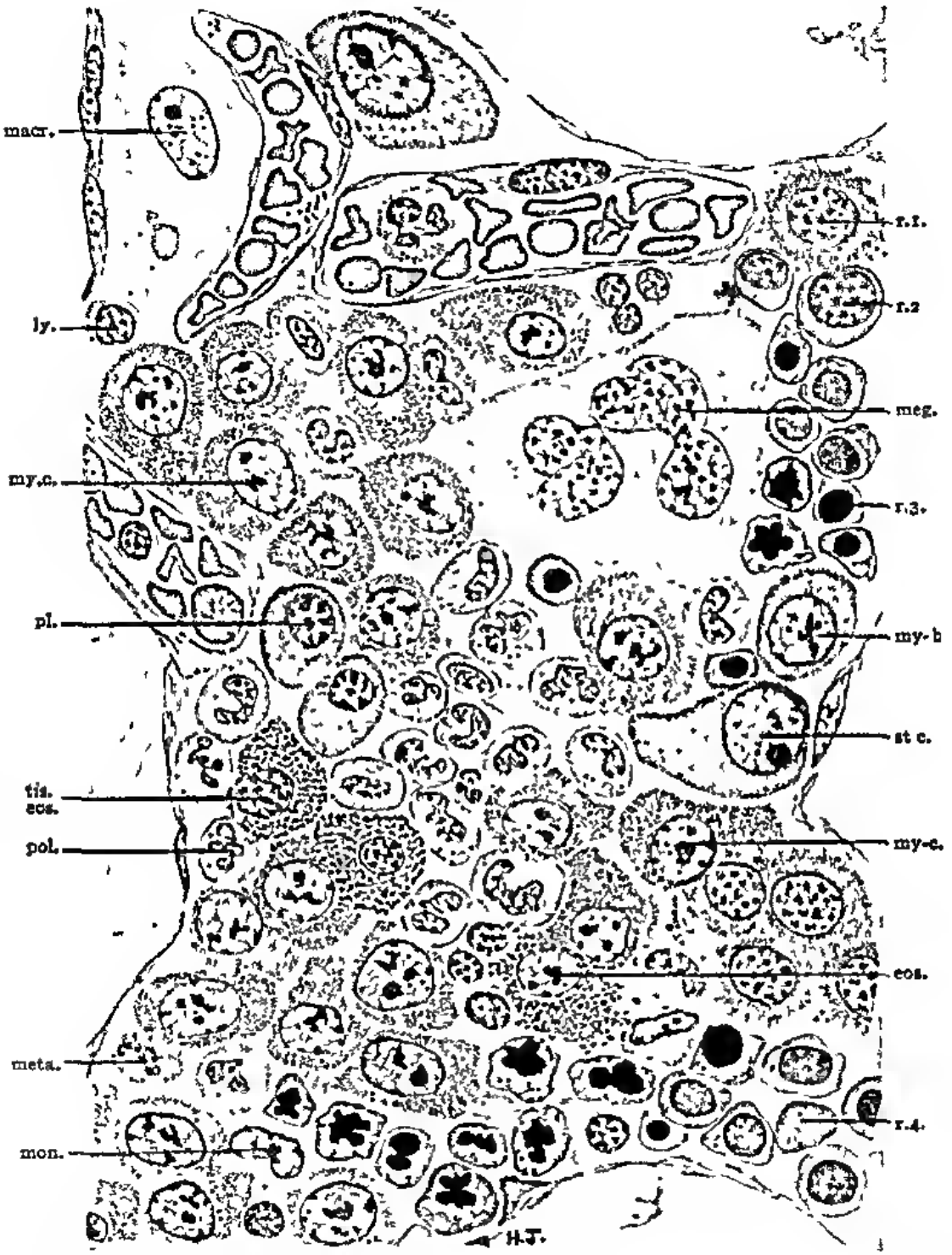


FIG 237 —NORMAL VERTEBRAL BONE MARROW, ADULT MAN.

Zenker fixation, decalcified and stained with phloxine-methylene blue In one field representative cells have been brought together in the proper proportion and relation one to another in order to illustrate the typical normal picture Zeiss ap obj 90, oc 10 Eos , eosinophil, ly , lymphocyte, macr , macrophage, meg , megakaryocyte; meta , metamyelocyte, mon , monocyte, my-bl, myeolblast, my-ce , myelocyte, pl., plasma cell, pol , polymorphonuclear leucocyte, r.1, r 2, r 3, r 4, stages in the red corpuscle formation; st. c , stem cell, tis. eos , tissue eosinophil.

in which the chromatin is in one or two large, heavily staining masses, and by a basophilic cytoplasm without specific granules. It is often seen in mitosis, thus assuring a constant supply; but it also may produce cells of other types, which all become progressively smaller as they mature. As to the definite characteristics of the various types of cells and their relation to each other in the early stages there is as yet no agreement.

In sections of human bone marrow fixed in Zenker's fluid and stained with phloxine-methylene blue (Fig. 237), as opposed to bone marrow smears stained by one of the Romanowsky stains, the cytoplasm of the stem cell appears distinctly granular, though still without specific granules. The cell is often angular in shape as it adapts itself to the reticular tissue network. Some believe it is possible to detect as the next step two cell types, one, the myeloblast, the other, the erythroblast. The former has the same type of nucleus as the stem cell, but less cytoplasm, and is round or oval, with smooth contour. The erythroblast is also spherical, but the cytoplasm is definitely irregular in outline, 'like a burr,' so that groups of these cells often seem stuck together; the nucleus is smaller, the chromatin in finer masses than in the stem cell. Thus by some the red cell series is recognized before the advent of the hæmoglobin, and for this reason the nomenclature has been shifted by them so that the cell with hæmoglobin and vesicular nucleus is called a normoblast, that with pycnotic nucleus a 'nucleated red cell.' The hæmoglobin appears rather suddenly, and as it increases the basophilia decreases and the cell becomes smaller; the nucleus also shrinks, becomes pycnotic, and then is extruded. The immature red blood cells are found usually in small groups.

Cells in developmental stages leading to any one of the three types of granular leucocytes are called myelocytes; the prefix neutrophilic or eosinophilic, etc., indicating the special type. The nucleus becomes smaller, the chromatin more finely divided; oval or indented shapes occur (myelocytes with rod-shaped nuclei are called *metamyelocytes*) and finally the subdivision into the beaded form may take place. The cytoplasm is reduced in amount with the reduction of the nucleus; and the characteristic granules appear in it. These may occur at first in one part only of the otherwise hyaline cytoplasm, and in any one cell are always of the same type. In the drawing, neutrophilic and eosinophilic myelocytes are shown. Cells with basophilic granules may appear, but according to Ringoen[1] these cells are merely an 'unripe' form of eosinophil, which change in the bone marrow to the adult form. The granules of true basophils are lost after Zenker fixation, and even in smears of bone marrow basophils are seldom seen. They are abundant in marrow smears

[1] Ringoen, 1915.

of the rabbit, however; in fact animal marrow differs greatly from human in the types of cells present.

As the granules appear the basophilia gradually disappears, the cells show increasing motility, and the nucleus becomes oval and then crescentic. On the successive proportions of these characteristics Sabin recognized three classes of myelocytes, A, B, and C. These cells and the more primitive ones are found in the blood stream only in diseased conditions, when for some reason there are too few granulocytes in circulation. The mature cells may remain for a while in the bone marrow, but soon enter the vessels. The normal proportions of the different cell types in the bone marrow are given by Custer.[1]

Lymphocytes are not a conspicuous element of the marrow, yet they are present and sometimes in disease become abundant. Young forms, such as are found in the lymph glands, may be present, showing that lymphocyte production is a minor function of this organ. Plasma cells are normally present in small numbers.

The giant cells of the marrow have a single polymorphous nucleus. They have therefore been named 'megakaryocytes,' in distinction from the multinucleate osteoclasts or 'polykaryocytes,' which may also be present in relation with bone spicules. The nucleus is so large that it may be cut into several slices, and by combining these it has been found that the entire nucleus is a hollow sphere with perforated walls; the nuclei, however, are very irregular, and some may be of other forms, thought to represent different growth phases of the cells.[2] With Wright's stain the cytoplasm clearly shows an outer hyaline exoplasm and an inner granular endoplasm. It has been said that the latter is divisible into two concentric zones, which differ from the protoplasm within the nuclear sphere. In ordinary preparations these details are not evident. A large number of centrosome granules (over one hundred) have been found, and pluripolar mitoses have been observed. Their origin is described by Kingsley,[3] in the liver of the fetal pig, as from 'hæmocytoblasts differing from other stem cells only by the presence of specific granules,' without passing through any non-granular lymphoid stage (Fig. 238). They are said not to be phagocytic, though cells may be pressed in to their cytoplasm. Their important function of producing blood plates has already been described (p. 277).

The circulation in the bone marrow comprises arteries and arterioles which empty into venous sinuses lined with phagocytic reticulo-endothelial cells connecting with the true vein radicles. By a combination of injection methods and clearing the tissue of most of the free cells (which

[1] Custer, 1935. [2] Kaufman, 1929. [3] Kingsley, 1935.

can be done by starvation or by repeated bleeding, since the younger cells are thus called into the blood stream), it was found[1] that in the pigeon the venous sinuses are interconnected by a capillary net, perhaps usually closed. In these capillaries, between collapsed segments, the formation of red blood corpuscles (erythropoiesis) is said to take place, stimulated perhaps by a lack of oxygen. The mature cells of such a colony would be projected into the blood stream by the opening up of the collapsed segments. Erythropoiesis would thus be intravascular, while the leucocytes arise extravascularly and must make their way into the vessels. Others maintain that the walls of the sinuses are incomplete and that all the blood cells, arising in the tissue spaces, migrate into them. In fixed normal bone marrow the capillaries are unrecognizable and the sinuses hard to define.

FIG. 238.—PHOTOGRAPH OF TWO YOUNG MEGAKARYOCYTES IN LIVER OF FETAL PIG, SHOWING SPECIFIC GRANULES. (Kindness of D. M. Kingsley.)

The functions of the marrow are the production and dissolution of bone, the storing of fat, the formation of granular leucocytes (neutrophils, eosinophils, and basophils), of red corpuscles, and to a less extent of lymphocytes; to these some would add the destruction of red corpuscles, as indicated by ingested fragments and intercellular granules.

LYMPH NODULES AND LYMPH GLANDS

The lymph glands arise as nodules of dense tissue in close relation with an artery, a vein and a lymphatic vessel, as seen in the photographs, Figs. 239 and 240. The first distinct lymph glands in the body are a pair *in the axillary region, a pair in the iliac region, and a pair or two in the* maxillary region. They are found in rabbit embryos of about 30 mm., and in human embryos of about 40 mm. These first glands are soon followed by others in their vicinity, producing axillary, inguinal and cervical groups, respectively; and scattered glands more peripherally situated along the vessels develop later. At the same time, the tissue around the jugular and mesenteric lymph sacs becomes transformed into dense lymphoid tissue, which is resolved into the chains of deep lymphatic glands. These acquire a structure similar to that of the superficial glands. There is no satisfactory evidence that the dense lymphoid tissue of which

[1] DOAN, CUNNINGHAM AND SABIN, 1925.

the glands are composed is produced by the emigration of cells from the arteries, veins or lymphatics associated with them.

In further development the lymph glands become organized as shown in the diagrams, Figs. 241 and 242. The left half of each diagram represents a younger stage than the right half. These instructive figures were prepared by Stöhr on the basis of Kling's studies.[1] In the youngest stage it is seen that the blood vessels enter and leave the gland on one side, at a place called the *hilum* (Lat., a small thing, applied to the eye of a bean, and to similar hollows in bean-shaped organs). The lymphatic vessel, as a plexiform *peripheral sinus*, encircles the entire structure. After the gland has enlarged, lymphatic vessels extend into the mass of

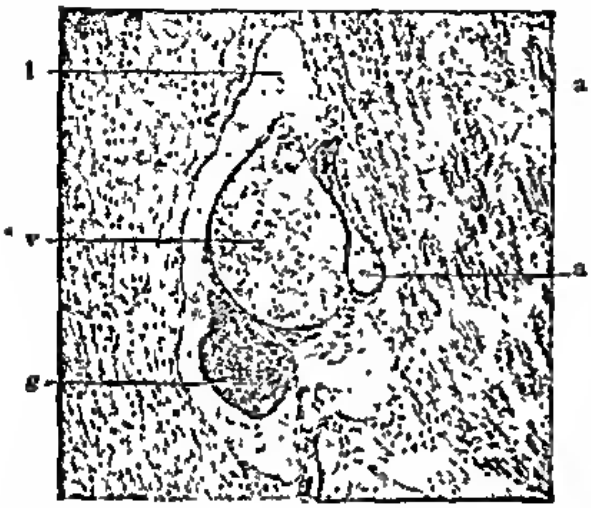


Fig. 239.—The First Axillary Lymph Gland of the Rabbit From an Embryo of Twenty Days, 29 mm. × 60

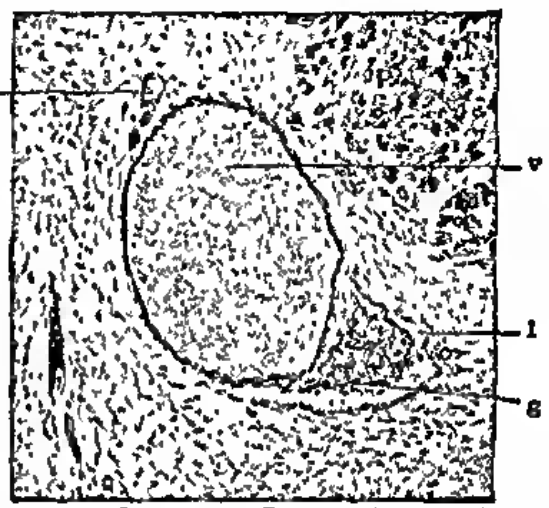


Fig. 240.—One of the Earliest Cervical Lymph Glands. From a Human Embryo of 42 mm. × 60

a, Artery, g, lymph gland, l, lymphatic vessel, v, vein

lymphoid tissue, as shown on the right of Fig. 241, and eventually they pass clear through it in a system of anastomosing sinuses. The lymph then flows into the gland from the periphery, and out at the hilum; both the afferent and efferent vessels are shown in Fig. 242. Finally a connective tissue capsule develops around the larger glands, and in some of them it extends into the interior, producing a system of supporting *trabeculæ*, either round or lamellar. These may unite with one another as shown on the right of Fig. 242. When present within the glands they are always found in the central axes of the lymph sinuses.

By the production of the internal lymph sinuses, the substance of the gland is subdivided into rounded *nodules* and elongated *cords* of lymphoid tissue. The nodules are found at the periphery of the gland and collectively they form its *cortex;* the cords constitute the *medulla*. Several other organs, *e.g.*, the kidney and suprarenal glands, are divided into an outer cortex (bark) and an inner medulla (pith). In the center of each cortical nodule there is often a light spot, seen with low power, which

[1] Kling, 1904.

constitutes the *germinal center*. These general features of a lymph gland are shown in Fig. 243. Certain of the nodules in the cortex are imperfectly separated from one another, and they are continuous below with the anastomosing medullary cords.

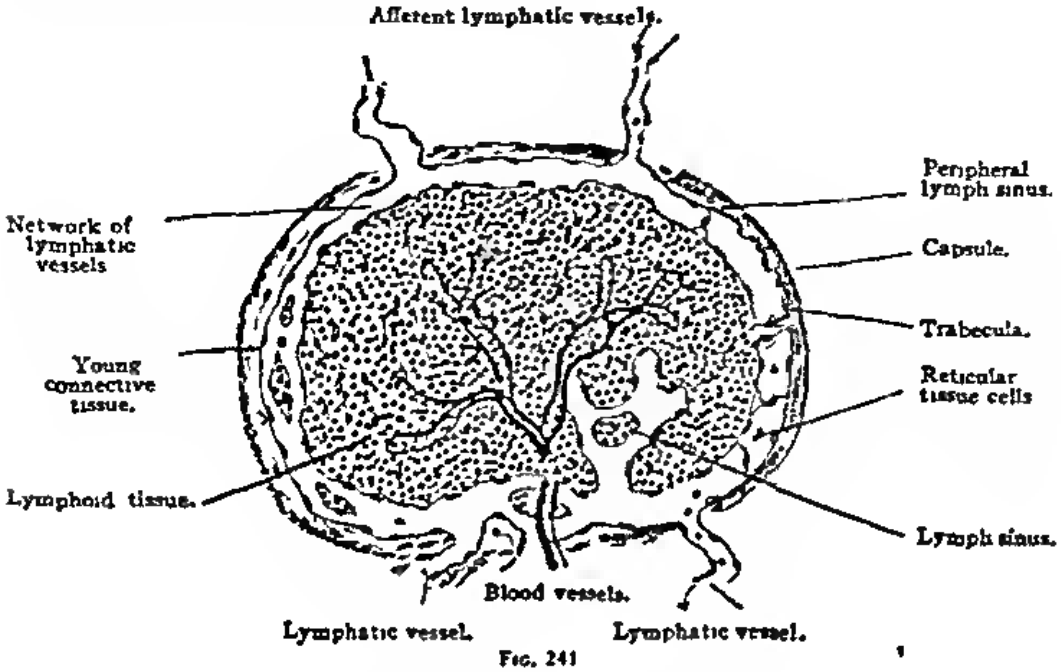


Fig. 241

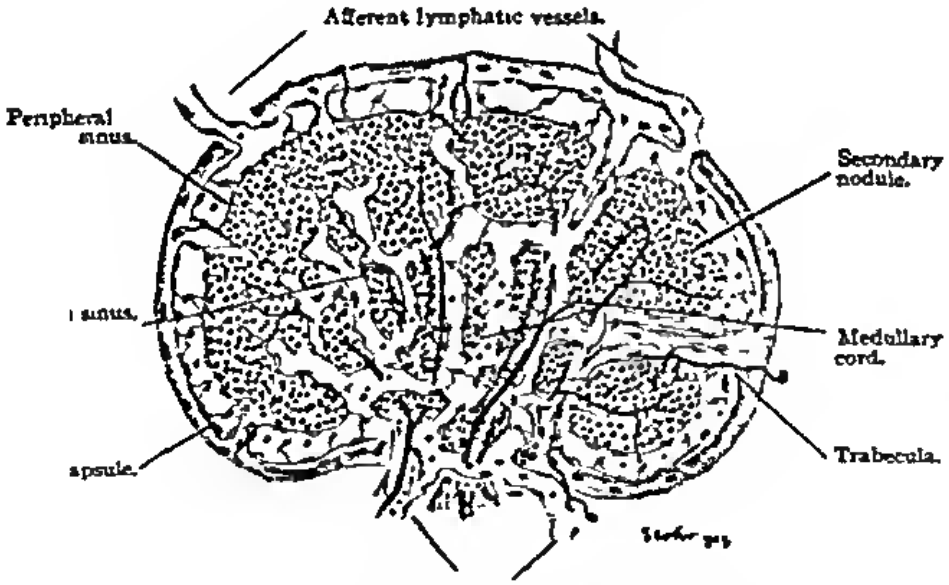


Fig. 242.

Figs. 241 and 242.—Diagrams Representing Four Stages in the Development of Lymph Glands.

The lymph glands of the adult (*lymphoglandulæ*, also called lymph nodes) are round or reniform structures varying in length from a few millimeters to a few centimeters. The largest of them show trabeculæ and are subdivided into cortex and medulla as above described; the small ones remain permanently in the various developmental stages. The smallest structures consist of but a single nodule.

Lymphoid tissue (formerly called adenoid tissue) consists of a framework of reticular tissue together with detached cells, chiefly lymphocytes, which fill its meshes. Stained with hæmatoxylin it is very dark, because of the preponderance of nuclear material, and its appearance, even under low magnification is quite characteristic. It is found in diffuse form, in

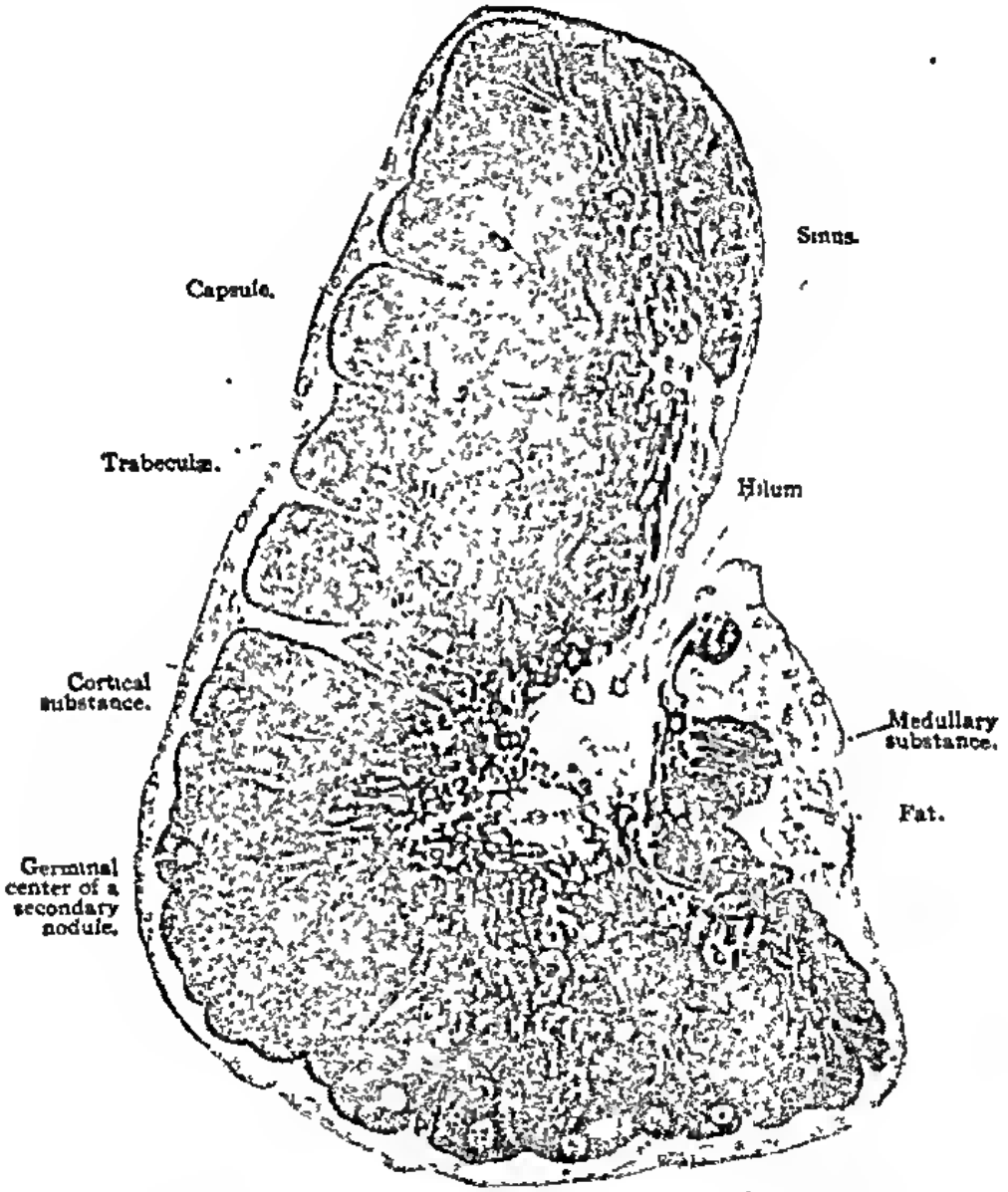


FIG 243.—LONGITUDINAL SECTION OF A HUMAN CERVICAL LYMPH GLAND. × 12.

cords, or in nodules, the grouping of the lymphocytes depending on the local fineness of the reticular mesh, since the free cells are readily floated out of a wide-meshed area. In sections the oval nuclei of the reticular cells are readily seen, but the anastomosing processes are so overlain by the lymphocytes that they become mostly invisible. Eosinophils and the various forms of blood corpuscles may leave the blood vessels and be present in the meshes in small numbers.

The lymph nodules are thought to be centers of lymphocyte production, perhaps active intermittently. They often show a lighter central area, called the 'germinal center' (Fig. 244) in which cells resembling the promyelocytes, with considerable cytoplasm and large nuclei, may represent the ancestral form of the lymphocyte, and various stages in lymphocyte development may be followed. Mitotic figures may be frequent. The ancestral cell, sometimes called a 'lymphoblast,' has a large, pale nucleus, with one or two prominent chromatin granules, and an abundant non-granular cytoplasm. The nucleus becomes progressively smaller, the chromatin increases and is more finely divided, finally

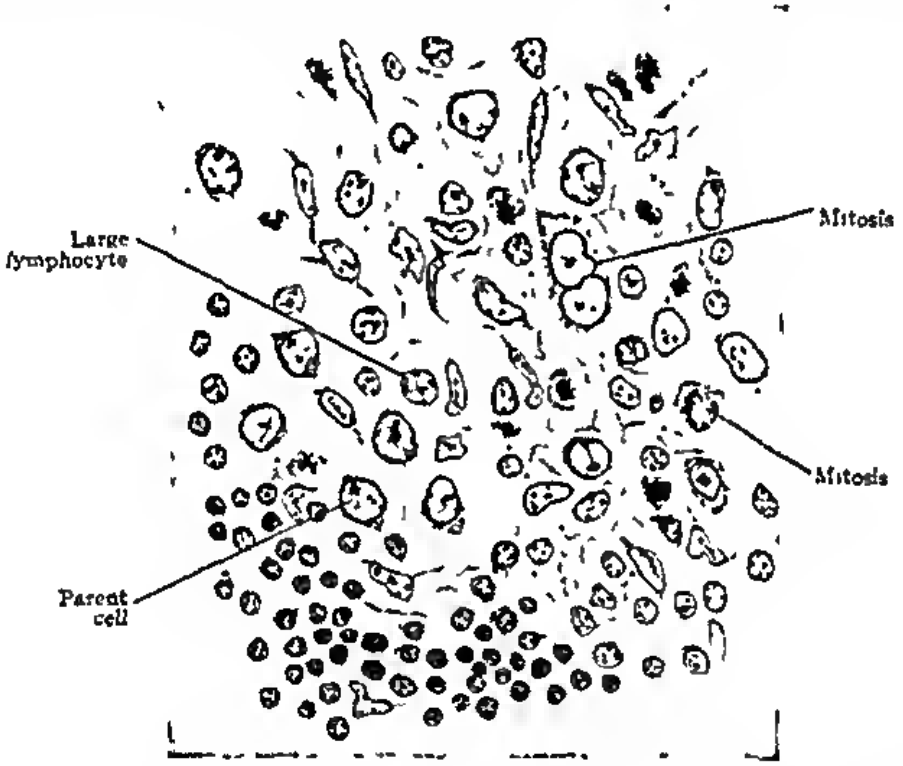


FIG. 244.—GERMINAL CENTER OF LYMPH GLAND, HUMAN.
Within the reticular tissue meshes are large clear cells, resembling promyelocytes, gradations can be followed to the large and small lymphocytes. Mitoses are frequent.

taking the characteristic lymphocyte arrangement. The cytoplasm is meanwhile reduced in amount, but remains without specific granules. The germinal center merges laterally into closely packed lymphoid tissue, with small adult lymphocytes which may leave the nodule. In certain organs containing germinal centers (*e.g.*, spleen), it is known that lymphocytes are produced. On the other hand germinal centers are absent from the early stages of embryonic lymph glands, when production of lymphocytes would be expected to be rapid. Latta[1] considers them areas of degeneration, and Ehrich[2] suggests that they may be 'looked upon as reserve depots of lymphoblasts, which, when lymphocytes are needed, change into small lymphocytes.'

[1] LATTA, 1922. [2] EHRICH, 1929

Some confusion has arisen in the use of the term 'secondary nodule.' His called the light areas in the center of the cortical nodules 'vacuoles'; Brücke used the term 'central spot' for the same structures. Flemming (1885), who first called attention to the active cell division in these regions, named them 'morphologically secondary nodules, physiologically germinal centers,' thus making the terms synonymous. Kolliker follows this usage, but Stöhr, probably thinking of the embryological grouping of lymphocytes as the primary nodule, designated the cortical nodule as secondary, the lighter central portion of which was the germinal center

Lymphoid tissue is found widely throughout the body, in lymph glands and spleen, and in the mucous membranes of various organs. Lymphoid nodules irregularly massed about epithelial pits become the essential tissue of the tonsils. Throughout the intestinal tract 'solitary nodules' occur, and in the lower part of the small intestine these become so numerous as to fuse, constituting the 'aggregate nodules' or 'Peyer's patches,' readily visible to the naked eye. The appendix is crowded with lymph nodules. Wherever it occurs, lymphoid tissue has essentially the same structure. The function of the lymphocytes is probably the elaboration of a material to counteract toxins in the blood or lymph.

The lymph sinuses are not well-defined endothelial tubes, but appear rather as washed-out portions of the reticular tissue. If the endothelial layer which lines the lymphatic vessels enters the lymph gland to form the sinuses, it must be considered that the cells separate and that strands of reticular tissue pass through them. Some authorities consider that the endothelial tissue blends freely with the reticular tissue, so that any distinction is here arbitrary. Such reticulo-endothelial cells in the sinuses are highly phagocytic (Fig. 67), and ingested fragments may be seen within them in sections.[1] Certain of these cells become detached, and there is reason to believe that they are one source of the large mononuclear leucocytes. Lymphocytes from the adjacent cords and nodules also enter the lymph as it passes through the sinuses, and thus they are added to the circulation. Within the cords and nodules they are enclosed in a closer-meshed reticulum than that of the sinuses, which may prevent them from escaping too freely.

The capsules of the lymph glands consist of fibrous connective tissue, containing elastic elements which increase in abundance with age. Smooth muscle fibers are present as scattered cells or as slender bundles. The trabeculæ, which are extensions of the capsule, are composed of the same tissues. They are completely surrounded by the lymph sinuses as shown in Fig. 245. The flat cells over their surfaces may be regarded as endothelial cells.

The blood vessels of a lymph gland enter chiefly at the hilum, but in the larger glands some of them come in from the periphery and run in

[1] Drinker, Wislocki and Field, 1933.

the trabeculæ; others, however, pass out through the trabeculæ into the capsule. The principal artery enters at the hilum and divides at once into several branches, which travel in the trabeculæ for a short distance, and then pass over into the medullary cords. They extend through the axes of the cords into the nodules, giving off small branches which form a venous network at the periphery of these structures. The veins which drain this network soon cross the sinuses and enter the trabeculæ, in which they travel toward the hilum alongside the arteries.[1]

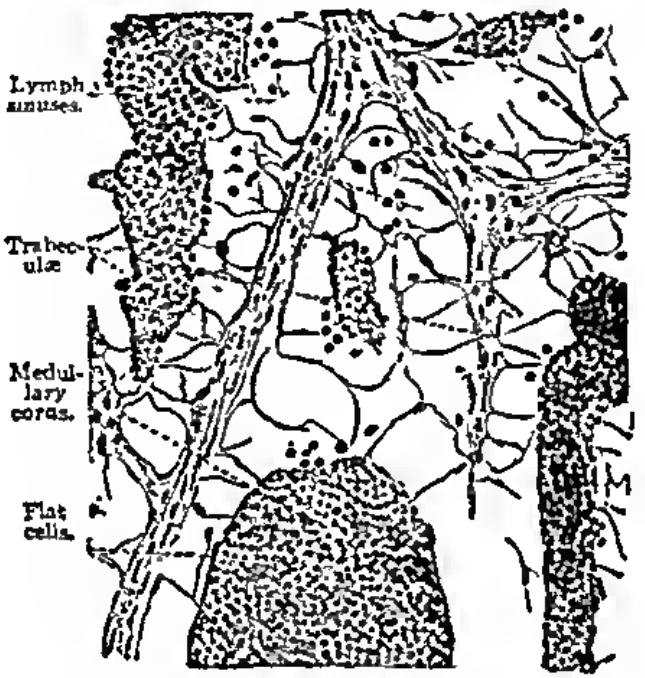


Fig. 243.—From the Medulla of a Lymph Gland of an Ox. × 240.

The arrangement of vessels shown in the nodules is called a vascular unit, and is frequently found in other organs. It represents the economical distribution to and drainage of an area of limited size, and can be understood by studying the blood supply of an intestinal villus as shown in Fig. 316, A. An artery runs in the center of this structure giving off capillary branches on all sides toward the periphery, where the capillaries unite to form two or more veins which return the blood to the base. In compact tissues, the peripheral veins drain the two neighboring units between which they lie. The diameter of the unit depends on the length of the capillaries, and this again is regulated by the rate of blood flow and the time necessary for exchanges of oxygen, etc., between blood and tissues. A shorter capillary would be inadequate, a longer one unnecessary. The length varies in different organs, but is rarely more than 0.5 mm.; the units therefore are 1 mm. or less in diameter.

Nerves to the lymph glands are not abundant. They consist of medullated and non-medullated fibers, which form plexuses about the blood vessels, and supply the muscle cells in the capsule and trabeculæ. They have not been found in the nodules and cords.

[1] Calvert, 1897.

The function of the lymph glands is not only to produce lymphocytes which enter the lymphatic vessels and are conveyed through the thoracic duct into the blood, but also to 'filter the lymph.' If certain poisonous substances, inert particles, or bacteria are brought to the gland in the lymph, they may be removed by the phagocytic endothelial or reticular tissue cells. The gland at the same time may become enlarged by congestion and by multiplication of its cells. Pressure on the nerves in the capsule causes tenderness.

Hæmal[1] Glands or hæmolymph[2] glands resemble small lymph glands, ranging in size from a 'pin-head to an almond.' They were first described by Heneage Gibbes in 1884, as 'structures found in the connective tissue between the renal artery and vein in the human subject.' They are also found elsewhere among the ordinary lymph glands, but their distribution differs in the various animals studied. They are said to be inconstant and variable in the rat and Guinea-pig, and absent in the pig. While resembling lymph glands in general structure, with capsule and trabeculæ, and a similar arrangement of lymphoid tissue with its blood vessels, hæmal glands are characterized by the presence of blood in the sinuses, derived from permeable vessels, either capillaries or veins, and of large hyaline cells, sometimes pigmented. The presence of secondary lymph nodules indicates that one function is the production of lymphocytes. Their connection with lymph vessels seems to vary. In the sheep they are said to have no connection with the lymphatic circulation, in other animals various gradations have been found from this condition to that of the ordinary lymph gland. Jordan[3] and others consider the hæmal glands as true lymph glands more or less isolated from the lymph stream by atrophy of the lymph vessels and utilized temporarily as organs of erythropoiesis; the large hyaline cells are interpreted as lymphoblasts or primitive blood cells. If this is so, the experimental bleeding of animals should cause greater hæmopoietic activity, in response to the body's demands, and an increase in the number of hæmal glands. Such experimental increase has been both confirmed and denied. Drummond[4] and Meyer[5] (sheep), on the other hand, find that they are organs entirely distinct from lymph glands in origin, distribution, and in minor structural details, such as the greater width of peripheral sinuses, and the presence of more smooth muscle in the trabeculæ. They are then thought of as organs for the removal of worn-out red corpuscles, which are ingested by the large cells (phagocytes) either whole or in fragments, and reduced to hæmoglobin pigments. Similar activities take place in the spleen, and the 'accessory spleens,' occasionally described, may be large hæmal glands.

[1] Clarkson, 1891. [2] Robertson, 1890. [3] Jordan, 1927.
[4] Drummond, 1900. [5] Meyer, 1918.

Macmillan,[1] while denying any proof of erythropoiesis, suggests that there are two types of nodes which contain erythrocytes in the sinuses: those with purely hæmal circulation, characteristic of the prevertebral region of the sheep, and those which are merely modified lymph glands. The possibility that blood may be brought to the sinuses from a peripheral or internal hæmorrhage should always be considered.

SPLEEN

The spleen, being five or six inches long and four inches wide, is much the largest organ of the lymph gland series. It is the first of them to develop, appearing in rabbits of 14 days (10 mm.) as a condensation of

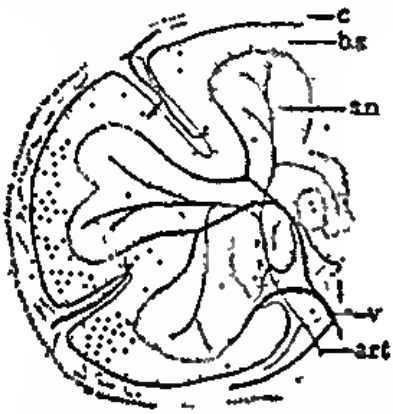


Fig. 246

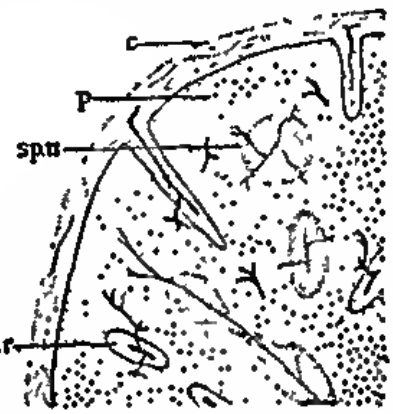


Fig. 247.

Figs. 246 and 247.—Diagrams of a Hæmolymph Gland and of the Spleen Respectively.
The arteries are shown as slender lines (art) and the veins as heavy ones (v), c, capsule, b. s, blood sinus, corresponding with the splenic pulp p, s. n, secondary nodule, s.p. n, splenic nodule, tr, trabecula

the mesenchyma in the dorsal mesentery of the stomach. At this stage the only lymphatic vessels in the embryo are those near the jugular vein. Lymph glands are not indicated until six days later. For the development see Thiel and Downey.[2] The blood vessels enter the spleen at its hilum and branch freely. In early stages they form an ordinary capillary plexus, but subsequently their walls become pervious. Surrounding the arterial branches there is a zone of lymphoid tissue, which arises rather late in embryonic life. In reptilian spleens it is so abundantly developed that the organs resemble mammalian hæmal glands. In the Guinea-pig the lymphoid sheath of the arteries is continuous, though narrow; in man it is so interrupted as to form a succession of spindle-shaped or spherical masses, called *splenic nodules* (Malpighian corpuscles). An arterial branch passes through each nodule. Thus, as compared with the hæmal gland, the spleen is deficient in lymphoid tissue (Fig. 247). The bulk of the spleen is composed of *splenic pulp*, which corresponds with the blood sinus of the hæmal glands. Its framework of reticular tissue is continuous with that of the nodules, and it contains blood cor-

[1] Macmillan, 1928. [2] Thiel and Downey, 1921.

puscles of all sorts, special phagocytic cells known as *splenic cells*, and the terminal branches of both arteries and veins. There are no lymphatic vessels within the spleen. The capsule and trabecular framework are highly developed as in the largest lymph glands, and contain smooth muscle fibers. The following features of the spleen may be described in turn—the blood vessels, the pulp, the nodules, the capsule and trabeculæ, and finally the nerves.

As shown in the diagram, Fig. 248, the splenic artery enters at the hilum and, accompanied by veins, its branches are found in the largest

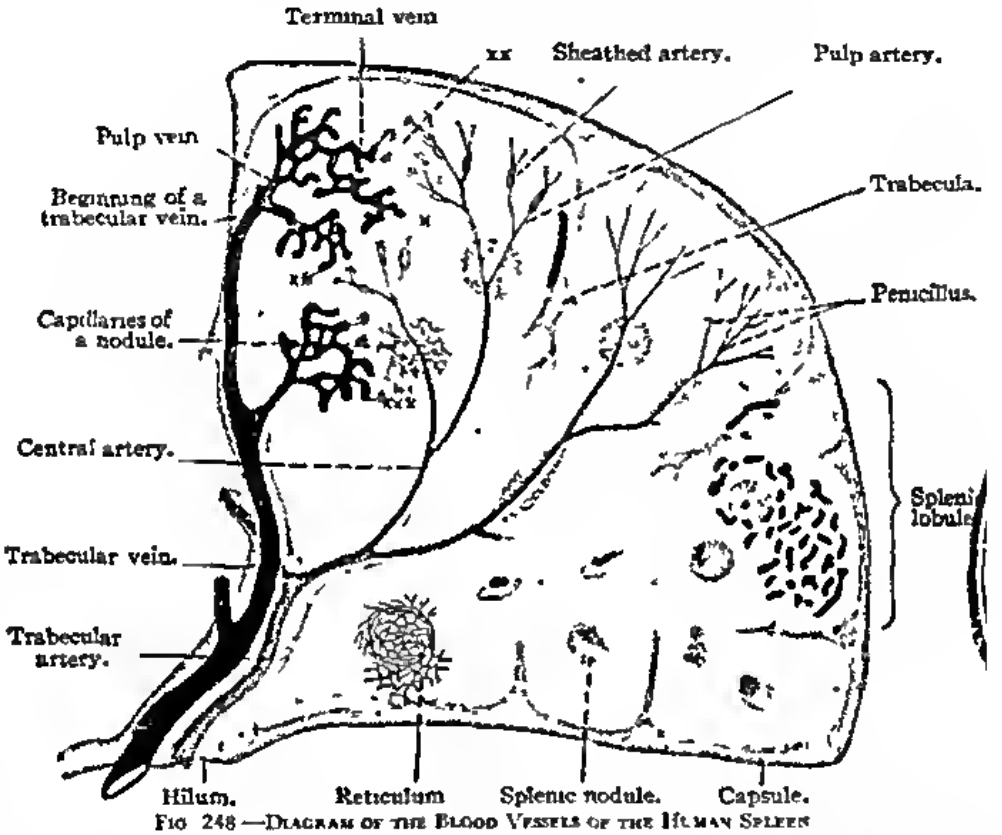


FIG 248.—DIAGRAM OF THE BLOOD VESSELS OF THE HUMAN SPLEEN

At x is shown the direction of terminal arteries with terminal veins (the existence of such a connection has been questioned) At xx and xxx are the free endings of the terminal veins in the pulp and near the nodules respectively

trabeculæ. When about 0.2 mm. in diameter the arteries leave the trabeculæ, in which the veins continue further. The arteries, however, are still surrounded by a considerable connective tissue layer, the outer portion of which at infrequent intervals becomes reticular and is filled with the lymphocytes of the nodules, in which the artery usually takes an eccentric position. Small lateral twigs ramify in the nodules, making a rich plexus and then passing out into the pulp. The arteries continue beyond the nodules and, when about 15 μ in diameter, form brush-like groups of branches (penicilli). These branches do not anastomose. For a short distance before their termination the walls of the branches possess ellipsoid thickenings, due to a longitudinal arrangement of closely applied fibers of reticular tissue. These 'sheathed arteries' are 6–8 μ in

caliber and have been supposed to regulate the amount of blood which enters the terminal portion of the artery beyond them. It is possible that some of these arteries connect directly with the veins, but the great majority of them give off capillaries. According to some authors[1] these capillaries, as well as those from the nodular plexus, open directly into the tissue spaces of the reticular tissue through ampullæ or end-bulbs. According to others the arterioles open into the *splenic sinuses*, which are modified capillaries forming a network and connected with the veins.

The splenic sinuses or *terminal veins* are dilated, irregularly branching and anastomosing spaces, occupying most of the pulp. Their endothelium

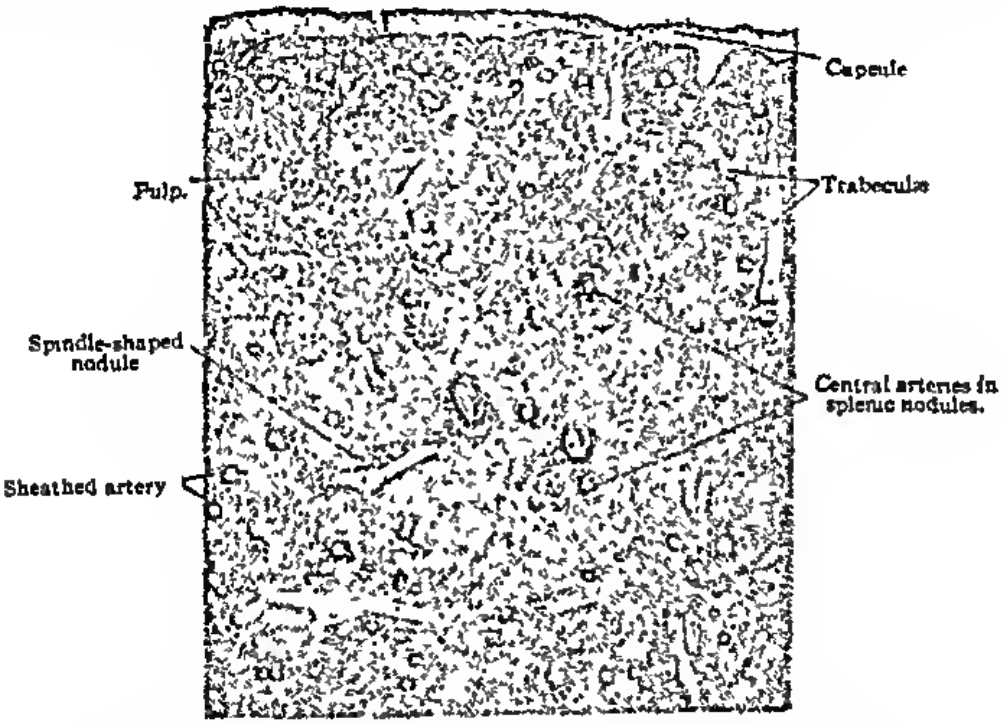


Fig. 249.—Part of a Section of the Spleen from an Adult Man. × 15.

has become perforated so that it shows longitudinally arranged narrow slits, which give the cells the appearance of elongated fusiform elements, resembling smooth muscle fibers. The transverse connections are inconspicuous in ordinary preparations, but are readily visible in sections of spleen from which the free cells have been washed out, stained by Bielschowsky's method or with Heidenhain's iron-hæmatoxylin. Foot,[2] however, considers them a part of the surrounding reticulum. The nuclei are distinctive, bulging far into the lumen and fitting to the main body of the cell by a notch on their outer side (Fig. 250). Presumably they are covered by a thin film of protoplasm. On surface view, the perpendicular walls of the notch may give the appearance of parallel lines within the oval nucleus. It has been stated that the cells are contractile, as are many other endothelial cells. The relation of the cells to

[1] MacNeal, 1929. [2] Foot, 1927.

the reticular tissue seems uncertain: they are said to rest on reticular fibers which form a basket work about the vessels, but such fibers are not readily recognized. Within the outer portion of their protoplasm longitudinal fibrils, like myofibrils, have been described, and might easily be mistaken for reticular fibers. A continuous basement membrane stretching across the intervals between endothelial cells probably does not exist, since a free passage through the clefts for cells of all kinds is essential to splenic function.

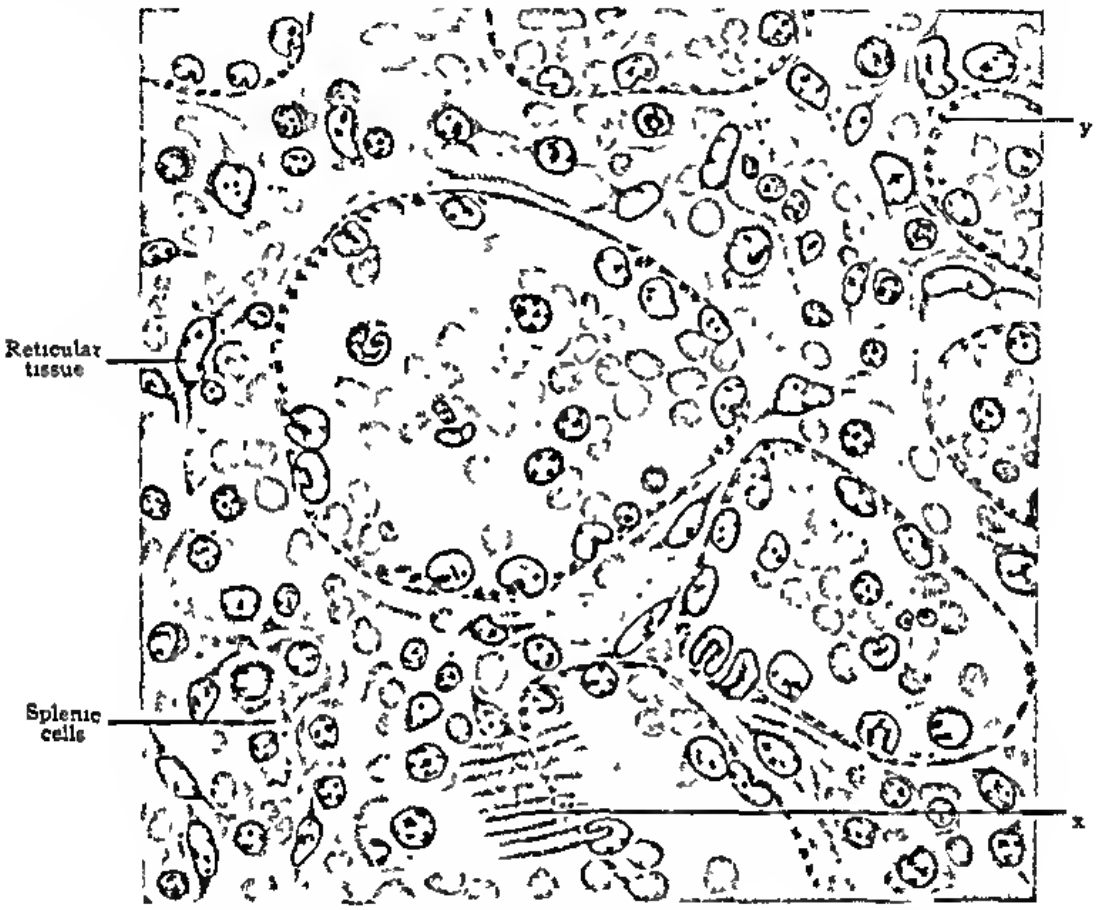


FIG. 250.—SPLENIC PULP, HUMAN

Terminal veins cut across and obliquely x, the endothelium cut lengthwise, resting on reticular fibers, y, same cut transversely

Several terminal veins unite to form a pulp vein, lined with ordinary endothelium, which enters a trabecula in which it passes toward the hilum. In the trabeculæ the veins are almost without coats of their own, relying on the trabecular connective tissue for support. The trabecular veins join to form the splenic vein.

The *splenic pulp* consists of a reticular tissue framework. It supports the terminal arteries and veins, and in its meshes are the white and red corpuscles passing between them.

The pulp appears as a diffuse mass of cells infiltrated with red corpuscles, and since the vessels within it are thin-walled and hard to follow, likewise containing corpuscles, it is often impossible in ordinary sections

to determine which cells are inside and which are outside of the vessels. The nodules are not sharply separated from the pulp, so that lymphocytes are abundant in their vicinity. These lymphocytes enter the terminal veins and thus are removed from the spleen. In the splenic vein the proportion of lymphocytes to red corpuscles is said to be seventy times as great as in the splenic artery. One to every four red corpuscles have been reported by two investigators, but later estimates are lower. It seems evident that lymphocyte production is an important function of

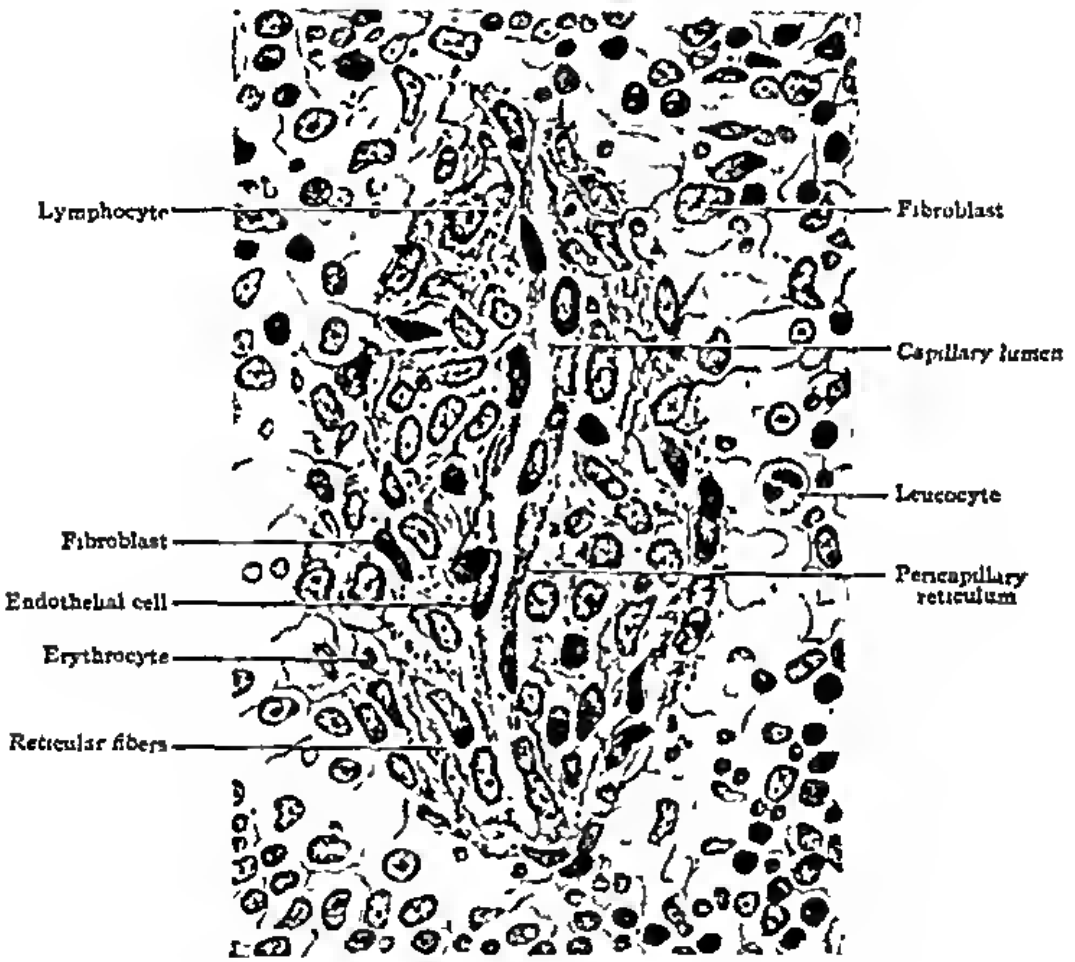


FIG. 251.—"SHEATHED ARTERY" IN THE SPLEEN OF A CAT. CONTRACTED ORGAN. Susa fixation, eosin-methyl blue. × 800. (Riedel.)

the spleen. Another is the destruction of old red blood corpuscles, which of course increases the lymphocyte percentage in the splenic vein. As in the hæmal glands, granular debris is present, and occasionally whole red corpuscles are found within the phagocytic cells which abound in the pulp, and are called *splenic cells*. They are often pigmented or eosinophilic, with large round nuclei and considerable cytoplasm. They vary in size, but the small forms are most numerous. Some resemble plasma cells, and may be related to the lymphocytes; others are probably derived from the reticular cells, which are themselves phagocytic. They may be

the common macrophages. Erythroblasts are not found in the normal adult human spleen; they occur, however, in certain blood diseases, and are normal in some adult mammals, as in the skunk. They are abundant in the spleens of human embryos. Giant cells are numerous in the spleens of young animals but are seldom found in the human adult. They are described as megakaryocytes, and are like those in bone marrow. The formation of granular leucocytes, which has been asserted, presumably does not occur.

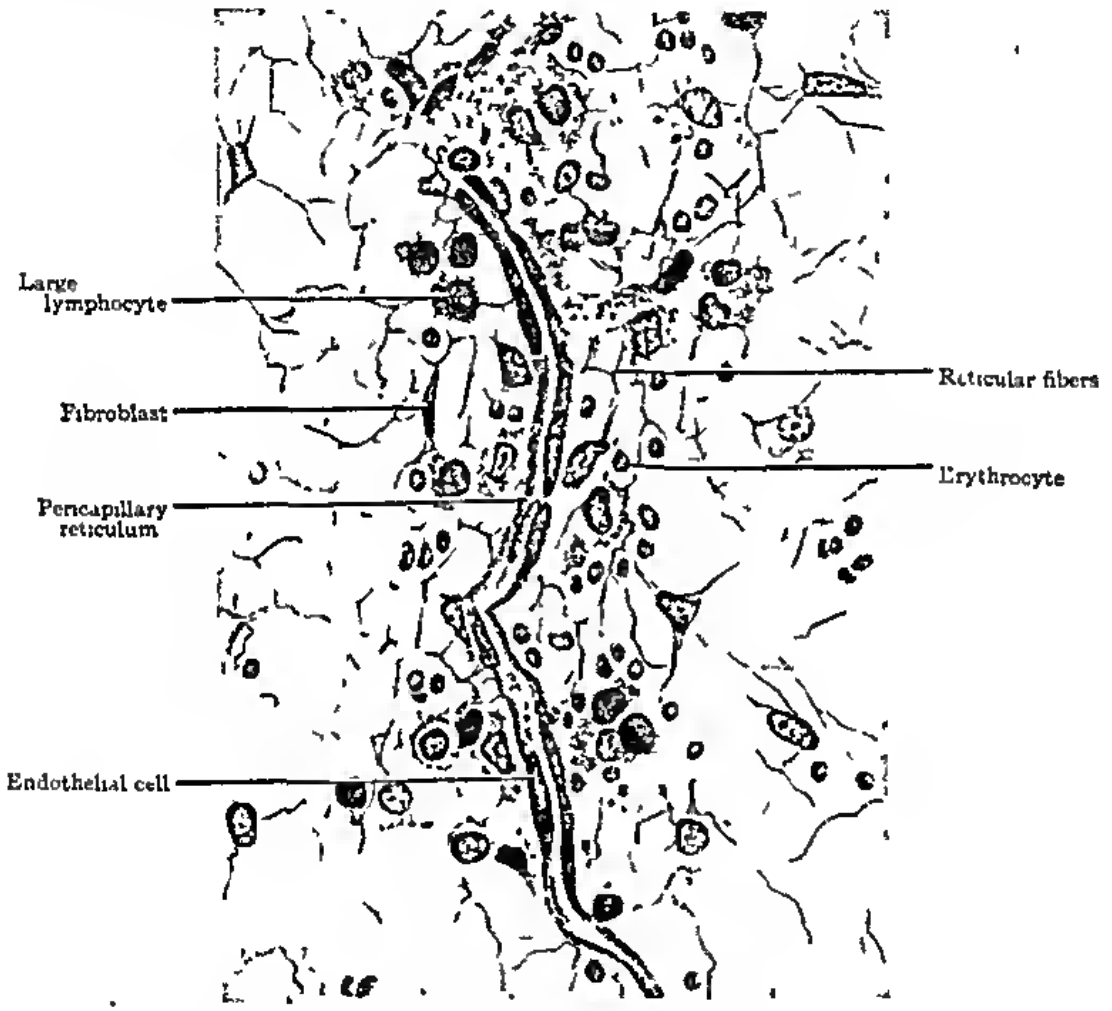


FIG 252.—"SHEATHED ARTERY" IN THE SPLEEN OF A CAT DISTENDED ORGAN
Susa fixation, eosin-methyl blue × 800 (Riedel)

It will be seen that there are at least two theories as to the circulation of the spleen. Either it is 'closed,' in that most of the blood stream continues through it enclosed within endothelial walls, although in the slow current of the relatively large sinuses the damaged or older red corpuscles may be sorted out and passed through to the phagocytes in the pulp; or it is 'open,' in that the arterial capillaries, at least in many instances, open into the reticular tissue from which the most vigorous blood cells return through the sinus walls to the main stream, after running the gauntlet of the phagocytes.

From the study of sections the latter theory would seem the most probable, for all of the blood cells are found both in vessels and in the pulp, yet it entails a circulation which is remarkable in that the blood leaves the vessels to flow freely in tissue spaces. In most other parts of the body this would lead to the formation of a clot. In the placenta

the same sort of extravasation without clotting occurs, and in the lymph gland the afferent vessels open into intercellular spaces. The presence of reticular tissue seems to be essential for this phenomenon, and perhaps shows the close relationship between this tissue and endothelium. Special provision is made also for the return of the blood elements from the tissue spaces to the veins. Sabin, in describing the closed sinusoidal circulation of the bone marrow, notes that an organ with open capillaries must be provided with muscles, like those in the splenic trabeculæ, to ensure the return of the blood to the veins, and will always show red corpuscles in the tissue spaces. The hæmal

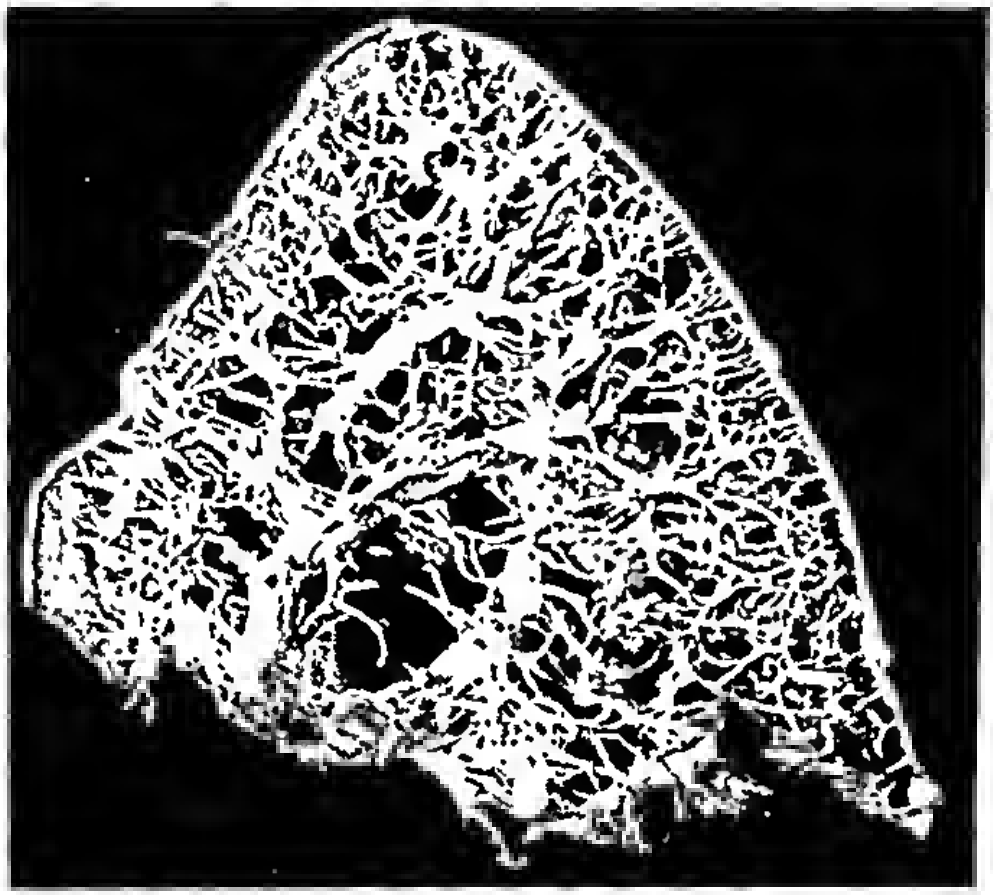


Fig 253.—Human Spleen.
Prepared to show the trabecular framework. Digestion in 1% solution of sodium carbonate at 42 degrees C. × 4 (*Schleicher—courtesy of the Wistar Institute*)

glands have no such muscles (Drummond disagrees with this statement, see p. 291), but the red corpuscles are there probably ingested, instead of re-entering the circulation.

The 'closed' circulation is advocated by Knisely,[1] from studies in the living animal. He finds that the sinuses are irregular sausage-shaped structures, expansions of direct capillary connections between artery and vein (cf. Fig. 255), emptying intermittently and retaining all or most of the blood cells. At the slightest injury or even disturbance the delicate mechanism breaks down and the sinus walls leak. This would account for the appearance in sections. An 'open' circulation becomes functionally 'closed' by splenic contraction.[2]

The *splenic nodules* are quite like the cortical nodules of lymph glands. They consist of a reticular tissue framework continuous with that of the

[1] Knisely, 1935.

[2] MacKenzie, Whipple and Wintersteiner, 1941.

pulp, but having finer meshes. Fine elastic fibers are associated with it. It contains lymphocytes, and near the central arteries germinal centers are sometimes distinct. The nodules have been regarded as varying in shape from time to time, being but transient accumulations of lymphocytes.

The *capsule* of the spleen is divided into two layers. The outer is the *tunica serosa* and the inner, the *tunica albuginea*. The serosa consists of the peritoneal mesothelium, which covers the spleen except at the hilum, and of the underlying connective tissue. The albuginea is a dense layer of connective tissue, containing elastic networks and smooth muscle fibers. Similar tissue is found in the trabeculæ. The muscle elements are less numerous in the human spleen than in those of many animals. They are found sparsely in rodents, very numerous in the dog and cat and in 'colossal amounts' in the pig.[1] By contraction they force blood from the pulp, constrict the capillaries and venous sinuses, and at the same time cause a suction in the thin-walled veins within the trabeculae.[2] When they are paralyzed, the pulp becomes filled with the blood corpuscles. A regular but very slow pulsation of the organ has been detected.

FIG. 254.—RETICULUM OF DOG'S SPLEEN. FREE CELLS WASHED OUT WITH RINGER'S SOLUTION, STAINED WITH ORCEIN.

The nerves of the spleen, from the right vagus and the cœliac sympathetic plexus, are medullated and non-medullated fibers, chiefly the latter. They form plexuses around the blood vessels and send fibers into the pulp, where they are said to become intra-protoplasmic in the syncytium of the reticulo-endothelium.[3] Besides supplying the muscles of the vessels and trabeculæ, some of them are thought to have free sensory endings. Lymphatic vessels are said to occur in the capsule and trabeculæ, but not in the pulp or nodules of the spleen.

The *spleen* is a large organ without obvious subdivisions. On its surface, when fresh, there is a mottled effect due to areas bounded more or less definitely by trabeculæ. Such areas, about 1 mm. in diameter, have been described by Mall as 'lobules,' and he states that they 'can easily be seen on the surface of the organ or in sections.' A lobule, as he describes it, has a central artery, and its base is where the lymphoid sheath of the artery terminates. It has peripheral veins, often three, enclosed in the trabeculæ. A

[1] RIFDEL, 1932. [2] MALL, 1903. [3] RIEGELE, 1932.

lobule is composed of some ten structural (or histological) units, imperfectly separated from one another by branches of the trabeculæ. Each unit contains a central terminal artery (branches of the lobular artery) and has peripheral veins (branches of those about the lobule). Apparently, therefore, the lobules shown in the diagram, Fig. 248, except along its lower border, represent groups or pairs of Mall's lobules. Stöhr notes that 'a division into lobules in the interior of the spleen is impossible.' MacNeal[1] recognizes a lobule the center of which is the splenic nodule, the ill-defined periphery made by a ring of larger venous sinuses. Lobes have also been described, corresponding with the main

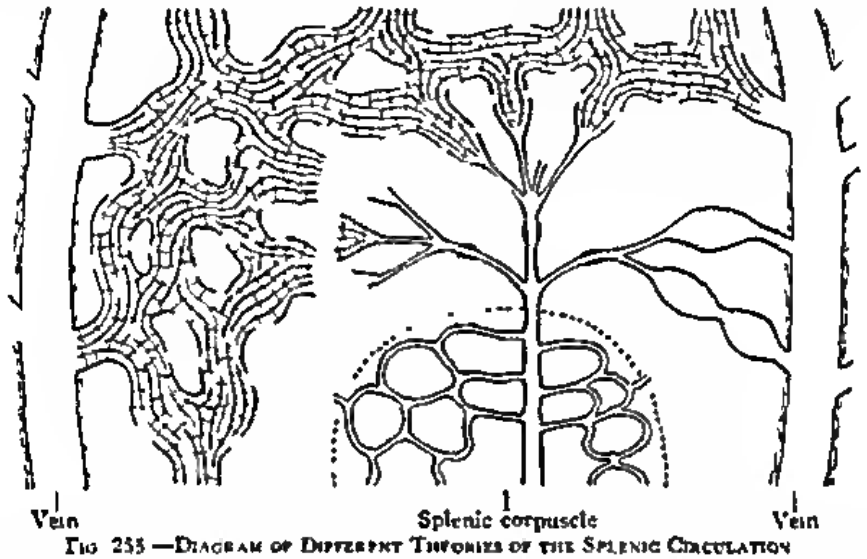


Fig. 255.—Diagram of Different Theories of the Splenic Circulation

In the splenic corpuscle looped capillaries are shown, in the left branch of the artery the circulation is shown as 'open' with fenestrated end-bulb, in the center branch as partly 'closed,' but with pervious sinuses, in the right branch as 'closed,' the sinuses also being normally impervious. The sinuses join veins as they enter trabeculæ.

branches of the splenic artery, but the lobes are not generally recognized. The spleen may present inconstant subdivisions, which sometimes produce detached portions called *accessory spleens*.

THE ENTODERMAL TRACT

MOUTH AND PHARYNX

Development. In a previous section the early development of the fore-gut or pharyngeal pocket of entoderm has been described and illustrated (Figs. 33 and 34). This fore-gut of the young embryo is to produce the pharynx, œsophagus, stomach and most of the small intestine of the adult. Its anterior extremity encounters the ectoderm at the bottom of a depression. The ectoderm and entoderm there fuse to make *the oral plate* or membrane (Fig. 256), which becomes thin, ruptures, and disappears. Just anterior to the plate, in the median line, the ectoderm sends out a gland-like projection which meets a similar outgrowth from the brain. Together these form the two lobes of a single organ, the *hypophysis*, lying in the *sella turcica* of the adult. The anterior lobe soon loses its connection with the oral cavity; its future development will be described later. The ectoderm in front of the oral plate forms also the epithelium

[1] MacNeal, 1927.

of the lips and of the peripheral part of the mouth, including the enamel organs of the teeth. The salivary glands are also considered ectodermal, but before they develop the oral plate has disappeared and the boundary between ectoderm and entoderm cannot be sharply drawn.

The entoderm of the mouth and pharynx is a layer of epithelium lining a broad, dorso-ventrally flattened cavity. From this cavity, a succession of paired out-pocketings, the pharyngeal pouches, grows out laterally. At corresponding positions on the sides of the head and neck deep grooves, the *branchial grooves*, appear as in-pocketings of the ectoderm. Where the pouches and grooves meet the two germ layers, entoderm and ectoderm, fuse in a series of plates, comparable with the oral plate. In fishes they rupture producing the *branchial clefts* (gill clefts).

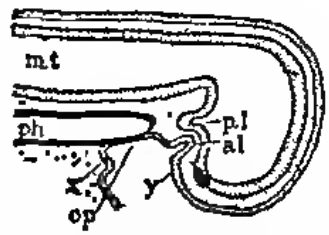


FIG 256.—DIAGRAM SHOWING THE RELATIONS BETWEEN ECTODERM AND ENTODERM IN THE MOUTH OF A MAMMALIAN EMBRYO

a. l, and p. l, Anterior and posterior lobes of the hypophysis, m. t, medullary tube; ph, pharynx; o. p, oral plate; x and y, ectoderm which produces the lip and enamel of the teeth in the lower and upper jaws respectively

Their arrangement in a young dog-fish is shown in Fig. 257. The mouth, *m*, leads into a cavity, the pharynx, which opens freely on the outer surface of the fish through five gill clefts, *g.c.* It also opens to the surface through the *spiracle*, *sp*, a structure similar to the gill clefts, but anterior to them and having a more dorsal aperture. In respiration water is taken in through the mouth and spiracle, and passes out through the gill clefts; but sometimes water is ejected through the spiracle. In mammals the corresponding structure is counted as the first gill cleft.

In mammalian embryos there are four well-defined pharyngeal pouches on each side, which reach the ectoderm at the bottom of corresponding grooves; but if their closing plates ever rupture they are soon restored, and permanent openings from the pharynx on the side of the neck are not found. The first pouch, corresponding with the spiracle, connects with the auditory groove (Fig. 258, *sp*). Around it the external ear develops, so that its position is always evident. The ectodermal depression which connects with the second pouch disappears, except in rare cases where it forms a cervical fistula.[1] This is a pit, or slender tube, in the skin of the neck, situated primarily between the hyoid bone and thyroid cartilage. The third and fourth pouches connect with the ectoderm at the bottom of a single funnel-shaped depression known as the *cervical sinus*. This also wholly disappears normally, but it may remain as a cervical fistula low down on the neck, and its deeper parts may give rise to branchial cysts. Thus all the ectodermal branchial grooves except the first normally disappear before birth.

The pharyngeal pouches, or entodermal portions of the gill clefts, as they occur in a mammalian embryo are shown in Figs. 259 and 260. The

[1] AREY, 1933.

pharynx opens to the exterior at the mouth, *m*, and divides posteriorly into the trachea, *tr*, and œsophagus, *oe*. In the median dorsal line the mouth gives rise to the anterior lobe of the hypophysis, cut off at *a. l*, and in the median ventral line the pharynx gives rise to the *thyroid gland, t*. This gland is a median structure, entirely separate from the pharyngeal

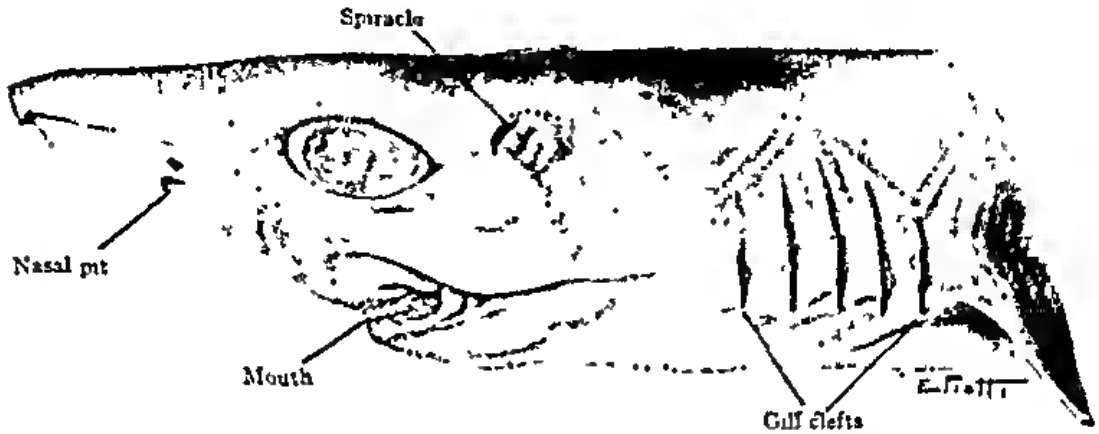


FIG 257.—HEAD OF A DOG-FISH. LATERAL VIEW SHOWING GILL CLEFTS

pouches. It grows down through the hind part of the tongue, acquiring a position in front of the trachea. Its branching terminal part becomes separated from its outlet by the obliteration of its duct (called the *thyro-glossal duct*). A blind pit, the *foramen cæcum*, permanently retained at the back of the tongue, marks the former outlet of the duct. (Fig. 260, *f.c.*) Thus the thyroid gland is a detached clump of entodermal tubules in front of the trachea.

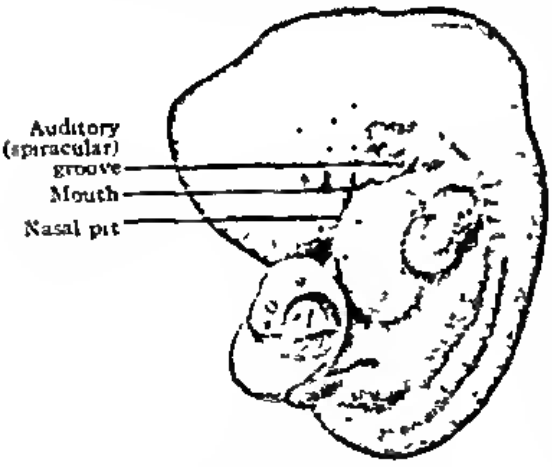


FIG 258.—HUMAN EMBRYO OF 10 MM

The entodermal portions of the gill clefts are four paired lateral outpocketings. The first (Fig. 260) extends to the auditory groove in the ectoderm, and becomes the *auditory tube* (Eustachian tube). The outer end of the tube expands to form the tympanic cavity of the ear, and will be further considered with the sense organs.

The second pharyngeal pouch (Fig. 260) loses its connection with the ectoderm and becomes a relatively shallow depression on the side of the pharynx. At a certain stage it is in close relation with the orifice of the auditory tube, and it has been thought to give rise to the *pharyngeal recess* (fossa of Rosenmüller), but according to Hammar[1] such is not the case. Instead, it produces only the *sinus tonsillaris*, into which a mound of

[1] HAMMAR, 1903.

lymphoid tissue, the *palatine tonsil*, later projects. Above the tonsil the *supratonsillar fossa*, which may readily be seen on looking into the mouth, is to be regarded as a remnant of the original second pouch.

The lingual and pharyngeal tonsils, which are similar in structure to the palatine tonsils, develop as median structures with no relation to the pharyngeal pouches Therefore the second pouches are to be regarded as the site rather than the source of the palatine tonsils· there are no tonsils in the second pouches of the rat (Hammar).

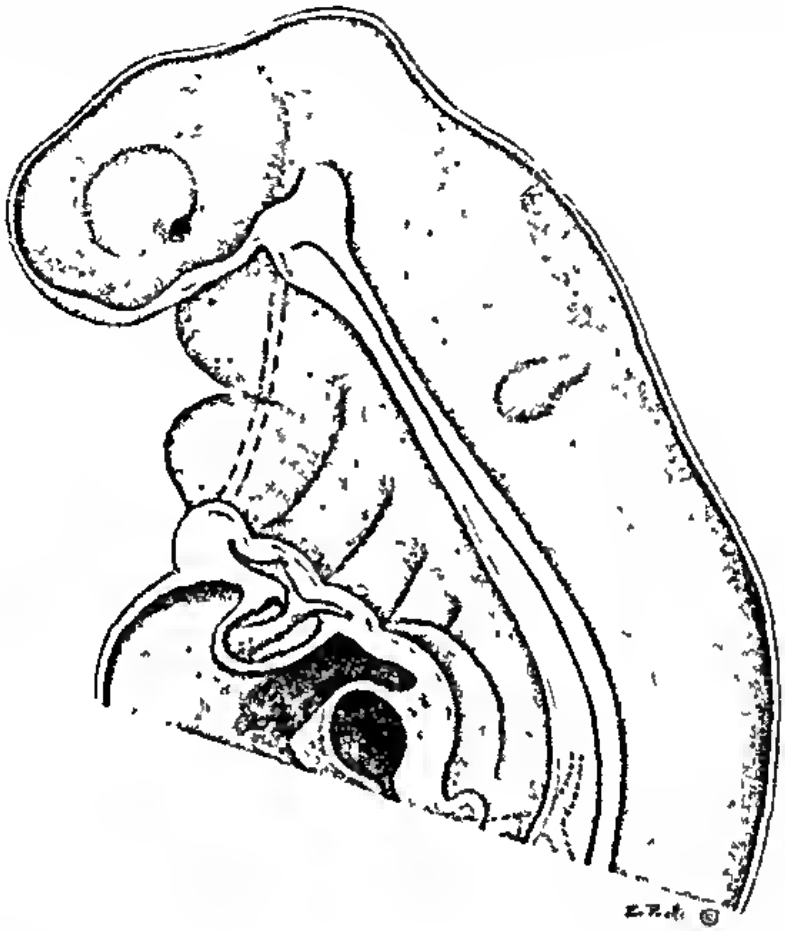


FIG 259.—A RECONSTRUCTION OF THE HEAD OF A HUMAN EMBRYO OF 4 2 MM (3 WEEKS) × 65 (Redrawn and enlarged from His)

The third pouch (Fig. 260, 3), near its junction with the ectoderm, sends a tubular diverticulum (*th*) down the neck behind the thyroid gland; it continues into the thorax, lying ventral to the arch of the aorta. This diverticulum loses its lumen, becomes detached from the pharynx, and unites with its fellow on the opposite side to form the *thymus*. Besides this elongated structure, each third pouch produces an *epithelial body*, or *nodulus thymicus*, which is a round clump of cells detached from the pouch at the upper end of the thymic diverticulum. Each epithelial body becomes attached to the posterior surface of the thyroid gland, forming the inferior pair of *parathyroid glands*, parathyroid III.

The fourth pouch on each side (Fig. 260, 4) gives rise to an epithelial body similar to the nodulus thymicus. These likewise become

detached as *parathyroid glands*, and they constitute the superior pair. Sometimes a parathyroid gland degenerates and disappears, and in other cases one of them may become subdivided, but typically there are four in the human adult. The rat develops no fourth pouch and has no parathyroid IV.

Behind the fourth pouch, on each side, there is a tubular prolongation of the pharynx variously known as the postbranchial, ultimobranchial or telobranchial body. As the fourth pouch becomes well formed, the postbranchial body is so closely associated with it that together they form a Y-shaped structure, attached to the pharynx by a common stalk. The postbranchial bodies then grow toward one another across the front of the neck, after the manner of the thymic diverticula. Their ventral ends become detached and embedded in the thyroid gland, to the substance of which they were formerly believed to contribute. There is, however, no satisfactory evidence that they produce thyroid tissue in man, and they are generally supposed to disintegrate.[1]

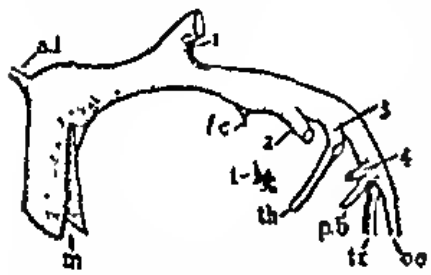


FIG 260.—DIAGRAM OF THE PHARYNX OF A MAMMALIAN EMBRYO

a l, Anterior lobe of the hypophysis, f. c, foramen caecum, m, mouth, oe, œsophagus, p. b, postbranchial body, t, thyroid, th, thymus, tr, trachea, 1, 2, 3, 4, pharyngeal pouches.

The first recognition of the significance of the mammalian gill clefts is credited to Rathke, in 1832, who published the following significant conclusions in his 'Untersuchungen über den Kiemenapparat der Wirbelthiere.'

'In all vertebrates without exception, in the earliest period of development, there are formed the beginnings of a branchial apparatus. Its elements vary in number in the different vertebrates, yet in tissue, form, position and connections they are very similar to one another, and are built upon the same plan. Their development, however, proceeds along different lines in the various animals. In some it is partly regressive, bringing about the most manifold and divergent modifications of these structures, not merely in form but also in tissue, type, and significance. Yet there always remains an analogy between them; and through easy transitions, the forms and types pass into one another from the bony fishes even to man. The branchial apparatus is most highly developed in fishes; in the other vertebrates its development is the less complete, the further, in general, these vertebrates are removed from the fishes.'

The mammalian gill clefts, although rudimentary as branchial organs, are of the utmost anatomical importance. A single large artery passes from the ventral aorta to the dorsal aorta between the successive pouches, and also in front of the first and behind the last. These *aortic arches*, therefore, number one more than the series of pouches; from them, portions of the aorta, carotid and subclavian arteries are produced, as described in works on embryology. The nerves send trunks down between

[1] KINGSBURY, 1935.

the pouches, the facial nerve being between the first and second, the glossopharyngeus between the second and third, and the superior laryngeal branch of the vagus between the third and fourth. Thus these structures determine the arrangement of the vessels and nerves.

On the basis of comparative studies the presence of a fifth pouch in mammals was predicted, and the posterior arm of the Y-shaped outgrowth, including the postbranchial body, is often described as such. A branch of the superior laryngeal nerve is said to pass between the arms of the Y, but a typical branchial relation between the nerves and the fifth pouch has not as yet been established. A 'fifth aortic arch' is often represented as passing between the fourth pouch and the postbranchial body, but it has been shown that this arch differs from all the others in its order of development (forming only after the 'sixth' is complete). Whereas the third, fourth, and last aortic arches

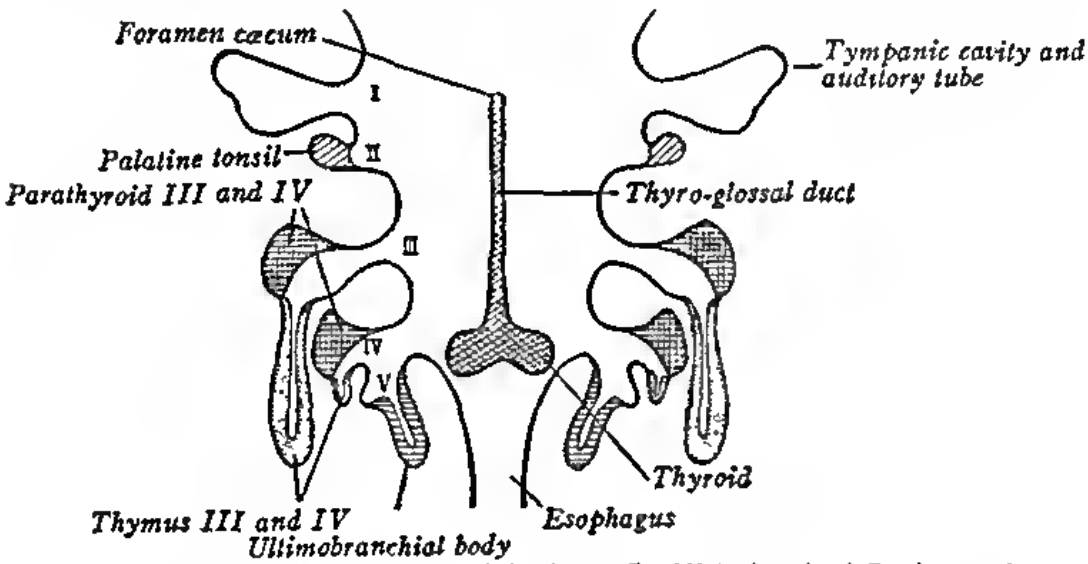


Fig. 261.—Diagram of Pharyngeal Derivatives. (After Groschuff and Kohn from Arey's Developmental Anatomy Courtesy W. B. Saunders Co.)

all produce very important vessels, the questionable 'fifth arch' is an insignificant plexiform anastomosis, which disappears rapidly. Small vessels, however, are always to be found near the postbranchial body in rabbit, pig and human embryos measuring 5–10 mm. The most convincing evidence of the presence of a fifth pouch is an actual contact with the ectoderm, posterior to the fourth pouch; this was recorded by Hammar in a 5-mm. embryo, but the contact on either side took place in only one 12 μ section. Grosser states that a closing membrane 'is perhaps not always formed, and is at all events very transitory.'[1] There are as yet very few observations to show that it ever occurs in mammalian embryos. The existence of a sixth pouch has been asserted on the basis of slight elevations which are perhaps inconstant.

TONSILS

The *palatine tonsils* are two rounded masses of lymphoid tissue, one on each side of the throat, between the arches of the palate. Frequently they have been called *amygdalæ* (almonds), but the older Latin term for them is *tonsilla* (a stake to which boats are tied). They are covered by the

[1] Grosser, 1912.

mucous membrane or *tunica mucosa*, which throughout the digestive tract consists of several layers. The soft moist entodermal *epithelium* rests on a connective or reticular tissue layer, the *lamina propria*. A structureless basement membrane, the *membrana propria*, is often present immediately beneath the epithelium. The epithelium, membrana propria, and lamina propria together form the mucous membrane, which in dissection would be stripped off as a single structure. Beneath it, and sometimes not clearly separable from the lamina propria, is the *submucous layer*, or *tela submucosa*. It is a vascular connective tissue, by which the mucous membrane is attached to underlying muscles or bones. All the layers named are

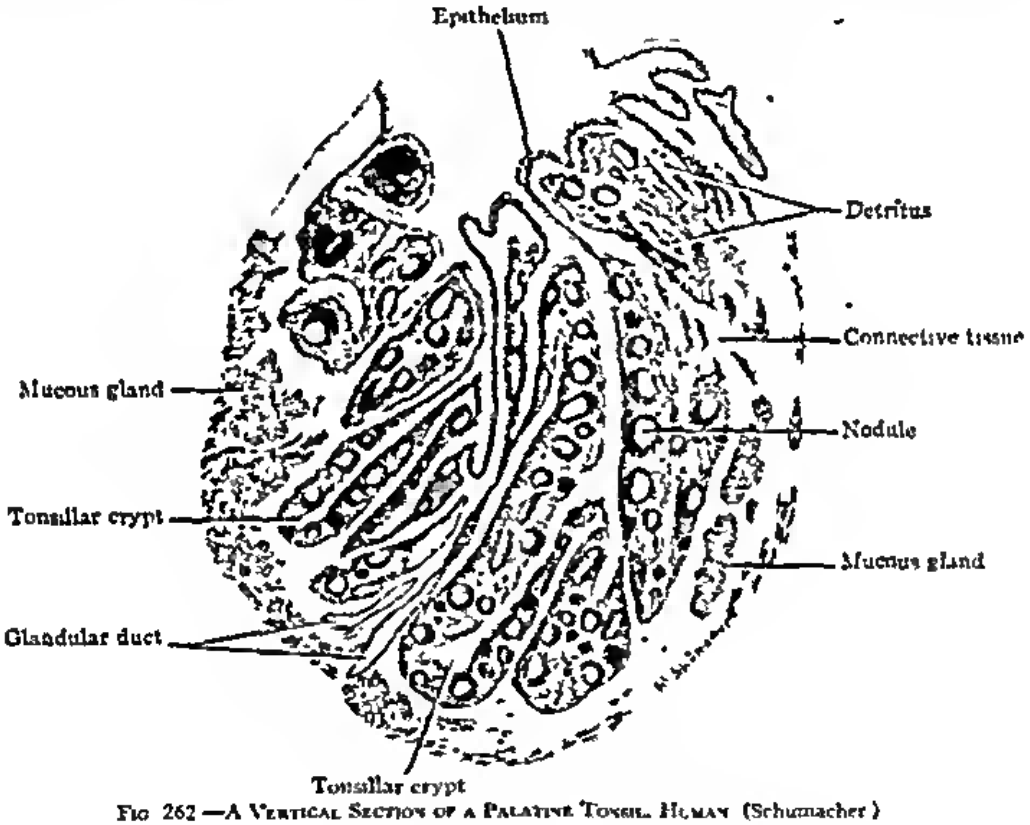


FIG. 262.—A VERTICAL SECTION OF A PALATINE TONSIL. HUMAN (Schumacher)

involved in the tonsils which, however, are essentially lymphoid accumulations in the lamina propria.

The epithelium of the palatine tonsils is a stratified epithelium of many layers, with flattened cells on its smooth free surface, and columnar cells beneath. Its attached surface is invaded by connective tissue elevations or papillæ, so that it appears wavy in sections (Fig. 262). The stratified epithelium lines from ten to twenty almost macroscopic depressions, called *tonsillar pits* or *fossulæ* (crypts). These are irregularly tubular and sometimes branched. Many lymphocytes penetrate between the epithelial cells and escape from the free surface into the saliva, becoming 'salivary corpuscles.' In places the tonsillar epithelium is so full of lymphocytes as to appear disintegrated, a condition which was first

described by Stöhr[1] (Fig. 263). It occurs also in the epithelium of the lingual tonsil. In the reticular tissue of the lamina propria, especially around the pits, there are many lymph nodules, some of which are well defined, with germinative centers, but many others are fused in indefinite masses. The lymphoid tissue constitutes the bulk of the tonsil.

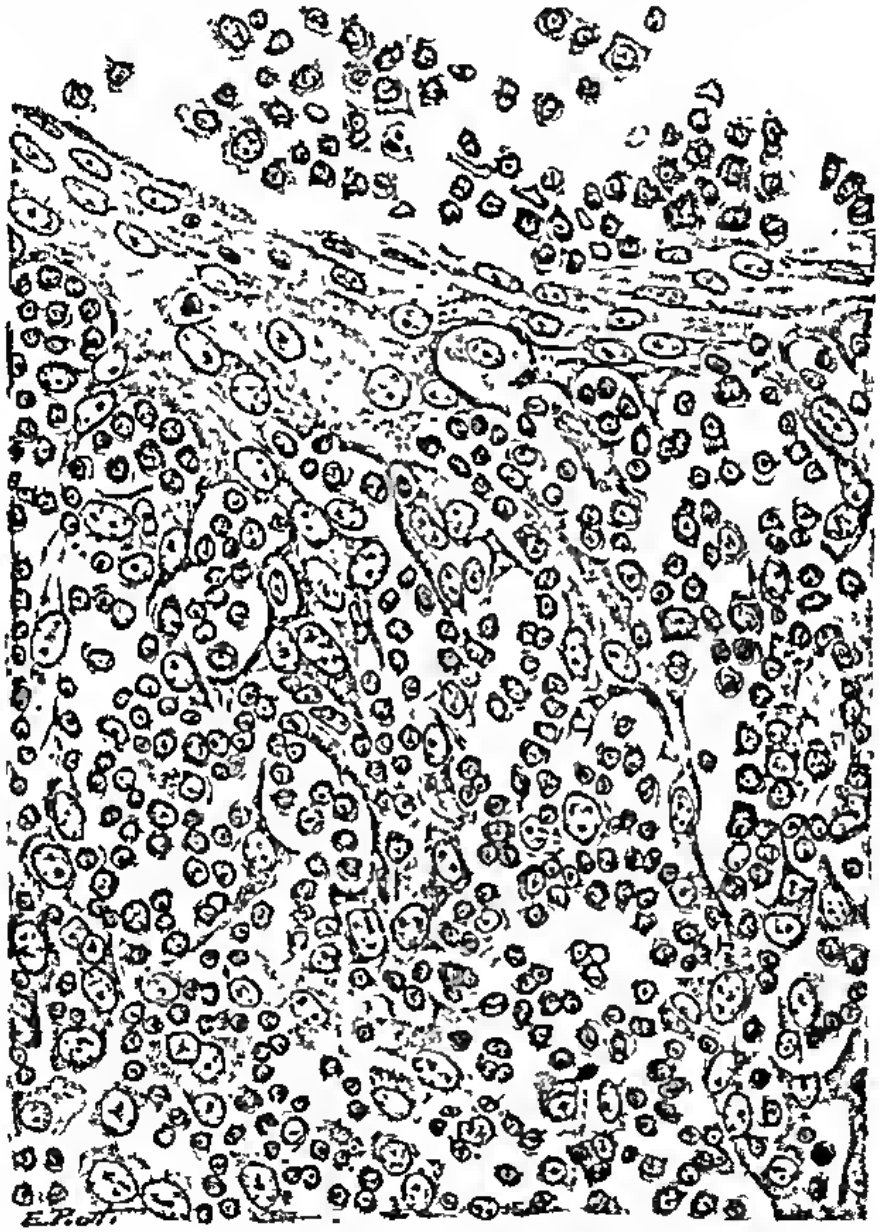


FIG. 263.—A VERTICAL SECTION THROUGH THE HUMAN PALATINE TONSIL.
Infiltration of the stratified epithelium in a crypt with leucocytes. Zenker fixation, hæmatoxylin and eosin.

The submucous layer forms a capsule for the organ, into which it sends trabecular prolongations. It contains many blood and lymphatic vessels, together with branches of the glossopharyngeal nerve and sphenopalatine ganglion which supply the tonsil. It contains also the secreting portions of small mucous glands, some of which empty into the

[1] STÖHR, 1882.

pits, but most of their ducts terminate in the mucous membrane surrounding the tonsil. They resemble other mucous glands of the mouth which will be described presently. Beyond the submucosa is striated muscle, belonging to the arches of the palate and to the superior constrictor of the pharynx; striated muscle fibers are therefore readily included in sections of the tonsil.

The *pharyngeal tonsil* is an accumulation of lymphoid tissue on the median dorsal wall of the pharynx, between the openings of the auditory tubes. In childhood it is liable to become irregularly enlarged so as to obstruct the inner nasal openings, thus forming the 'adenoids' of clinicians. It is covered with stratified epithelium, which is ciliated in embryonic life; and in the adult cilia may be found upon the epithelium

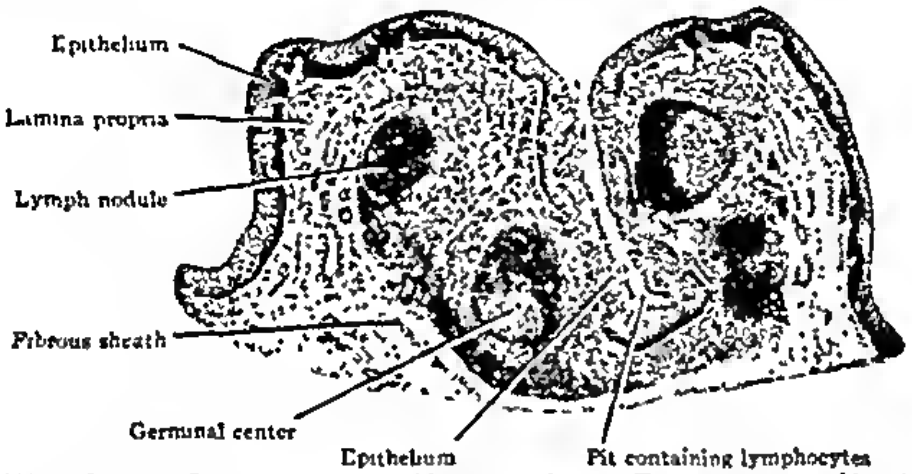


FIG. 264.—A VERTICAL SECTION THROUGH THE MIDDLE OF A LINGUAL TONSIL OF AN ADULT MAN. ×26.

within the pits. The pits and lymphoid tissue are quite like those of the palatine tonsils. Their development in the cat has been described by Kingsbury[1] and by Ramsay.[2]

The *lingual tonsil* is an aggregation of pits surrounded by lymphoid tissue (Fig. 264). It is found in the back part of the tongue, the surface of which is very different in texture from the front part, presenting low mounds with central depressions. Each depression is the outlet of a pit. Lymphocytes pass through the epithelium and become salivary corpuscles, which are said to produce substances protecting the tissue from bacterial infections.

As a group the three sets of tonsils form an incomplete ring of lymphoid tissue guarding the entrance to the pharynx. They may be considered as a special accumulation of the lymphoid nodules found throughout the intestinal tract. The special features of the tonsils are the arrangement of the nodules about the pits, which may be considered as the ducts of glands or abortive glands, and the passage of the lymphocytes to the

[1] KINGSBURY, 1933. [2] RAMSAY, 1935.

surface, even through a stratified epithelium. The pits may form convenient pockets for the growth of bacteria, and it is seldom that some evidence of their action is not shown by the resulting necrosis, even in tonsils considered normal.

Development and Structure of the Tongue. The tongue consists of two parts, an anterior and a posterior, which differ in origin and adult structure. Separating the pharyngeal pouches from one another there are columns of tissue known as *branchial arches.* They come together in the median ventral line to form the floor of the mouth (Fig. 265). In this figure the upper jaw and roof of the pharynx have been cut away; the pharyngeal pouches are seen as dark depressions bounded laterally

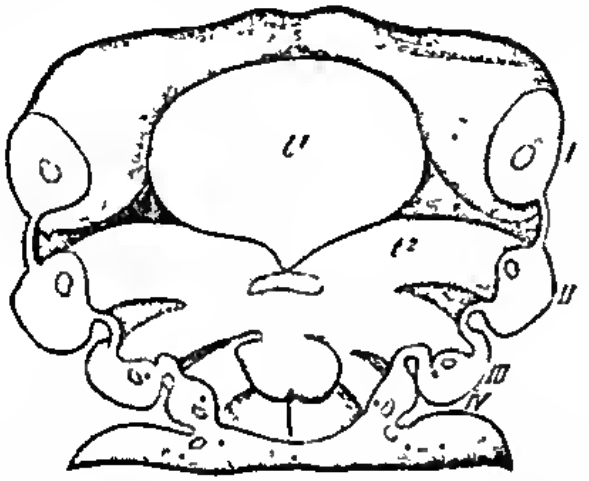


Fig 265.—Floor of the Pharynx of a 10-mm Human Embryo

I-IV. Branchial arches, t^1, anterior part of the tongue, t^2, second arch, joining the posterior part of the tongue toward the median line The thyroid gland is dotted The epiglottis extends over the fourth arch (From McMurrich after His)

by thin plates. The first branchial arch (1) is between the oral and auditory clefts. In the median ventral line an elevation (*tuberculum impar*) arises between this arch and the second; it becomes continuous with a larger elevated portion of the mandibular arch to form the anterior part of the tongue (t^1). The second and third arches unite toward the median ventral line and there produce the posterior part of the tongue (t^2). Between the anterior and posterior parts is the opening of the thyroglossal duct, later the foramen cæcum. The epiglottis is an elevated part of the third arch separated from the posterior part of the tongue by a curved groove.

In the adult (Fig. 266) the *dorsum* of the anterior part of the tongue is roughened with elevations or *papillæ.* These are chiefly the slender *filiform papillæ* and *conical papillæ;* but knob-like forms, the *fungiform papillæ*, are scattered among them over the entire surface, and in life they can be easily distinguished owing to their red color. Near the junction of the anterior and posterior parts of the tongue there is a V-shaped row of larger papillæ, generally six to twelve in number, called *vallate*

papillæ. Their name refers to the deep narrow depression which encircles them. Behind the apex of the V, which is directed toward the throat,

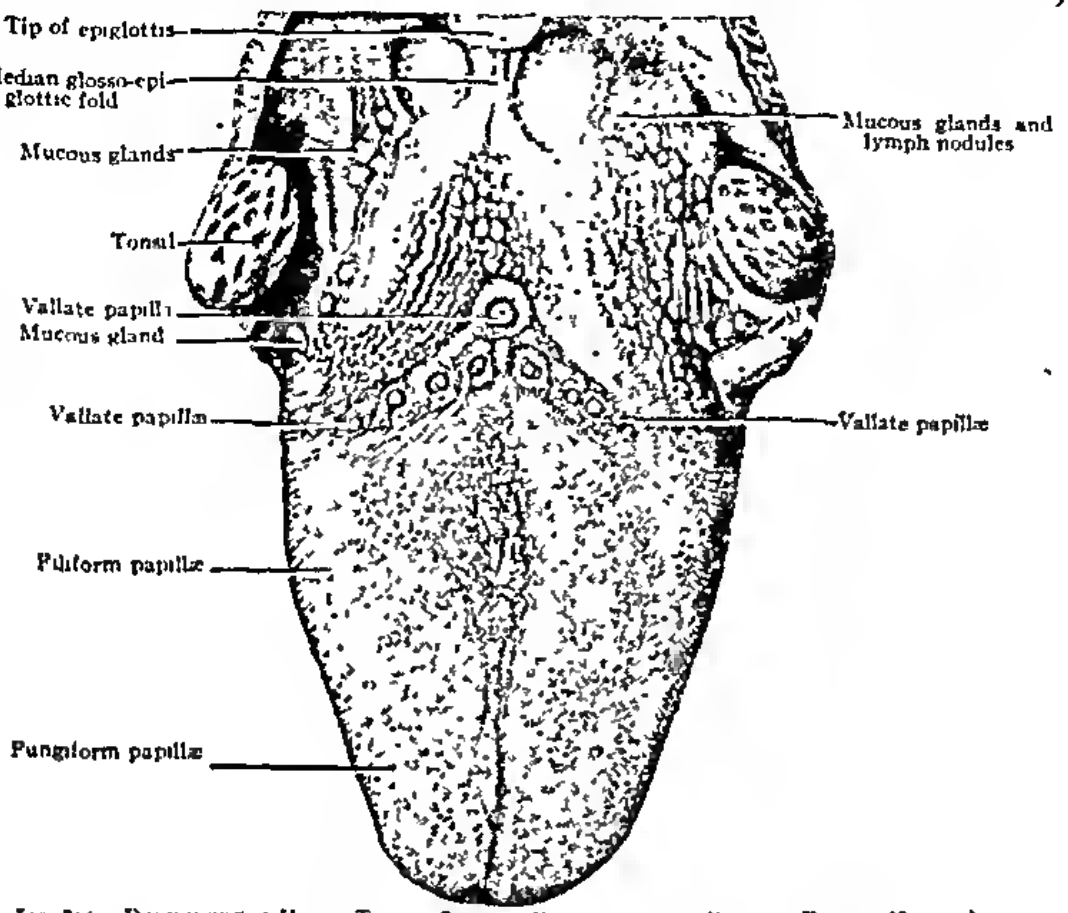


FIG. 266.—DORSUM OF THE HUMAN TONGUE SHOWING PAPILLÆ AND THE PALATINE TONSILS. (Sappey.)

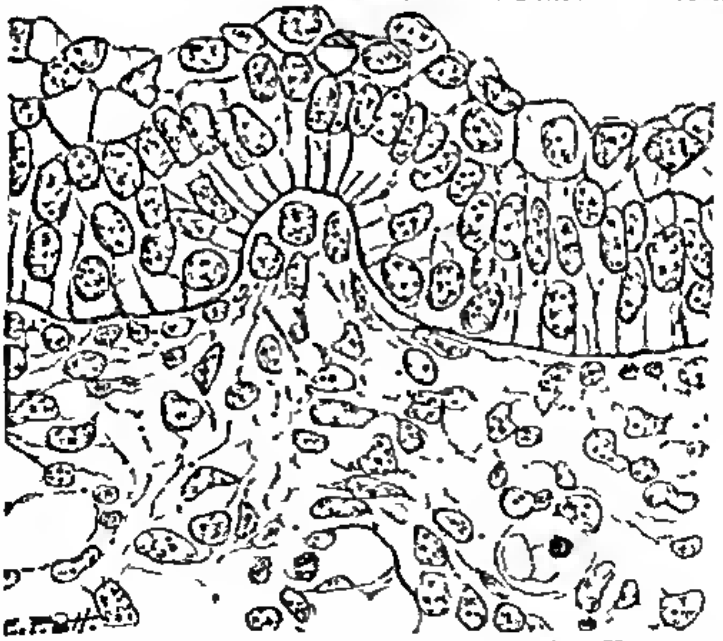


FIG. 267.—DEVELOPMENT OF A PAPILLA OF THE TONGUE IN A 36 MM HUMAN EMBRYO. Zenker fixation, cochineal and orange *C*.

is the foramen cæcum. On each side of the tongue, as indicated in the figure, there are from three to eight parallel vertical folds (2–5 mm. long)

occurring close together; these are the *foliate papillæ*. In the foliate and vallate papillæ the organs of taste are most numerous. The under surface of the tongue is free from epithelial papillæ; its mucosa resembles that which lines the mouth. The posterior part of the tongue has a nodular

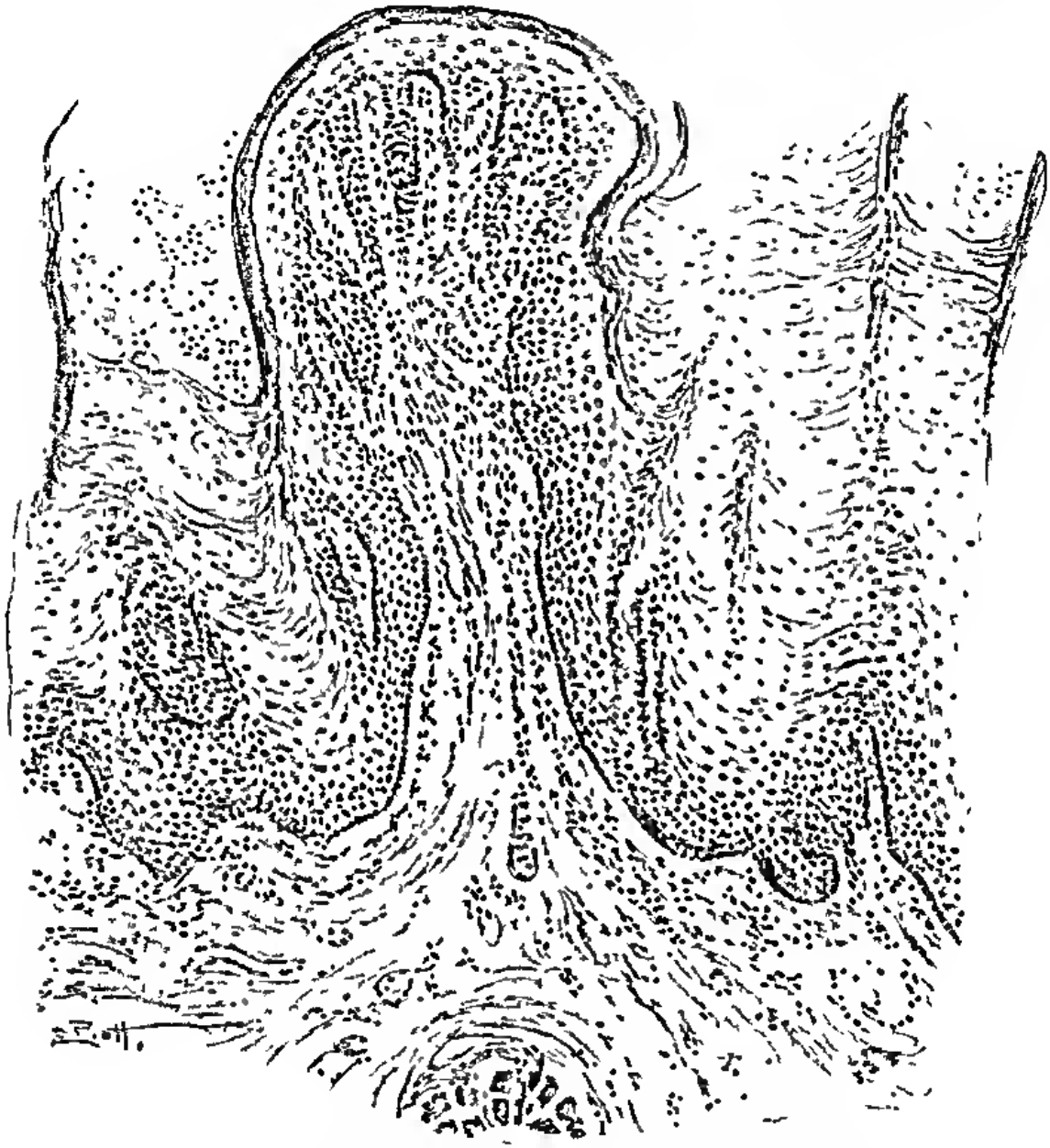


FIG. 268.—A FUNGIFORM PAPILLA ON THE HUMAN TONGUE
Note the primary and secondary papillæ. Zenker fixation; hæmatoxylin and eosin.

surface covered with soft epithelium and contains the lingual tonsil, which has already been described. Laterally it presents fold-like elevations called *lenticular papillæ*.

Filiform papillæ are slender, cornified, epithelial projections, composed of pointed cells which are described as stacked like superimposed hollow cones. The cells have undergone a horny, hyaline degeneration. These projections are arranged in clumps which rest upon a group of

from five to twenty connective tissue elevations, or secondary papillæ; and these in turn are at the summit of a cylindrical or conical primary papilla, composed of vascular connective tissue with numerous elastic fibers. These primary papillæ form the basal portions of the filiform papillæ. They are well shown in Fig. 268 and 269, along with the secondary papillæ, but the cornified processes of the thick epithelium above them have undergone post-mortem disintegration. Most of the papillæ of the tongue are of the filiform type.

Fig. 269.—A Filiform Papilla of the Human Tongue

Zenker fixation, hæmatoxylin and eosin

Fungiform papillæ are rounded elevations with a somewhat constricted base, varying in height from 0.5 to 1.5 mm. In life they are red, since their epithelium is not cornified and transmits the color of the blood beneath. Each contains a primary connective tissue papilla, with but few elastic fibers, beset on all sides with secondary papillæ.

The primary connective tissue papillæ cause corresponding elevations on the surface of the tongue, while the secondary papillæ cause differences in thickness of the stratified epithelium. The latter may be considered as a means of increasing the area of the basal layers in proportion to the surface, and thus providing more cells capable of reproduction and of replacing the loss from the surface. The number and height of the secondary papillæ in any stratified epithelium may be taken as a measure of the normal surface loss of dead cells. The secondary papillæ also provide an economic arrangement for the blood supply, each representing a vascular unit with central artery.

The vallate papillæ resemble broad fungiform papillæ. They are from 1 to 3 mm. broad and 1 to 1.5 mm. tall, each being surrounded by a deep groove (Fig. 270). Their connective tissue often contains longitudinal, oblique, or encircling smooth muscle fibers, the last named being found near the lateral walls. Secondary papillæ are confined to the upper wall. Occasionally the epithelium sends branched prolongations into the

underlying tissue. These may become detached from the surface and appear as concentric bulb-like bodies such as are generally known as 'epithelial pearls.' There are also branched serous glands which grow down from the epithelium, having ducts which open into the deep grooves. The foliate papillæ are parallel folds of mucous membrane, in the epithelium of which there are many *taste buds*. These structures, which occur also in the lateral walls of the vallate papillæ, will be described with the nerves of the tongue.

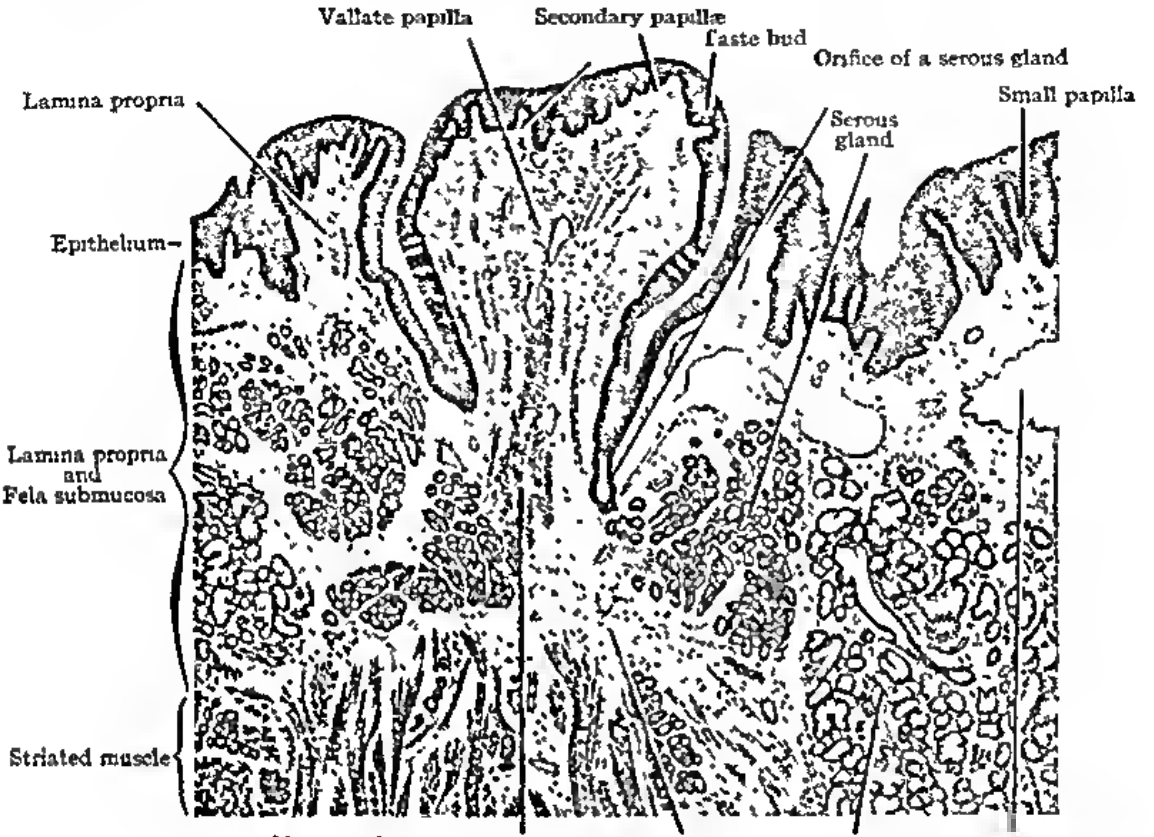


FIG 270.—VERTICAL SECTION OF A HUMAN VALLATE PAPILLA. × 25

The lamina propria of the mucous membrane is a loose connective tissue layer containing fat. It is not sharply separated from the denser submucosa. At the tip of the tongue, or *apex linguæ*, and over the dorsum, the submucosa is particularly firm and thick, forming the *fascia linguæ*. Three sorts of glands branch in the submucosa and may extend into the superficial part of the muscle layer. These are the serous glands found near the vallate and foliate papillæ; mucous glands occurring at the root of the tongue, along its borders, and in an area in front of the median vallate papilla; and the two mixed *anterior lingual glands*, from half an inch to an inch long, each of which empties by five or six ducts on the under surface of the apex. The structure of these types of glands will be described in the section on oral glands.

The muscular layer consists of interwoven bundles of striated fibers which are inserted into the submucosa or into the intermuscular connective tissue. Some of these striated fibers are branched. The musculature of the tongue is partly divided into right and left halves by a dense median connective tissue partition, the *septum linguæ*, which begins low on the hyoid bone, attains its greatest height in the middle of the tongue, and becomes lower anteriorly until it disappears. It does not extend clear through the tongue since it ends 3 mm. beneath the dorsum. The

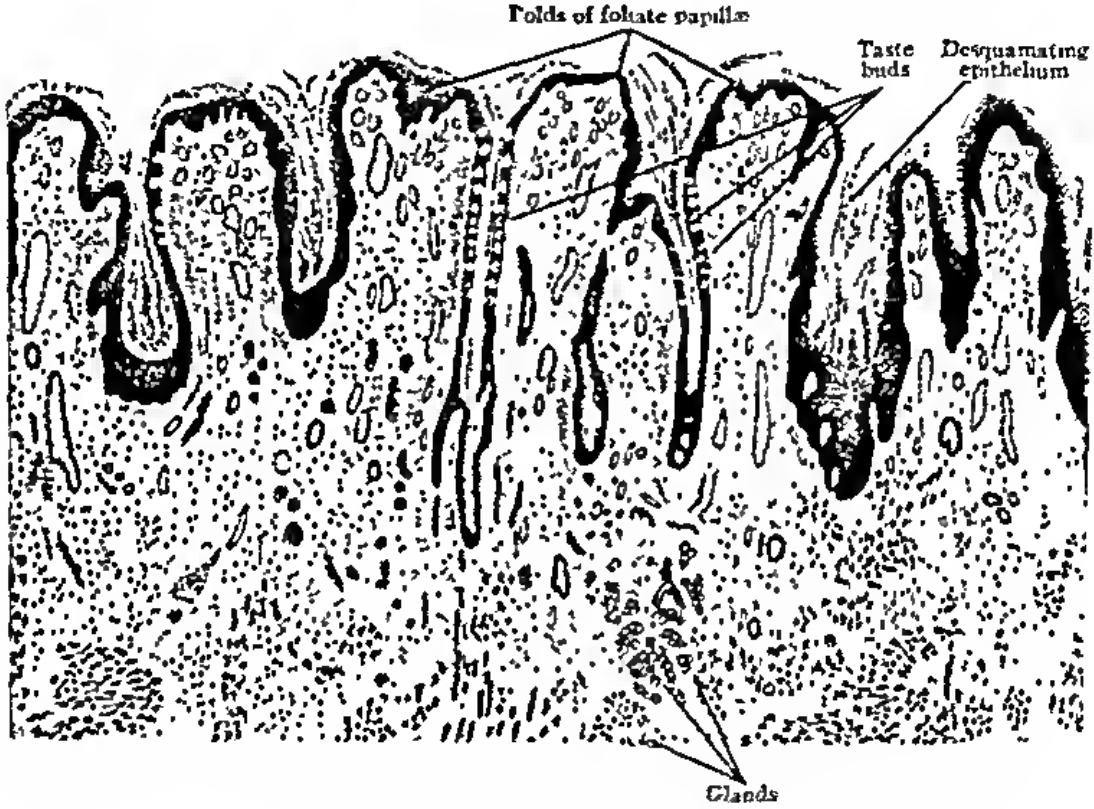


FIG. 271.—VERTICAL SECTION OF A HUMAN FOLIATE PAPILLA. Zenker fixation, hæmatoxylin and eosin. (Sobotta.)

muscles of the tongue are partly vertical (*Mm. genioglossus, hyoglossus,* and *verticalis linguæ*), partly longitudinal (*Mm. styloglossus, chondroglossus, superior* and *inferior longitudinalis linguæ*), and partly transverse (*M. transversus linguæ*). The *glossopalatine muscle* of the palatine group also enters the tongue. Some of the muscle fibers are oblique but many of the bundles cross at right angles. In the connective tissue between them, medullated nerves are abundant. Some are sensory nerves to the mucosa, but many of them are the lingual branches of the hypoglossal nerve which supply all the tongue muscles except the inferior longitudinal; the latter is supplied by fibers from the chorda tympani. Sensory spindles have been found in the lingual muscles.

Blood vessels are numerous in the submucosa and form extensive capillary networks in the lamina propria of both primary and secondary

papillæ. Small lymphatic vessels also form a network in the lamina propria, and this is continuous with a coarser net in the submucosa.

The sensory nerves are the terminations of the lingual branches of the mandibular nerve anteriorly, and of the lingual branches of the glossopharyngeus posteriorly. In the submucous connective tissue they form a plexus of medullated and non-medullated fibers, and in some places, notably beneath the vallate papillæ, nerve cells are found, grouped in

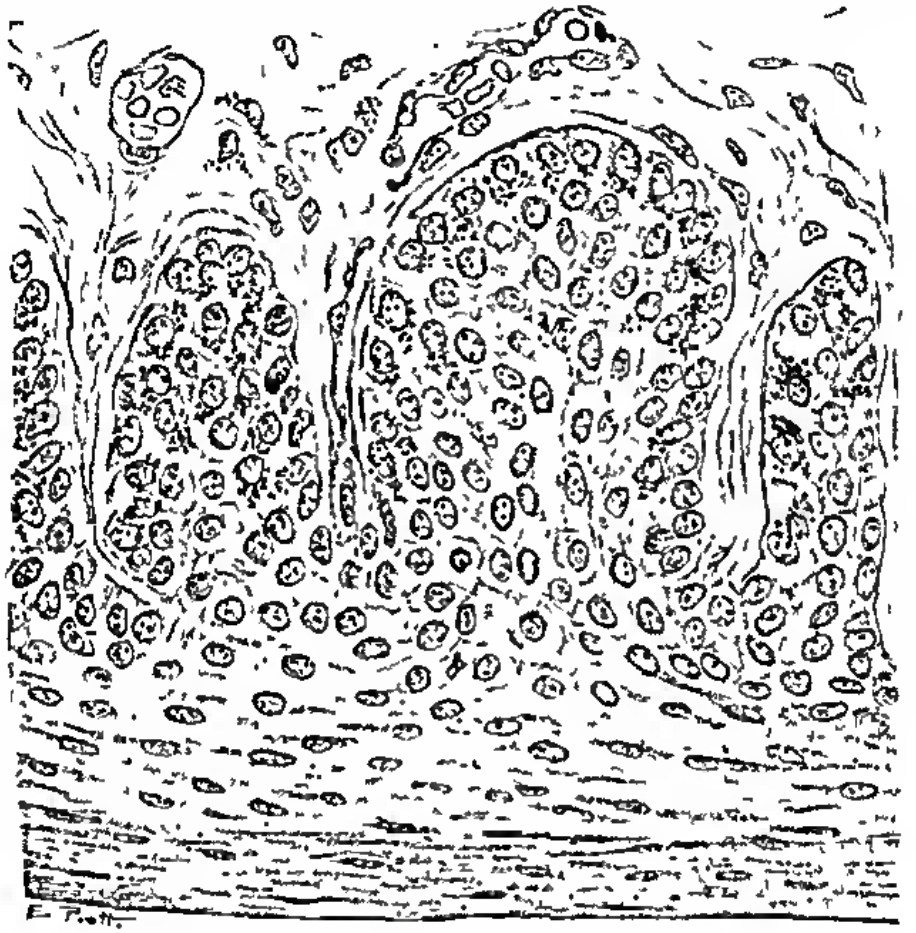


FIG. 272.—A VERTICAL SECTION OF THE HUMAN TONGUE

Under surface near the tip. The lowermost cells of the epithelium contain a yellow-brown pigment. Zenker fixation; hæmatoxylin and eosin.

small ganglia. The terminal branches of these nerves probably end in part in bulbous corpuscles, but most of them, as non-medullated fibers, enter the epithelium and extend to the outer epithelial cells, generally without branching. Others enter the groups of specialized epithelial cells, known as *taste buds*, which are believed to be the special organs of taste. Within the buds the nerves divide into coarse varicose branches which end freely, without uniting with the cells or anatomosing with one another.

Taste buds[1,2] are round or oval groups of elongated epithelial cells, most of which extend from the basal to the free surface of the epithelium. In embryos of from five to seven months they are more numerous than in

[1] LOVÉN, 1868. [2] SCHWALBE, 1868.

the adult, occurring in many filiform papillæ, in all the fungiform, vallate and foliate papillæ, and also upon both sides of the epiglottis. Subsequently they degenerate with an infiltration of leucocytes, except on the lateral walls of the vallate and foliate papillæ, on the laryngeal surface of the epiglottis, and a small portion of those on the anterior and lateral fungiform papillæ. These remain in the adult. In the outer half of each bud the cells converge like the segments of a melon, so that their

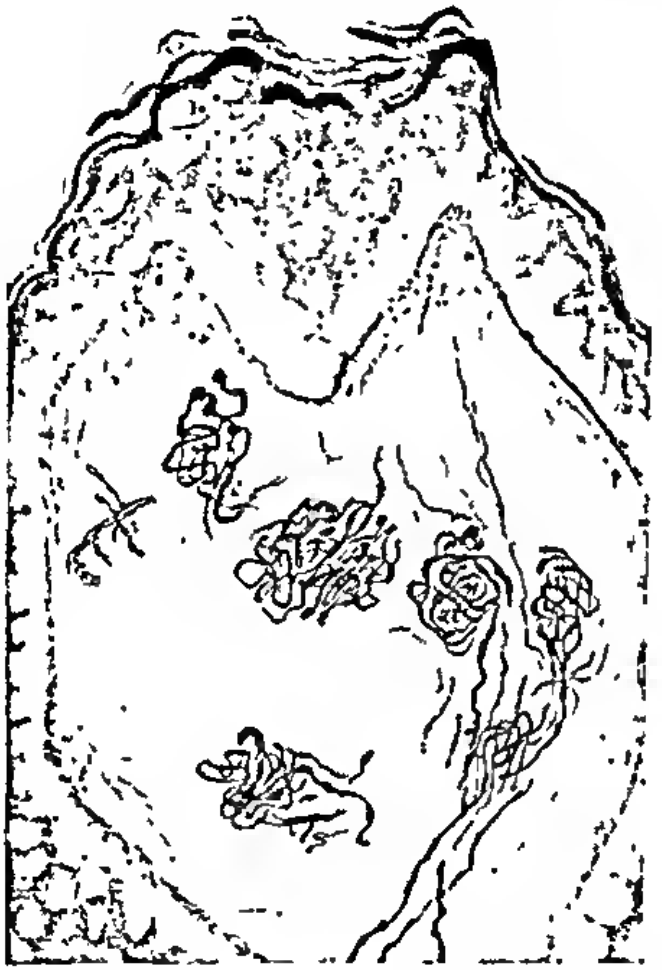


Fig. 273.—Sensory Nerve Endings in the Tip of the Human Tongue. Bielschowsky Method. (Kadanoff)

ends are brought together in a small area. This area is at the bottom of a little *pore* or short canal found among the outermost flat cells of the epithelium. The taste pore opens freely to the surface, as in Fig. 274, but in oblique sections it may appear bridged.

Within the bud three types of elongated cells may be distinguished; *pillar cells* which are peripheral, *supporting cells* and *taste cells* which are central. There are also certain cells which lie wholly in the basal part of the bud, and lymphocytes which have entered the bud from below are frequently seen among the other cells. The supporting cells are paler than the taste cells, and may be uniform in diameter or tapering toward their ends; they are sometimes forked or branched below. Their

nuclei are round and pale. The taste cells are darker and more slender, being thickened to accommodate the narrow nucleus which is usually near the middle of the cell. At the taste pore these cells end in a stiff refractive process which is a cuticular formation. The processes extend into the deeper part of the pore but do not reach its outlet. These cells are believed to transmit the gustatory stimuli to the nerves which branch about them. To a less extent the nerves are said to ramify around the supporting cells, which perhaps have other functions than their name implies. Heidenhain[1] considers both types as taste cells, more or less differentiated.

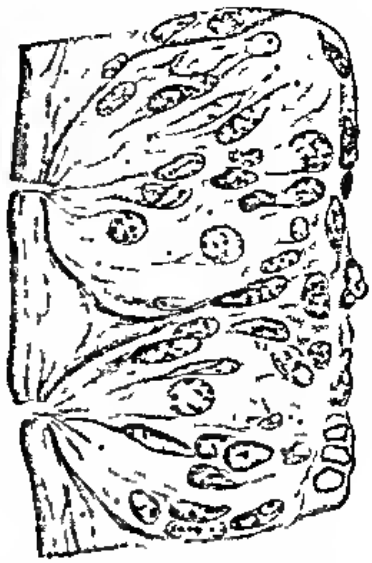


FIG. 274.—TASTE BUDS IN WALL OF FOLIATE PAPILLA, RABBIT

The processes of the taste cells are protected by their position within the pore from any injury by friction on the surface They can only be stimulated by substances in solution which can enter the pore A dry tongue, as after atropin which inhibits the secretion of the oral glands, cannot taste dry salt or sugar.

LINING OF ORAL CAVITY

The lining of the mouth, like the covering of the tongue, consists of epithelium, lamina propria, and submucosa. At the lips, toward the line

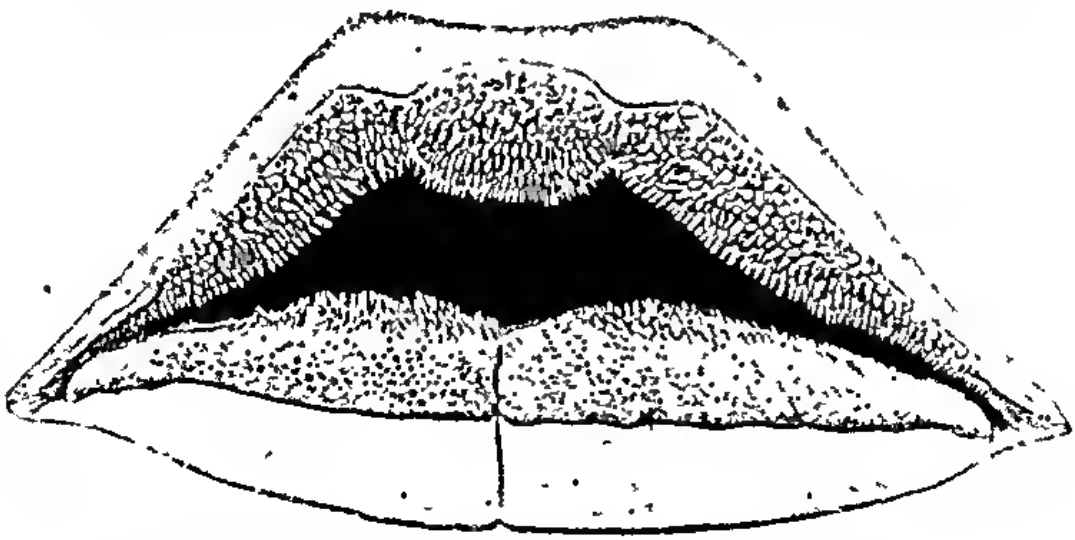


FIG 275.—LIPS OF THE HUMAN NEW-BORN FROM IN FRONT.

The smooth Pars glabra is sharply differentiated from the Pars villosa with papillæ The Tuberculum lateralis superior appears clearly as a part of the Pars villosa The dark points in the middle of the villi are blood vessels appearing *through the epithelium (Malka Ramm)*

of transition from skin to mucous membrane, hairs, disappear from the skin. The epithelium becomes thicker but more transparent as it crosses the line (Fig. 276). Its outer cells are still cornified, but they are not so

[1] HEIDENHAIN, 1914.

flat and compactly placed as in the skin. The deeper cells appear vesicular. Within the mouth, except on the tongue, cornified cells are absent, but granules of the refractive horny substance, *keratohyalin*, are said to occur in the outer cells, even in the œsophagus. The free surface of the epithelium is generally smooth, but its under surface is indented by many

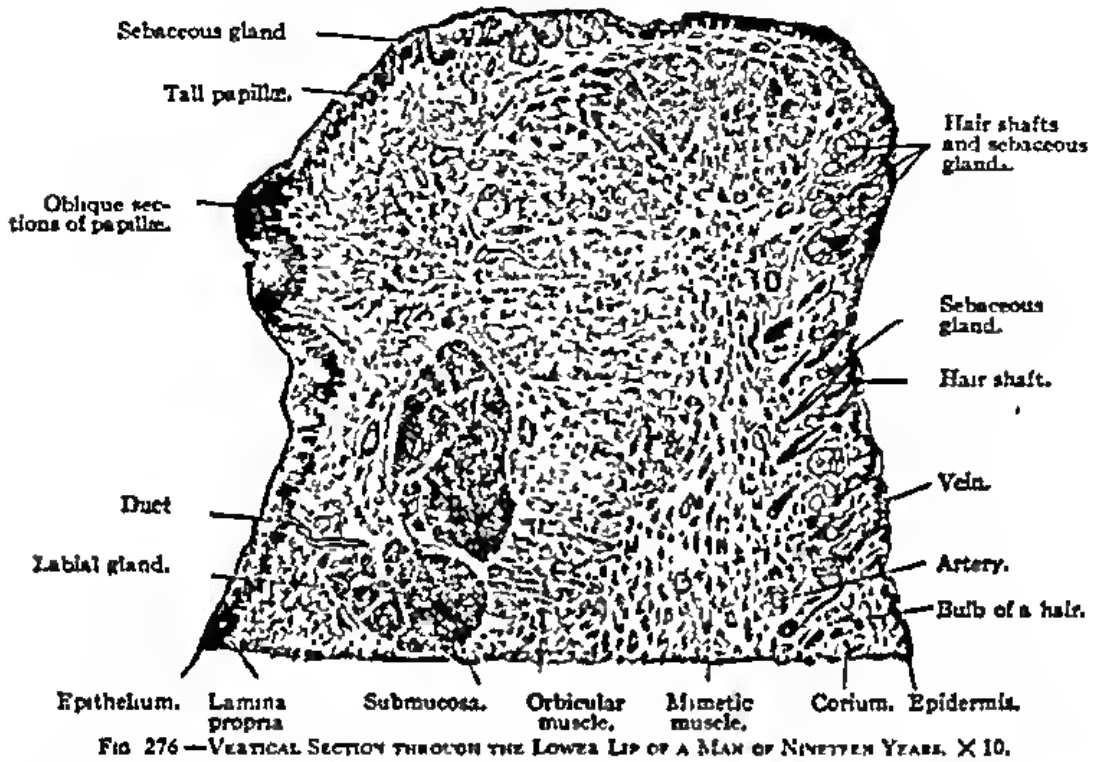


FIG. 276.—VERTICAL SECTION THROUGH THE LOWER LIP OF A MAN OF NINETEEN YEARS. × 10.
Epidermis and corium constitute the skin; epithelium, l. propria, and submucosa form the oral mucous membrane

connective tissue papillæ, which are particularly long and slender in the gums and lips. On the lips of still-born infants, after the epithelium has been macerated by the amniotic fluid, they may appear as slender villi.[1] Cilia are found on the oral, pharyngeal and œsophageal epithelia in the embryo, but in the adult cilia persist only in certain parts of the pharynx.

The lamina propria in the mouth, as is generally the case in the digestive tract, has few elastic fibers. Some of its tissue is reticular, and in it lymphoid accumulations are frequent; they may extend into the submucosa. On the oral surface of the soft palate there is a layer of elastic tissue between the propria and submucosa. A similar layer is found in the œsophageal end of the pharynx. It increases in thickness upward, at the expense of the submucosa, so that it forms a thick layer in the back of the pharynx in contact with the muscles, among the fibers of which it sends prolongations. This elastic layer, as the *fascia pharyngobasilaris*, is attached to the base of the skull.

[1] WIERRY AND ANSON, 1936.

In most of the oral region there is no sharp line of separation between the propria and the submucosa. The latter may be a loose layer contain-

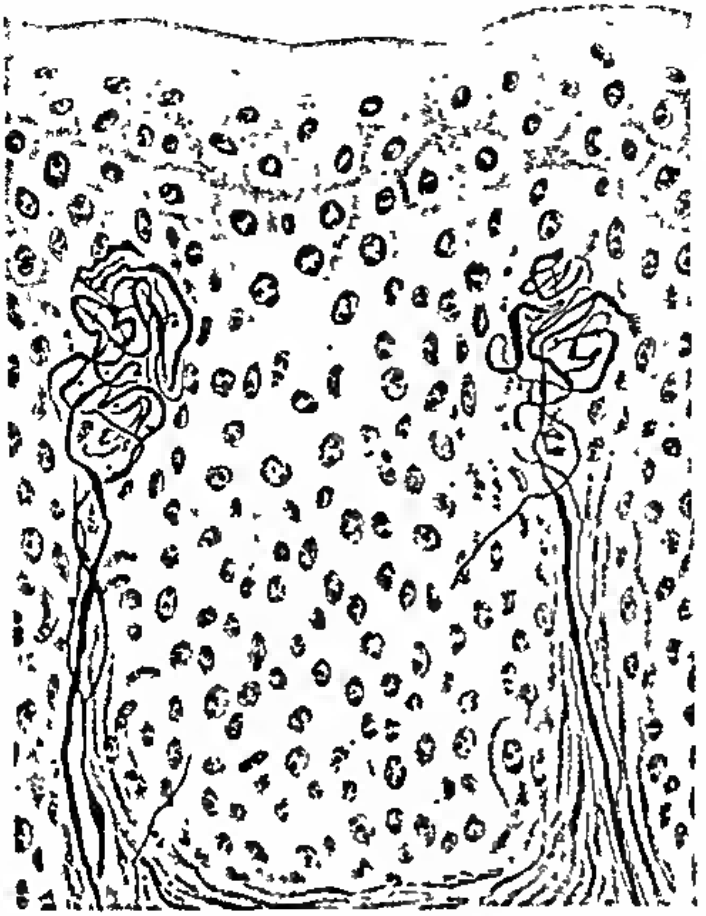


Fig 277.—Sensory Nerve Endings in the Human Lip. Bielschowsky Method. (Kadanoff)

ing fat, and allowing considerable movement of the mucosa, or, as in the gums and hard palate, it may be a dense layer binding the membrane closely to the periosteum. In the submucosa are the branches of various glands. On the inner border of the lips and the inner surface of the cheek, there are *sebaceous glands* without hairs, which first develop during puberty. This type is described with the skin. The other oral glands are considered in the following section.

Fig 278.—A Ruffini End-corpuscle in the Stratum Subpapillare of the Mucous Membrane of the Human Cheek. Golgi Method. (Cercherelli)

GLANDS OF THE ORAL CAVITY

The oral glands include serous glands, mucous glands and mixed glands, to be described in turn. In the general account of the processes of secretion (p. 83) the mucous and serous cells have already been described. Their arrangement in the particular oral glands follows.

Serous Glands. The serous (or albuminous) oral glands are the parotid glands and the serous glands of the tongue (v. Ebner's glands). The latter are branched tubular glands limited to the vicinity of the vallate and foliate papillæ. Generally they open into the grooves which bound these papillæ. Their ducts are lined with simple or with stratified epithelium, which is occasionally ciliated. Their small tubules consist of a delicate *membrana propria* or basement membrane, which surrounds the low columnar or conical serous cells. In this simple epithelium distinct cell boundaries are lacking. With special stains a dark granular zone toward the lumen may be distinguished from the clear basal portion of the cell which contains the nucleus (Fig. 279). The lumen of the tubules is very narrow and receives the still narrower intercellular secretory capillaries (Fig. 280).

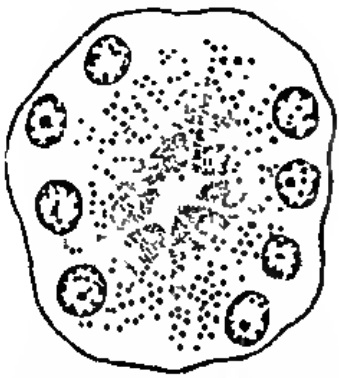


Fig. 279.—Tubule of Serous Gland from the Human Tongue. × 750.

Secretory granules toward the lumen are finer than those further out. The light intercellular lines represent the secretory capillaries.

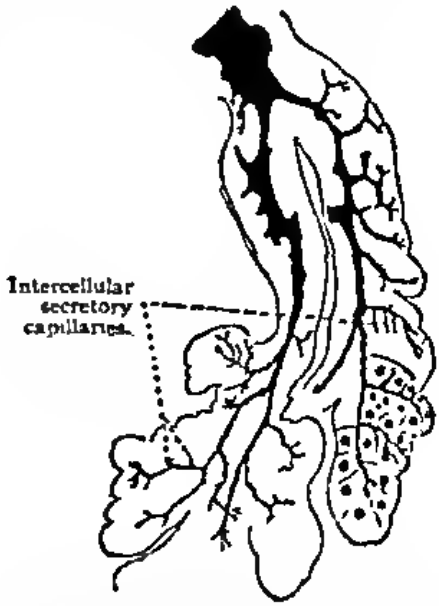


Fig. 280.—Section of a Serous Gland from the Tongue of a Mouse. × 240.

Prepared by Golgi's method, a precipitate has formed in the ducts. The right lower part of the figure has been completed by adding the cell outlines.

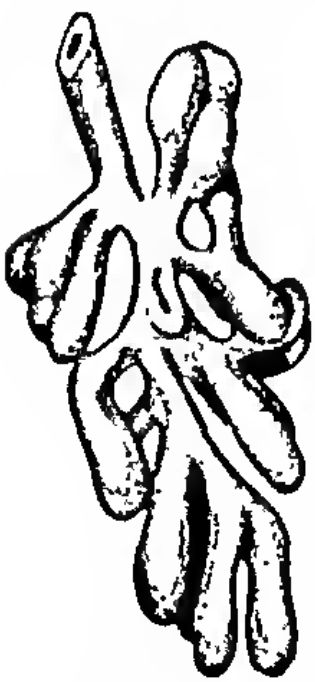


Fig. 281.—Reconstruction of a Portion of von Ebner's Gland from the Tongue of a 14 Yr. Old Boy (Mazurski).

The *parotid glands* are the largest oral glands. Each is situated in front of the ear and is folded around the ramus of the mandible; its duct, the *parotid duct* (Stensen's), empties into the mouth opposite the second molar tooth of the upper jaw. The parotid gland is an organic, branched serous gland, subdivided into lobes and lobules. The *accessory parotid gland* appears as a lobe separated from the others. The parotid duct is characterized by a thick membrana propria, and consists of a two-layered

columnar epithelium with occasional goblet cells. As the duct branches repeatedly, the epithelium becomes a simple columnar epithelium, after being pseudo-stratified, with two rows of nuclei. This *excretory* portion of the duct is followed by the *secretory* part, formed of simple columnar cells with basal striations, perhaps indicative of secretory activity. As shown in the diagram (Fig. 282) the secretory ducts become slender, forming the *intercalated ducts*. These are lined with flat spindle-shaped cells which are continuous with the large cuboidal serous cells of the terminal acini. The gland cells when empty of secretion are small and darkly granular, and when full are larger and clearer. They rest upon a basement membrane containing stellate or 'basket' cells. Intercellular secretory capillaries end blindly before reaching the basement membrane. Up to two years of age mucous cells are also present.

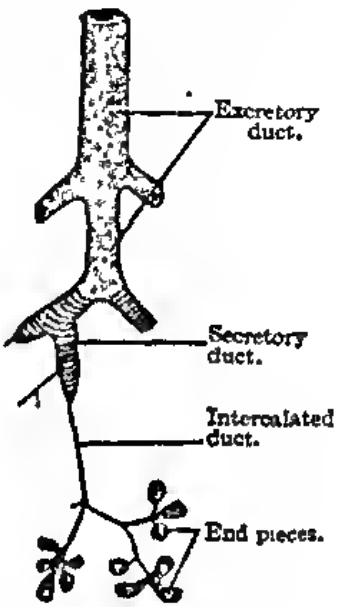


Fig. 282.—Diagram of the Human Parotid Gland.

Between the acini, which are somewhat elongated and branched, there is vascular connective tissue containing fat cells. In denser form it surrounds the lobules and lobes of the gland, and the larger ducts. The ducts which are found in the connective tissue septa are called interlobular ducts, in distinction from those which are surrounded by the acini in which they and their branches terminate. The latter are intralobular ducts. They are smaller and have less connective tissue around them than the interlobular ducts, of which, however, they are continuations.

Vessels and Nerves. The arteries generally follow the ducts from the connective tissue septa into the lobules, where they produce abundant capillary networks close to the basement membranes. The veins derived from these soon enter the interlobular tissue, and may then accompany the arteries. The lymphatic vessels follow the ducts, and branch in the interlobular connective tissue, in which they terminate. Only tissue spaces have been found within the lobules. The nerve supply is from several sources. Sympathetic nerves from the plexus around the carotid artery accompany the blood vessels into the parotid gland, and by controlling the blood supply have an important bearing upon secretion. The nerves which reach the gland cells are in connection with the tympanic branch of the glossopharyngeal nerve. This branch extends to the otic ganglion, from which fibers pass to the parotid gland by way of an anastomosis with the auriculo-temporal branch of the mandibular nerve. Within the gland the nerves pass along the ducts, where they are associated with microscopic ganglia, and form plexuses beneath the

basement membranes of the acini. From these plexuses fibers penetrate the basement membranes and form simple or branched, varicose endings in contact with the gland cells. Other nerves enter the substance of the gland, either to pass through it or to contribute to its nerve supply; these include branches of the trigeminal, facial and great auricular nerves, the last coming from the second and third cervical nerves. Free sensory endings of medullated fibers are said to occur in the epithelium of the ducts.

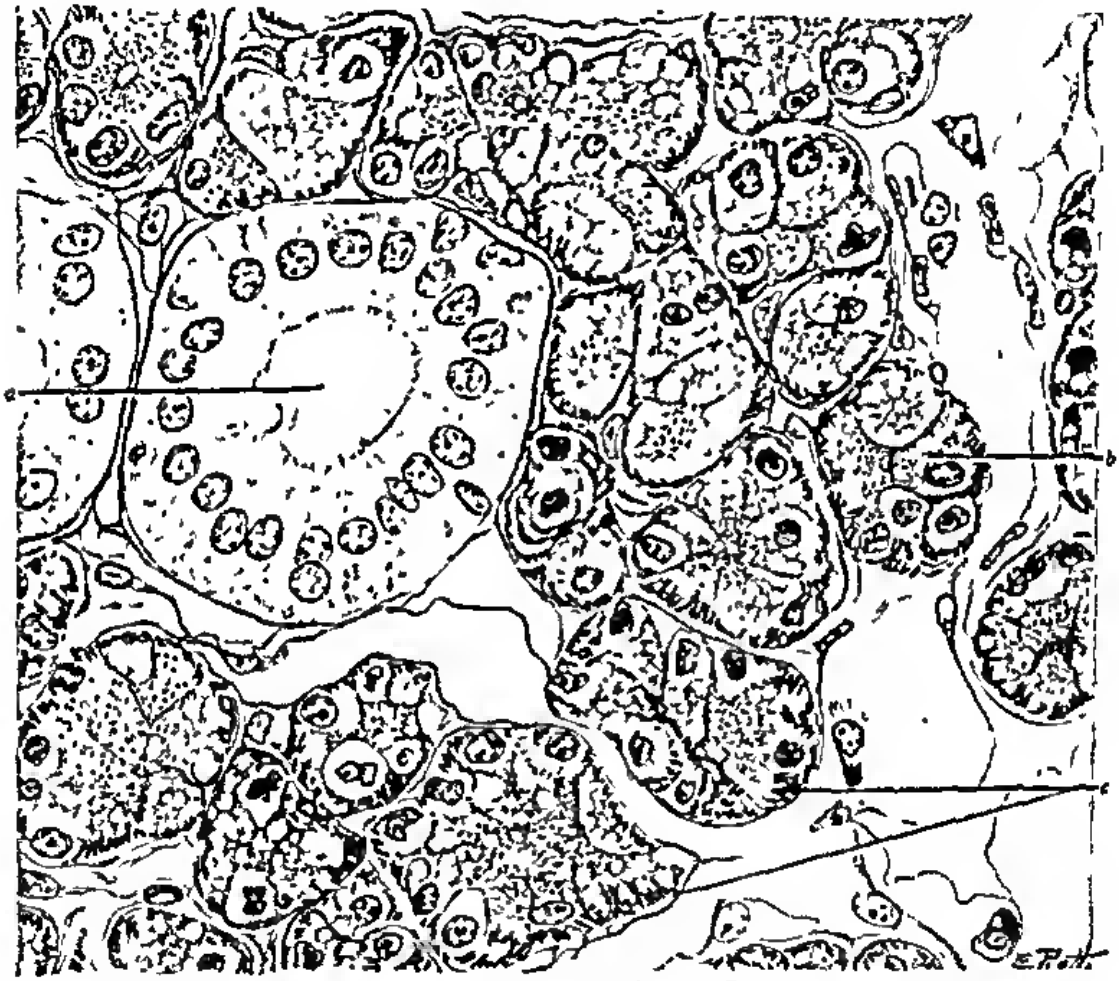
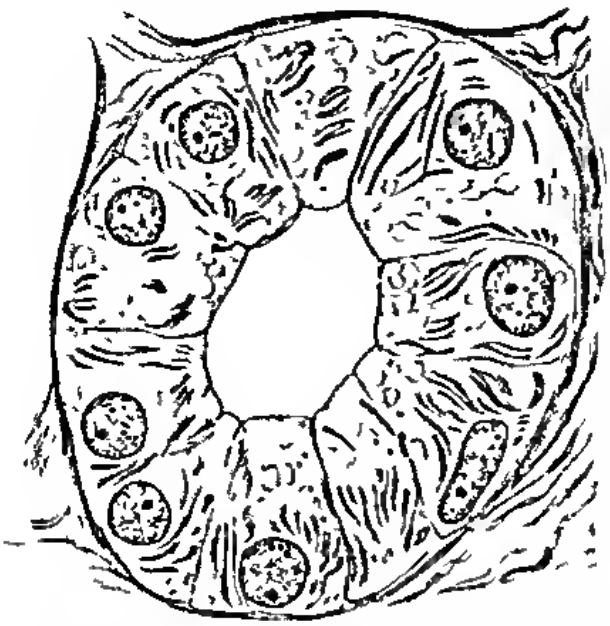


Fig. 283.—Section of a Human Parotid Gland

Zenker fixation, methylene blue and eosin. *a*—Section of a salivary duct. *b*—Secretory cell. *c*—Basal striations.

Mucous Glands. The purely mucous glands of the mouth are simple branched alveolotubular glands found on the anterior surface of the soft palate and on the hard palate (*palatine glands*), along the borders of the tongue (*lingual glands*), and in greater numbers in the root of the tongue. There they may open into the tonsillar pits through ducts lined with columnar epithelium, sometimes ciliated. The wall of the tubules consists of a structureless basement membrane and of columnar mucous cells, varying according to their functional condition. The empty cells are narrower than the others, and the nuclei, though at the base of the

cell and transversely oval, are not so flat as in cells full of secretion. Seldom can cells be found completely occupied by unaltered protoplasm. A single gland, or even a single alveolus, may contain cells in different



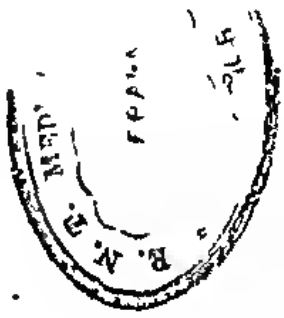


Fig. 284.—A Section of the Glossopalatine Gland of a 40 Yr. Old Executed Man. The blue spindle-shaped bodies in the cells are the 'attractosomes.' Alcohol-formaldehyde fixation, iron-hæmatoxylin and Mallory's connective tissue stain. (Clara.)

phases of secretion, as is clearly seen when special mucin stains are used. Secretory capillaries are not found in the purely mucous glands.

Mixed Glands. The mixed oral glands are the sublingual, submaxillary, anterior lingual, labial, buccal, and molar glands. The parotid gland also is said to contain mucous end pieces in the new-born, which disappear later in childhood.

The *sublingual glands* are two groups of glands, one on each side of the median line, under the mucous membrane in the front of the mouth. The largest component is an alveolotubular structure emptying by the *ductus sublingualis major* on the side of the *frenulum linguæ*. The main stem and the principal branches of the large sublingual duct are lined by a two-layered or pseudo-stratified columnar epithelium, as in the parotid duct. They are surrounded by connective tissue containing many elastic fibers. Ducts less than 0.05 mm. in diameter have a simple columnar epithelium, which in a few places becomes low and basally striated to form the secretory ducts. As shown in the diagram, Fig. 285, the secretory ducts are very short, and they are accordingly infrequent in sections;

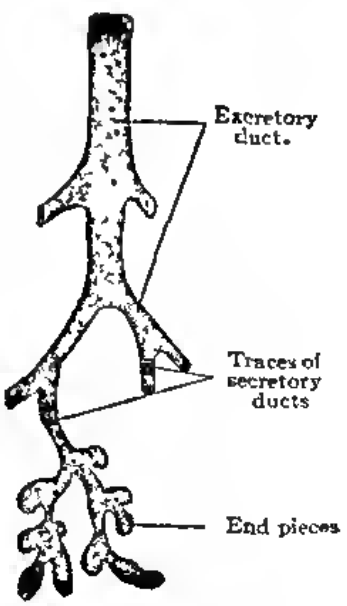


Fig. 285.—Diagram of the Human Sublingual Gland.

the slender intercalated ducts may be absent. The terminal secreting portions of the gland are somewhat tortuous structures, often presenting out-pocketings. They consist of mucous and serous cells quite evenly mixed, so that the gland has a characteristic appearance under low magnification. The serous cells sometimes border upon the lumen, but often they are separated from it by the mucous cells so that they form crescents or demilunes. Only the serous cells are provided with the

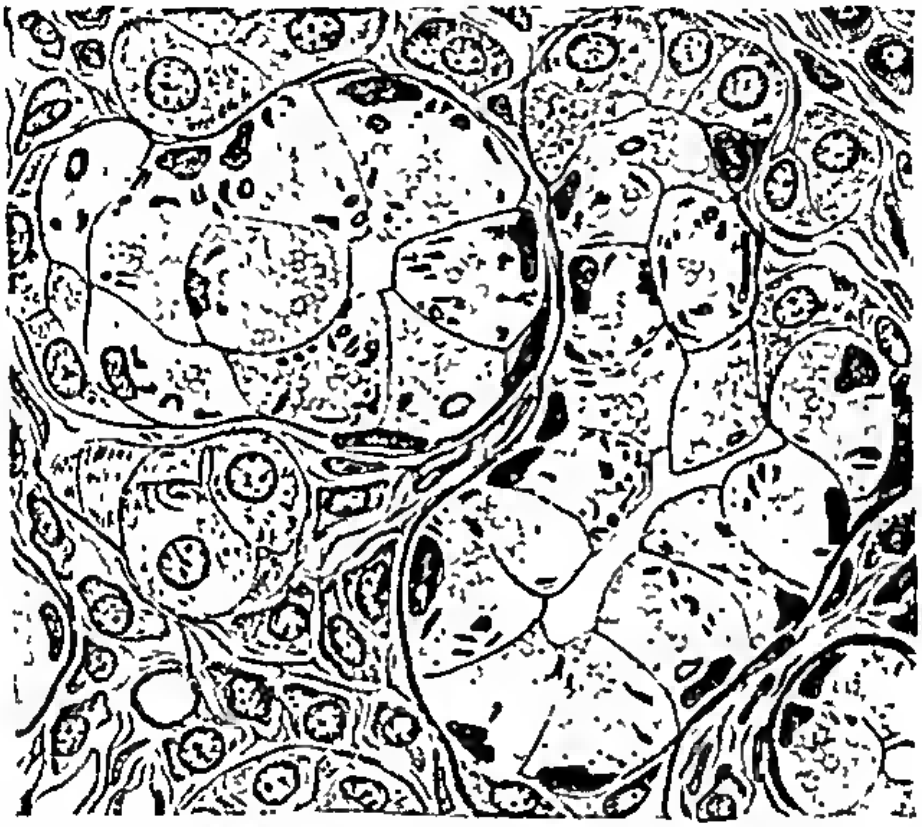


FIG. 286.—A SECTION OF THE SUBLINGUAL GLAND OF A THIRTY YEAR OLD EXECUTED MAN.
The mucous secreting cells are stained blue and show 'attractosomes'; the serous cells are gray. Zenker fixation; iron-hematoxylin and Mallory's connective tissue stain. (Clara.)

branched intercellular secretory capillaries. Around the tubules there is a basement membrane including certain stellate cells, which are considered by some as basal epithelial cells. The interlobular connective tissue contains many lymphocytes.

Near the gland just described, but apparently quite distinct from it, there is a group of 5 to 20 alveolotubular glands which open by separate ducts, the *ductus sublinguales minores*. These glands consist almost exclusively of mucous cells.

The sublingual gland as a whole receives fibers from the submaxillary ganglion, and so from the chorda tympani, which passes to this ganglion by way of an anastomosis with the lingual branch of the mandibular nerve. Its ducts are said to have sensory fibers, probably derived from the lingual nerve. Sympathetic fibers from the superior cervical ganglion,

which have ascended the neck as perivascular plexuses, extend to the sublingual gland around its arteries.

The *submaxillary glands* are a pair of branched alveolar glands, in part tubulo-alveolar, found in the floor of the mouth, each being drained by a *submaxillary duct* (Wharton's) which opens on the sides of the frenulum linguæ near its front margin. Sometimes this duct is joined by the *ductus sublingualis major* so that the two have a common outlet. Its orifice may be lined by stratified epithelium, but this soon gives place to the two-layered form. Secretory ducts are well developed (Fig. 287), and their basally striated cells contain a yellow pigment. The intercalated ducts, which are lined with simple cuboidal epithelium, lead to terminations of two sorts. Most of these consist entirely of serous cells.

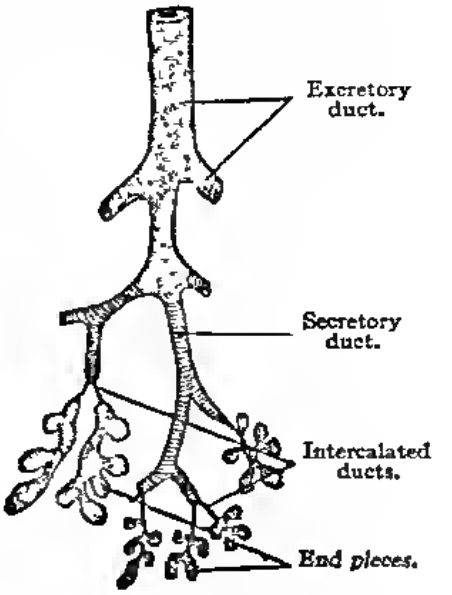


Fig. 287.—Diagram of the Human Submaxillary Gland

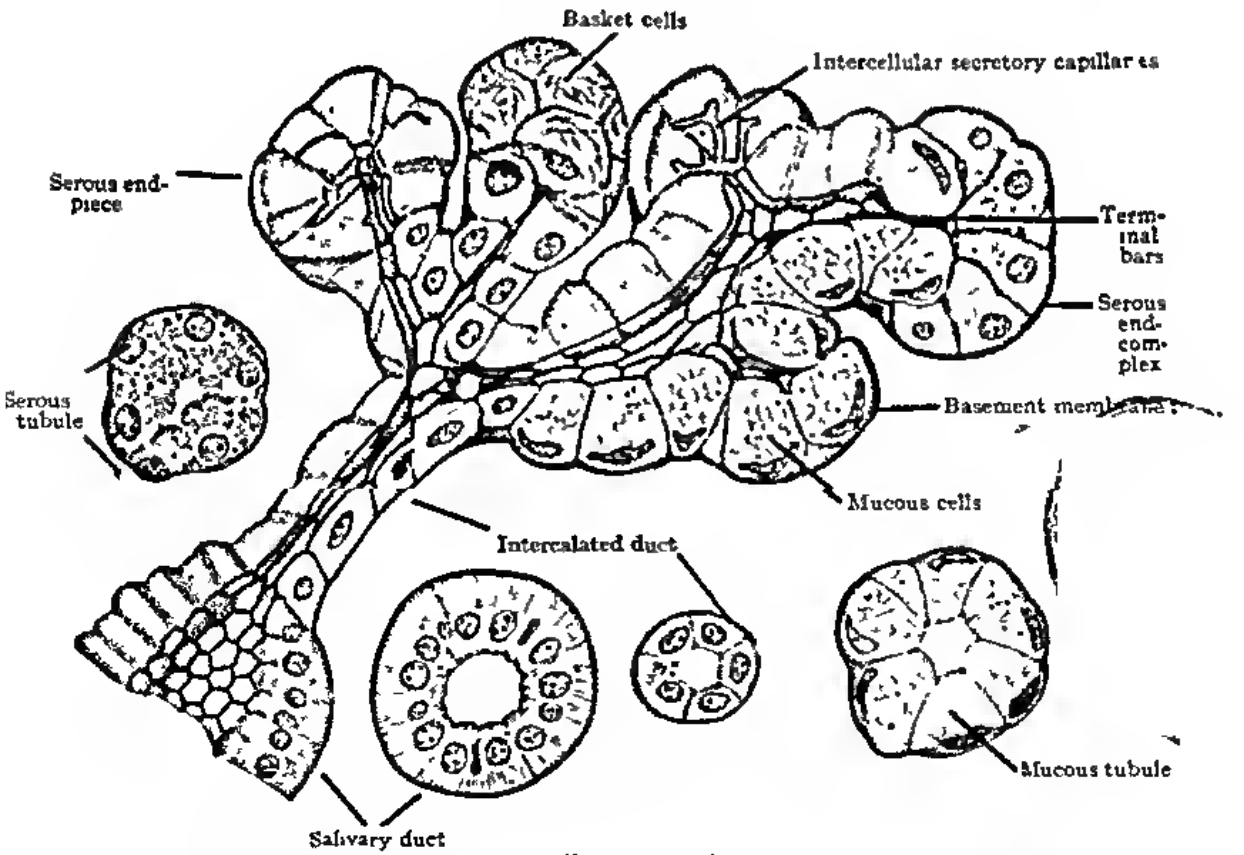


Fig. 288.—A Reconstruction of Salivary (Submaxillary) Glandular End-pieces. (Braus)

The others are mixed, but the crescents are small, composed of only a few

or even of single serous cells (Figs. 288 and 289). Secretory capillaries, such as have already been described, are related only to the serous cells. Elastic tissue surrounding the alveoli has been thought to aid in expelling the secretion through the ducts. The nerves have the same origin as those of the sublingual gland.

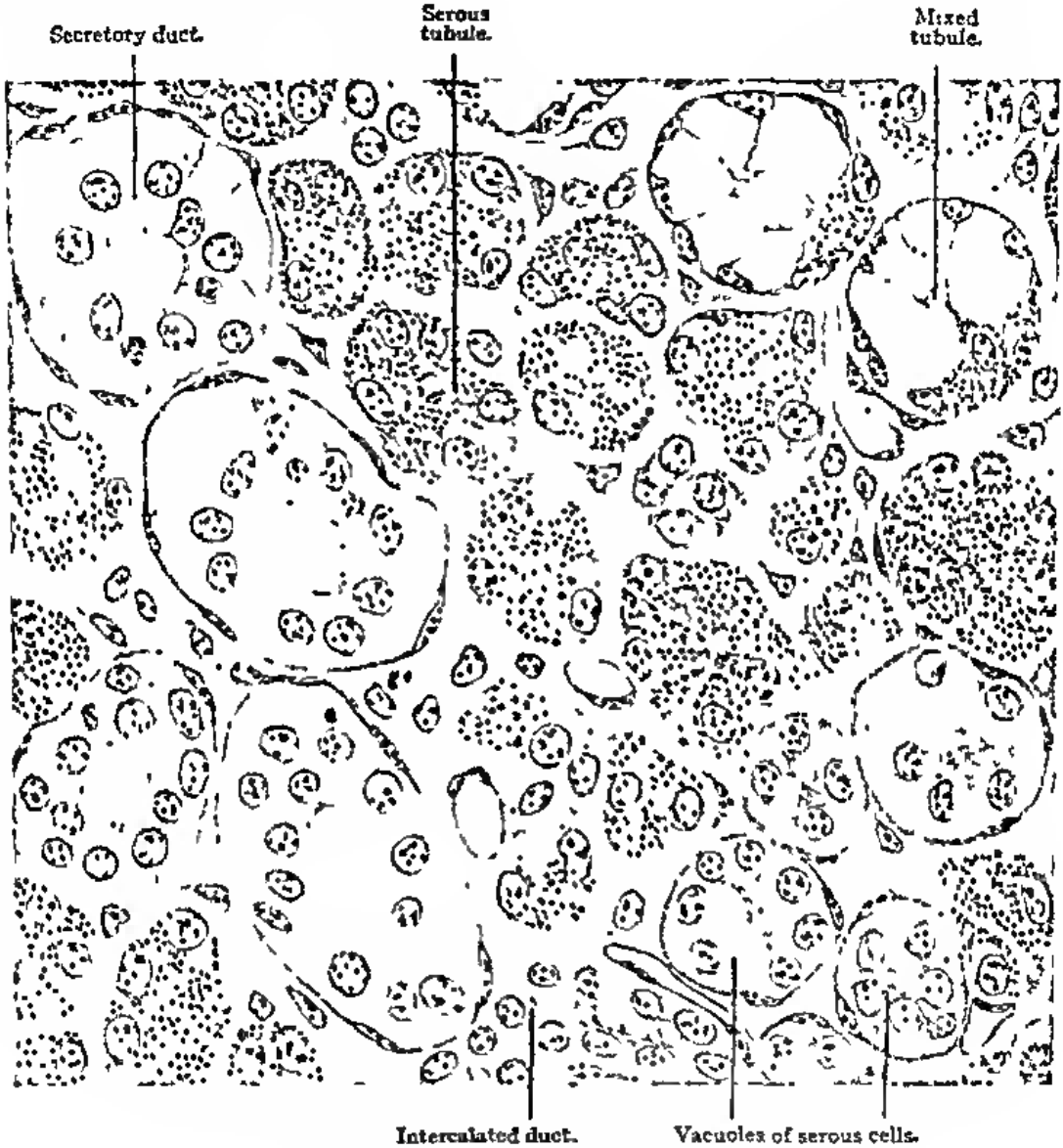


FIG. 289.—SECTION OF SUBMAXILLARY GLAND OF ADULT MAN, MUCOUS AND SEROUS CELLS.

In the oral glands, not infrequently degenerating lobules occur, characterized by abundant connective tissue between tubules with wide lumens and low gland cells. Sometimes they are surrounded by leucocytes.

The study of the demilunes in mixed glands has led to different theories in explanation of their function. They have been considered as cells ready to replace worn out mucous cells (Ersatztheorie), as cells in an early phase of secretion, which later will become mucous cells (Phasentheorie); and as cells fixed in the process of forming new growing buds for further expansion (Adenomerentheorie). For a discussion of these

theories see Zimmermann.[1] In all of them it is implied that the same cell may at one period produce a serous secretion and at another a mucous secretion. It has been shown that even two groups of mucous cells may produce mucus with differing staining qualities, and some cells apparently serous in form and granule content may respond slightly to mucous stains. Heidenhain,[2] on the other hand, accounts for the mucous cells by supposing that they are cells of the intercalated ducts which have taken on a mucous function (Verschleimungstheorie).

DIGESTIVE TUBE

Development. The digestive tube of mammals arises as two outgrowths from the yolk-sac—the fore-gut and hind-gut respectively. They are shown in Fig. 290, A, which represents a young rabbit embryo placed in a vertical position. Most of the spherical yolk-sac has been cut away. Anteriorly the fore-gut (*ph*) is seen extending from the yolk-sac to the oral membrane (page 301); posteriorly the sac has given rise to a short hind-gut from which a tubular ventral outgrowth, the *allantois,* has begun to develop. The allantois will be described with the membranes which surround the embryo. In an older stage (Fig. 290, B) the fore-gut and hind-gut have elongated, and the connection of the tube, which they form, with the yolk-sac is becoming reduced to a slender yolk-stalk. The entodermal tube within the stalk is called the *vitelline duct.* The caudal end of the hind-gut, behind the allantois, is called the *cloaca;* it approaches the surface to form the *cloacal membrane,* similar to the oral membrane. A short tail gut may extend beyond this. (The marked bend in the intestinal tube shown in Fig. 290, B, which is often seen in human embryos, is exaggerated, if not produced altogether, by a post-mortem sagging of the yolk-sac.)

In the later stage (Fig. 290, C) both the fore-gut and hind-gut have greatly elongated; together they form a loop of intestine extending out into the cavity of the umbilical cord. Near the bend in this loop the yolk-sac is still attached to the intestine by a stalk; the sac itself has been cut away in the figure. In addition to the pharynx already described, the fore-gut has given rise to an expanded portion or *stomach.* Between the stomach and pharynx it remains tubular and becomes the *œsophagus;* posterior to the stomach it is likewise tubular and there it forms a part of the small intestine. The first portion of the small intestine is called the *duodenum,* and is followed by the *jejunum* which passes without demarcation into the *ileum.* The ileum includes the portion to which the yolk-stalk is attached, and terminates at a bulbous enlargement (Fig. 290, C, *cae*) which gives rise to the *cæcum* and *vermiform process.* This *bulbus coli* (Johnson) marks the beginning of the large intestine or *colon,* and the cæcum and vermiform process are parts of the large intestine. Toward

[1] Zimmermann, 1927. [2] Heidenhain, 1921.

the cloaca the colon becomes the *rectum*, and near its termination it forms an elongated bulbous enlargement, the *bulbus analis*. This bulb forms essentially the *zona columnaris* in the anal part of the rectum. The *anus* is produced after the cloaca has separated into dorsal and ventral portions. The ventral division, which carries with it the allantois, becomes expanded to form the bladder, but its outlet remains relatively narrow and becomes the urethra. The outlet of the rectum is the anus, which is at

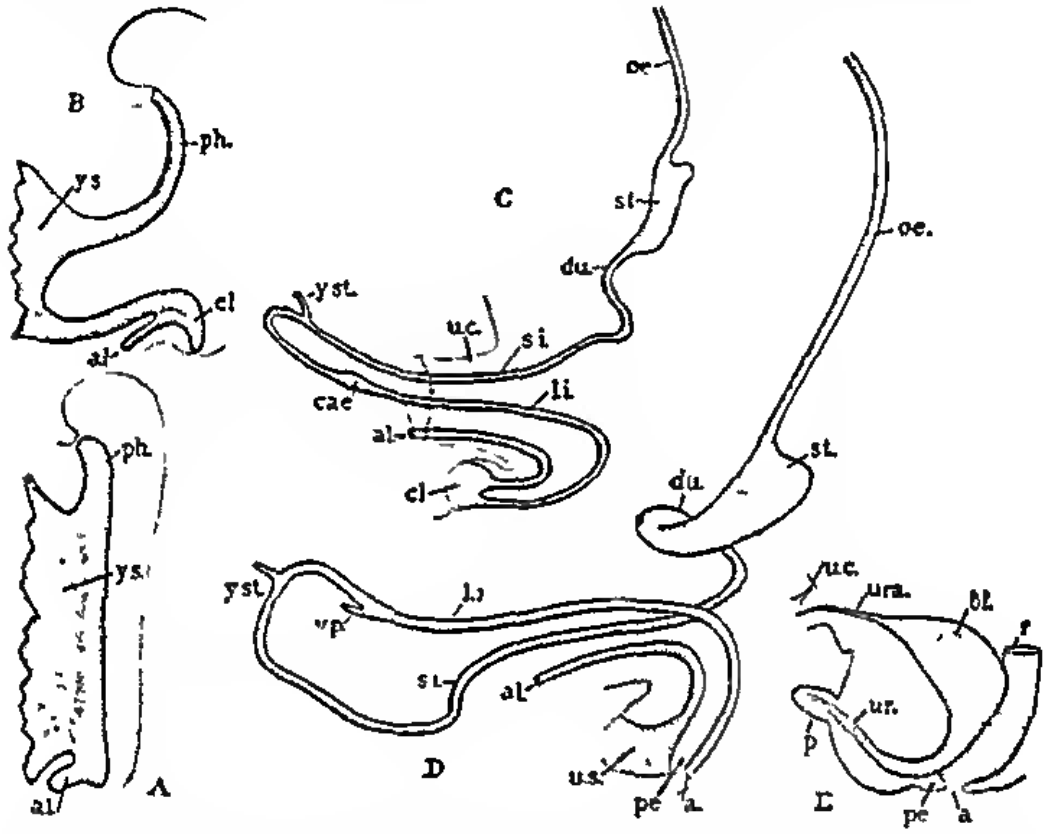


Fig. 290.—Stages in the Development of the Digestive Tube.

A, Rabbit of nine days. B, Man 2·15 mm. (after His). C, Pig, 12 mm. D, Man, 17·8 mm. (after Thyng). E, Man, about five months. a., Anus. al., allantois. bl., bladder; cæ., bulb of the colon, cl., cloaca, du., duodenum, l. i., large intestine, oe., œsophagus, p., penis, pe., perineum, ph., fore-gut, r., rectum, s. i., small intestine; st., stomach, u. c., umbilical cord, ur., urethra, ura., urachus, u. s., urogenital sinus; v. p., vermiform process, y. s., yolk-sac, y. st., vitelline duct within the yolk-stalk.

first closed by the *anal membrane;* this membrane ruptures in embryos measuring from 20 to 30 mm., except in the occasional cases of imperforate anus. The tissue which subdivides the cloaca reaches the surface and constitutes the perineum.

In human embryos of about 10 mm. the intestinal loop becomes twisted on itself (Fig. 290 D), and the large intestine is carried across the small intestine in the duodenal region. The vermiform process thus comes to lie on the right side of the body, and the colon, after it is withdrawn from the umbilical cord into the body, is so bent as to form *ascending*, *transverse*, and *descending* portions, below which, as the convoluted *sigmoid colon*, it connects with the rectum. The disposition of the

adult intestines depends chiefly upon this primary torsion of the intestinal loop, and upon the subsequent elongation of the small intestine, which forms many loops and coils.

Meanwhile the yolk-sac has become detached, and its stalk has disappeared, usually leaving no indication of its former position. The stalk does not become the vermiform process as was once supposed, but occasionally it produces a blind pouch of the ileum, 3–9 cm. long, situated about three feet above the beginning of the colon. This is the *diverticulum ilei*, described and correctly interpreted by Meckel in 1812.

The division of the intestine into six parts is a heritage from the Arabians. Duodenum, jejunum, ileum, cæcum, colon and rectum were well recognized in the fifteenth century, when, following Hippocrates, they were counted from below upward. The various names which have been applied to them are discussed by Hyrtl.[1] Those which are now adopted have the following significance. The rectum is the *straight* terminal portion. 'Colon is the κῶλον of Aristotle, which according to Pliny is a great source of pain (colic).' The cæcum, or blind intestine, was so named by Galen, who did not practice human dissection and so referred to the more elongated pouch in lower animals. The name has generally been considered inappropriate for the human cæcum. The Greek synonym τυφλὸν (blind) is used in the medical term *typhlitis* (inflammation of the cæcum). The ileum (from εἱλέω) is the coiled portion, and is arbitrarily defined as the lower three-fifths of the small intestine. The jejunum (Lat., fasting) is the portion generally found void and empty (Avicenna), since food passes through it rapidly. The duodenum, which has no free mesentery, was originally considered a part of the stomach; its name indicates that its length is twelve fingerbreadths. Hyrtl notes that the same term has sometimes been applied to the rectum.

Layers of the Digestive Tube. The wall of the digestive tube is composed of four layers—(1) tunica mucosa, (2) tela submucosa, (3) tunica muscularis, and (4) tunica adventitia or tunica serosa. The parts which are covered with peritoneum have a serous coat for their outer layer; the parts embedded in connective tissue have the adventitious coat instead.

The *tunica mucosa* consists of *epithelium*, *lamina propria*, and the *lamina muscularis mucosæ*. The epithelium is the entodermal lining of the tube, and is folded and in-pocketed so as to form innumerable pits and glands, varying in their nature in different parts of the tube. The lamina propria consists of reticular tissue, which in places becomes characteristic lymphoid tissue. It is set apart early in development as a layer with abundant nuclei, thus differing from the underlying mesenchyma. At a later stage the *lamina muscularis mucosæ*, or muscle layer of the mucous membrane, develops beneath it, separating it from the submucosa. The muscularis mucosæ is a thin layer of smooth muscle fibers.

The *tela submucosa* (*tela*, tissue) is a connective tissue layer which contains many blood and lymphatic vessels, and the ganglionated *plexus submucosus*.

[1] Hyrtl, 1879.

The *tunica muscularis* usually consists of an inner circular and an outer longitudinal layer of smooth muscle fibers, separated by a thin layer of connective tissue the *lamina intermuscularis* which contains the ganglionated *plexus myentericus*.

The *tunica serosa* is a connective tissue layer, covered by the peritoneal epithelium.

The wall of the digestive tube comprises portions of all the germ layers. The epithelium lining the cavity and glands is entodermal, the muscles and connective tissue are of mesenchymal origin and therefore mesodermal, as is also the peritoneal epithelium when that is present; the nerve cells and nerve fibers within the walls are of ectodermal origin.

The layers enumerated are to be examined in the œsophagus, stomach and intestine, which differ from one another histologically, since these layers are variously modified.

ŒSOPHAGUS

The œsophagus is a tube about nine inches long, the several layers of which are continuous anteriorly with those of the pharynx, and poste-

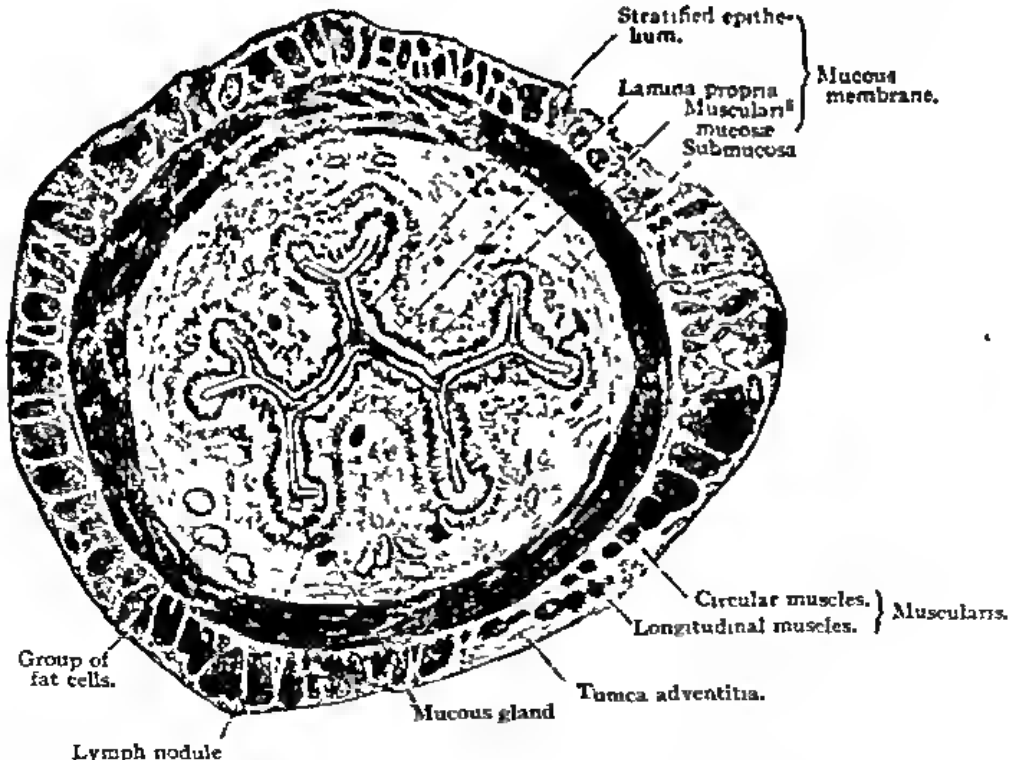


FIG. 291.—TRANSVERSE SECTION OF THE UPPER THIRD OF THE HUMAN ŒSOPHAGUS. × 5.

riorly with those of the stomach. The mucous membrane is thrown into folds, except when the tube is distended by the passage of food; but the muscularis merely thickens on contraction, so that it always forms a smooth round layer (Fig. 291).

The epithelium is thick and stratified like that of the pharynx (see Fig. 46, p. 72). Its outer cells are flattened in the adult, but in the embryo they include numerous islands of tall ciliated cells, some of which are found at birth. The basal surface of the epithelium rests upon connective tissue papillæ or ridges.

The glands of the œsophagus are of two sorts, superficial and deep. The deep glands (*glandulæ œsophageæ profundæ*) develop as scattered tubular downgrowths which pass through the lamina propria and muscularis mucosæ into the submucosa, where their blind ends expand and branch, producing a cluster of tubuloalveolar end pieces. The terminal portions at birth are still poorly developed. The human tubules are

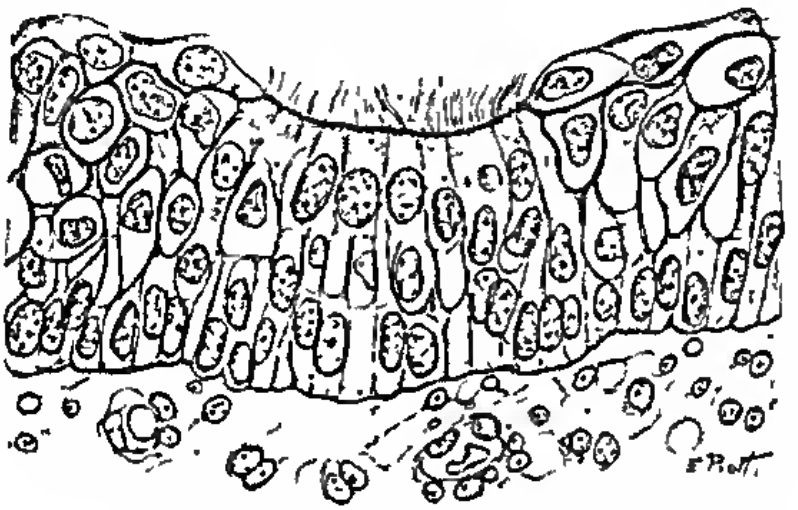


Fig. 292.—A Section of Epithelium from the Œsophagus of a Four Months Old Fetus Showing Ciliated and Non-ciliated Areas

Zenker fixation, iron-hæmatoxylin and orange O

composed wholly of mucous cells, although the basal cytoplasm sometimes simulates crescents. In some animals serous cells are frequent. The ducts are slender tubes generally lined with simple epithelium. They tend to slant toward the stomach, and they enter the epithelium where it dips down between the connective tissue papillæ. The cells of the ducts become continuous with the basal layer of the epithelium. Large ducts are sometimes lined with stratified epithelium, often ciliated, and they may present cyst-like dilatations. Lymphocytes tend to accumulate around the ducts and occasionally they form nodules in the lamina propria. The glands may show signs of infiltration and degeneration. The number of deep glands varies greatly in different individuals. They are usually more numerous in the upper half of the œsophagus.

The superficial glands (*glandulæ œsophageæ superficiales*) are limited to two rather narrow zones near the ends of the œsophagus. They are always found at the entrance of the stomach, extending from 1 to 4 mm. up the œsophagus; and generally (in 70% of the cases examined by Schaffer) they occur between the level of the cricoid cartilage and fifth

tracheal ring. They develop in the embryo much earlier than the deep glands, and appear as small areas of tall mucous cells which pass clear through the stratified epithelium. These islands of simple epithelium become depressed into shallow pockets from which a cluster of widely branching tubules grows out, but they never pass through the muscularis mucosæ into the submucosa. In the adult the upper group may be seen with the naked eye as an 'erosion' of the mucous membrane. The glands

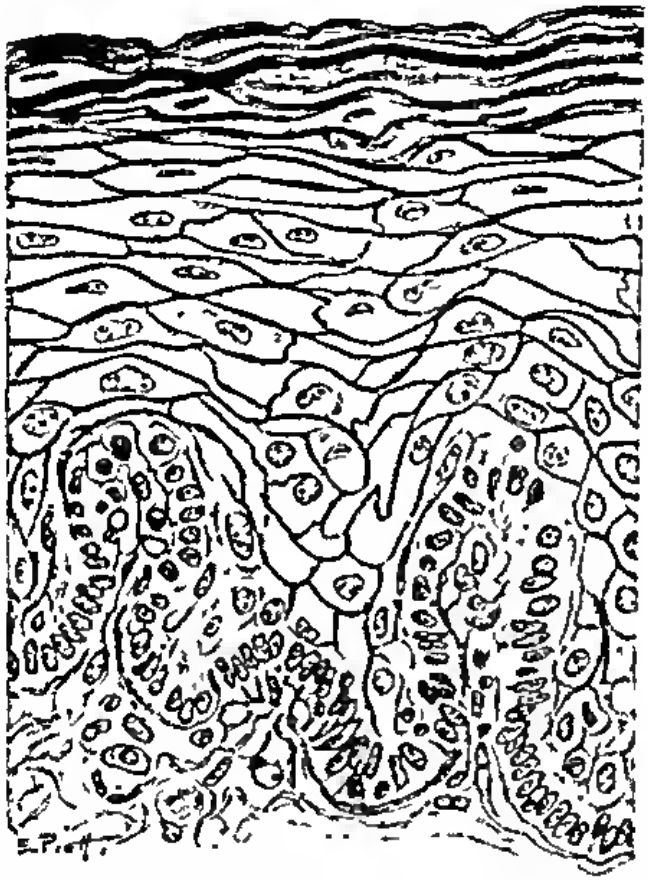


FIG. 293.—STRATIFIED SQUAMOUS EPITHELIUM FROM THE ŒSOPHAGUS OF A NINE YEAR OLD CHILD. Zenker fixation, hæmatoxylin and eosin

produce a form of mucus which stains less readily with the mucin stains than that of the deep glands. No special function has been assigned to this secretion. Glands of the lower group are shown in Fig. 295. They are freely branching mucous glands, the ducts of which open at the tops of connective tissue papillæ. They very frequently show cystic enlargements.

The lamina propria in the œsophagus has fewer cells in its meshes than that of the lower parts of the digestive tube. In places it includes solitary lymph nodules. The muscularis mucosæ is very wide in the œsophagus. It is a layer of longitudinal smooth muscle fibers, which is thrown into longitudinal folds when the œsophagus is contracted. It begins anteriorly at the level of the cricoid cartilage, arising as scattered bundles inside the elastic layer of the pharynx. As the muscles increase

to form a distinct layer, the elastic lamina terminates. The submucosa is a loose connective tissue layer, containing many vessels and nerves,

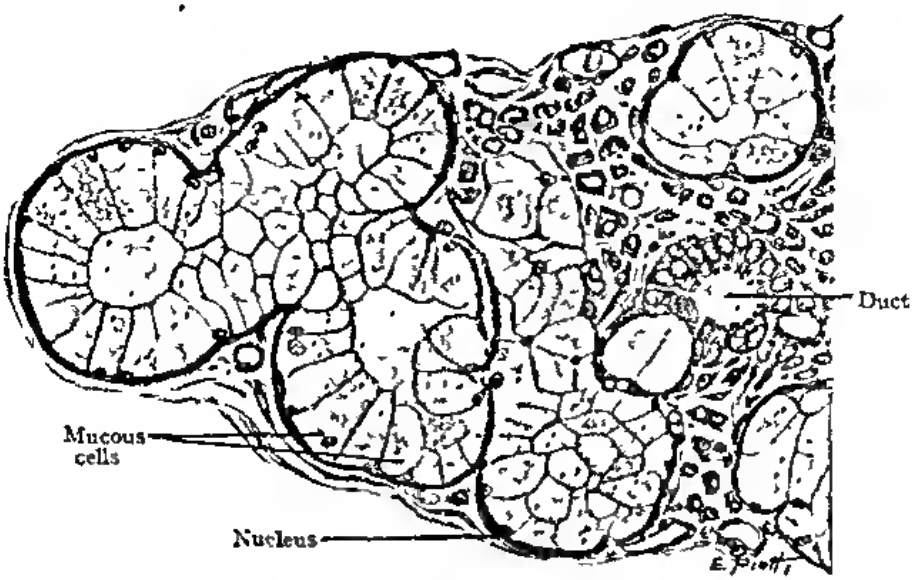


Fig. 294.—A Section of a Deep Gland from the Œsophagus of a Nine Year Old Child. Zenker fixation, hæmatoxylin and eosin.

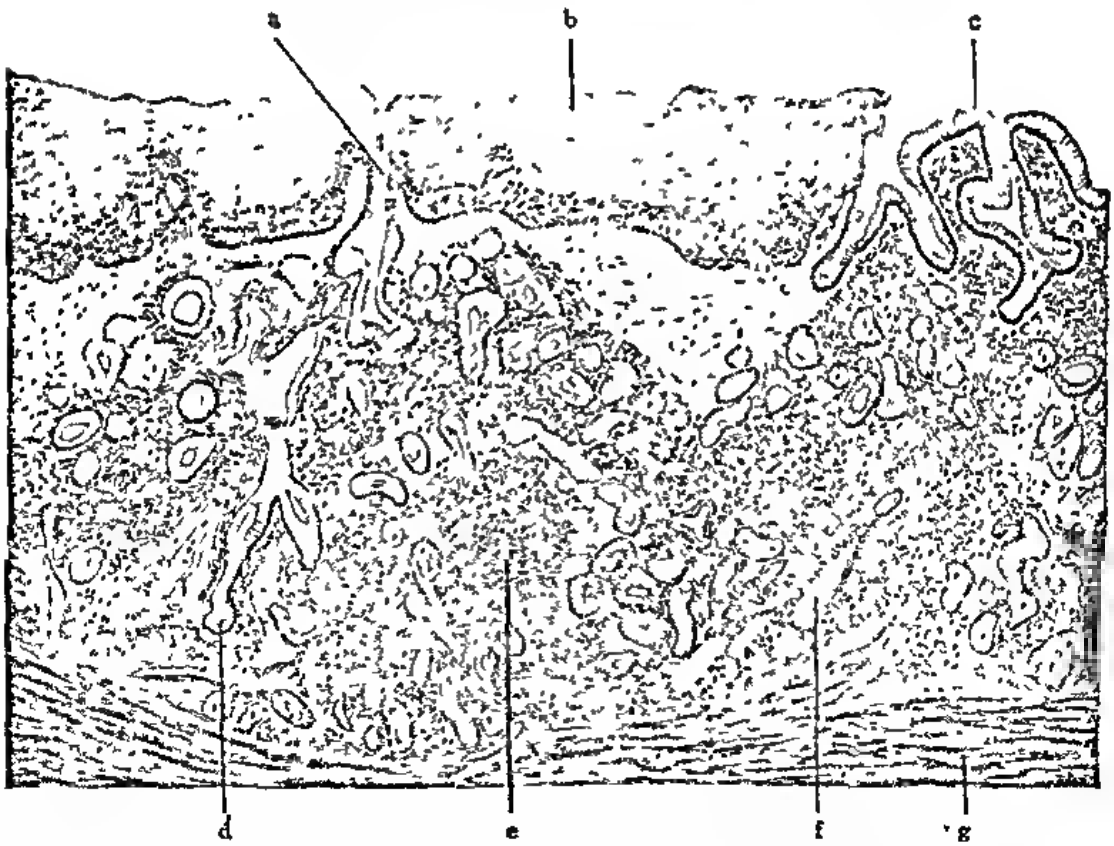


Fig. 295.—Longitudinal Section through the Junction of the Human Œsophagus and Stomach. a, Duct of a superficial œsophageal gland, b, œsophageal epithelium; c, gastric epithelium; d, tubule of the gland a; e, lymphoid nodule; f, lymphatic vessel, g, lamina muscularis mucosæ

groups of fat cells, and the bodies of the deep mucous glands. The muscularis consists of an inner circular and an outer longitudinal layer, as elsewhere in the digestive tube, but in the upper part of the œsophagus

the layers are composed of striated muscle fibers. These fibers are not a downward extension of the striated pharyngeal constrictors, but apparently develop from exactly such mesenchymal cells as produce smooth muscle further down. The striated muscles in man are limited to the upper half of the œsophagus; in the rabbit they extend its whole length. Striated and smooth muscles intermingle in the middle region in man.

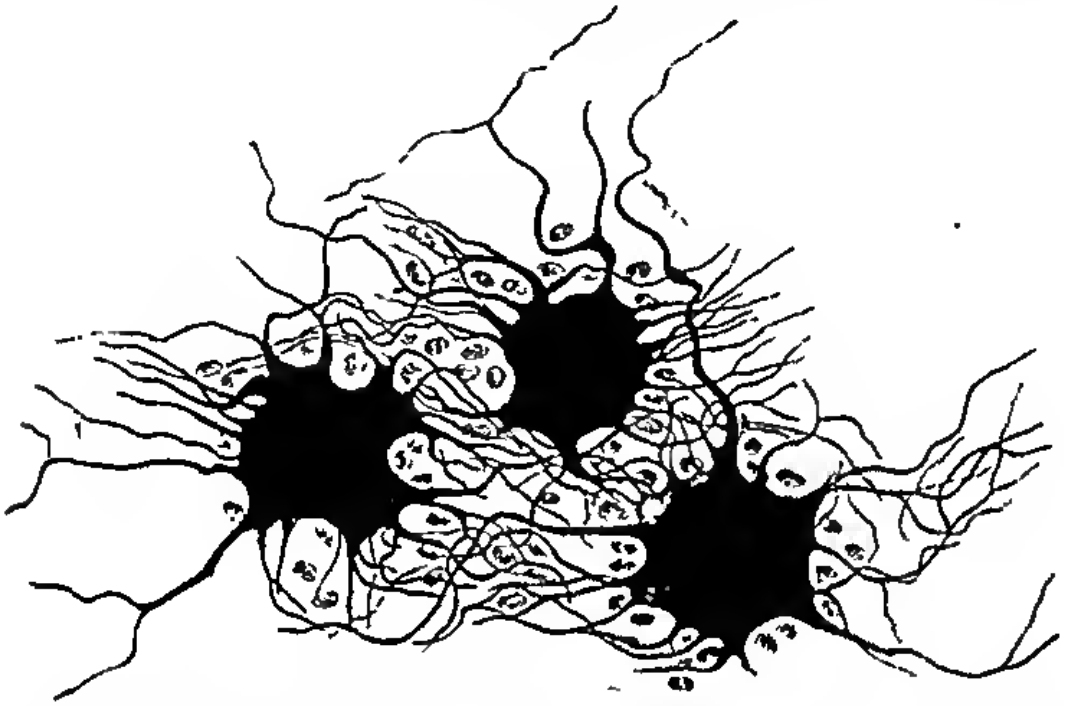


FIG. 296.—GANGLION CELLS FROM THE ADVENTITIA OF THE HUMAN ŒSOPHAGUS. BIELSCHOWSKY METHOD. × 410. (Greving.)

The adventitia is loose connective tissue, containing many vessels and the plexiform branches of the vagus nerves. From these nerves, medullated and non-medullated fibers enter the œsophagus and form a ganglionated myenteric plexus between the muscle layers, and the plexus submucosus in the submucosa. Medullated fibers proceed from the vagus trunks to the motor end plates of the striated muscles, which are thus stimulated reflexly from the central nervous system. Other fibers pass from the myenteric plexus to the plexus submucosus and thence to the epithelium, in which free nerve endings have been found. Such fibers, together with those to the smooth muscles, provide for local reflex action, whereby the contents of the œsophagus causes contraction above, and relaxation below, the place of stimulation. This takes place independently of the central system, and is the form of innervation characteristic of the intestine.

STOMACH

Form and Subdivisions The opening through which the œsophagus connects with the stomach is the *cardia* (Gr. **καρδία**, heart) and the opening from the stomach to the

intestine is the *pylorus* (Gr. πυλωρός, gate-keeper). The pylorus received its appropriate name from Galen (in the second century), who recognized that through its sphincter muscle it controlled the exit of food. The significance of 'cardia' was discussed by Fabricius (1618) who cites Galen as stating that the upper orifice of the stomach is called the heart because the symptoms to which it gives rise are similar to those which sometimes affect the heart, sometimes even the brain; but for Fabricius, 'cardia,' as applied to this orifice, merely indicates a chief part of the body. The stomach as a whole is termed *gaster*, from the Greek, but the Latin *ventriculus* was generally used by the early anatomists. Although flaccid and shapeless when seen in the dissecting room, the stomach has a very characteristic form. Its epithelium, from an embryo of 44.3 mm., is shown in Fig. 297, and an adult stomach is seen in Fig. 299. It is a tube which is greatly distended toward the left, where its border forms the greater curvature; its right border is the lesser curvature.

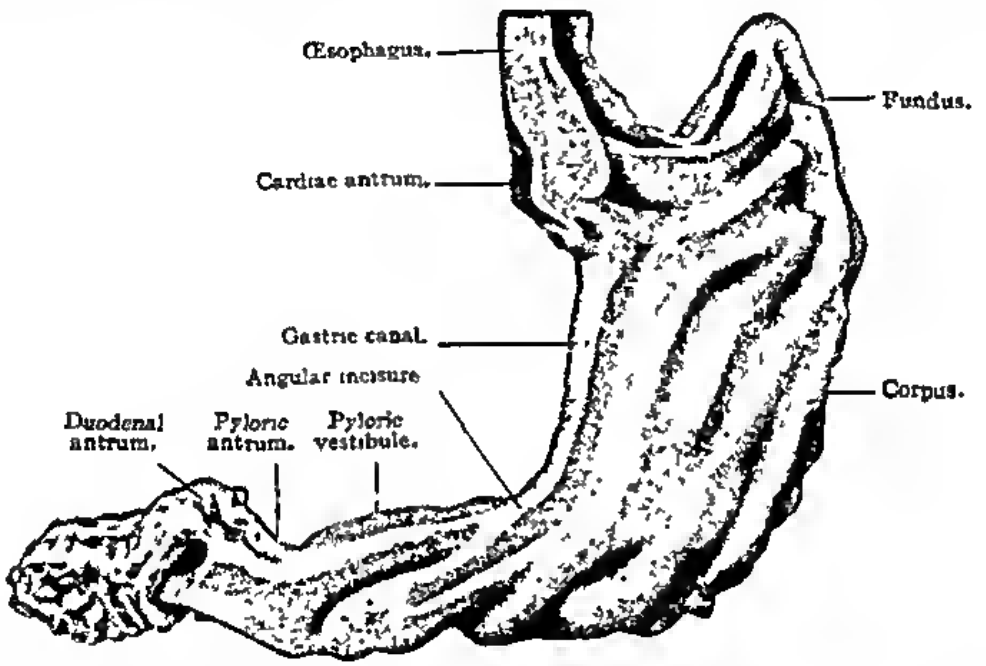


FIG 297.—MODEL OF THE GASTRIC EPITHELIUM IN A HUMAN EMBRYO OF 44.3 MM. × 18 diam.

These two borders are morphologically dorsal and ventral respectively, misplaced by the spiral rotation caused by the twist of the intestinal loop (Fig. 290, D). As a whole the stomach is divided into two parts, the cardiac portion (*pars cardiaca*) and pyloric portion (*pars pylorica*). This fundamental subdivision occurs in many animals, as was recognized by Sir Everard Home in 1814. The pyloric part is relatively long in the embryo. It becomes subdivided into the *pyloric vestibule* and the *pyloric antrum*. The latter is its smaller part extending to the pylorus; between the two, on the greater curvature, is the *sulcus intermedius*. (The term *pyloric antrum* has been variously employed, since in its original description by Willis (1674) the vestibule is not recognized; Cowper (1698) applies *antrum* to the terminal subdivision as above defined.) The cardiac part of the stomach is divided into a main portion, or body of the stomach (*corpus gastri*), and a blind pouch, formerly called the saccus cæcus, but now less appropriately known as the *fundus gastri* (the bottom of the stomach). Recently the gastric canal (*canalis gastri*) has been recognized along the lesser curvature of the human stomach. It is a channel, highly developed in ruminants, which conveys liquids from the cardia to the pars pylorica, when the stomach is filled with more solid contents. Ordinarily open toward the interior like a groove, it may become closed as a tube during its physiological activity. Beyond

the cardia there is a conical expansion of the œsophagus, not always well defined, known as the *cardiac antrum*, and beyond the pylorus is the first part of the duodenum or *duodenal antrum*. (A further account of the development of these subdivisions is given by F. T. Lewis.[1])

The inner surface of the stomach presents macroscopic longitudinal folds, which become coarse and prominent as the organ contracts. They are sinuous, and anastomose in an irregular network. As finer markings,

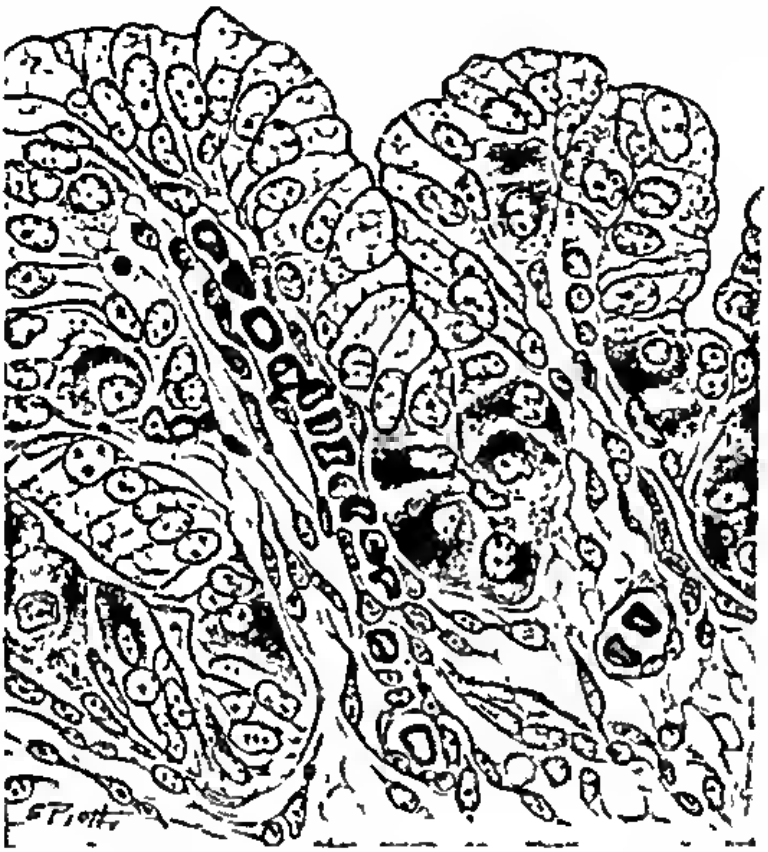


Fig. 298.—A Vertical Section of the Mucous Membrane of the Stomach of a 4 Months Old Human Fetus. Note the differentiation of cellular types. Zenker fixation; hæmatoxylin and eosin.

there are rounded or polygonal areas, 2–4 mm. in diameter, which may appear as elevations or depressions. They have been ascribed to the contraction of muscle fibers in the mucous membrane, to varying amounts of lymphoid tissue, and to the varying height of the glands. Toward the pylorus there are small leaf-like elevations, the *plicæ villosæ*, which may connect with one another in a network. The epithelium of the stomach is thin enough to transmit the color of the underlying tissue, and appears pinkish-gray; whereas the color of the œsophagus, with a thicker epithelium, is white.

The gastric epithelium, like that of the entire intestine, is a single layer of columnar cells. In the stomach the cells are tall and contain mucus, but they do not ordinarily acquire the bulging goblet shape,

[1] Lewis, F. T., 1912.

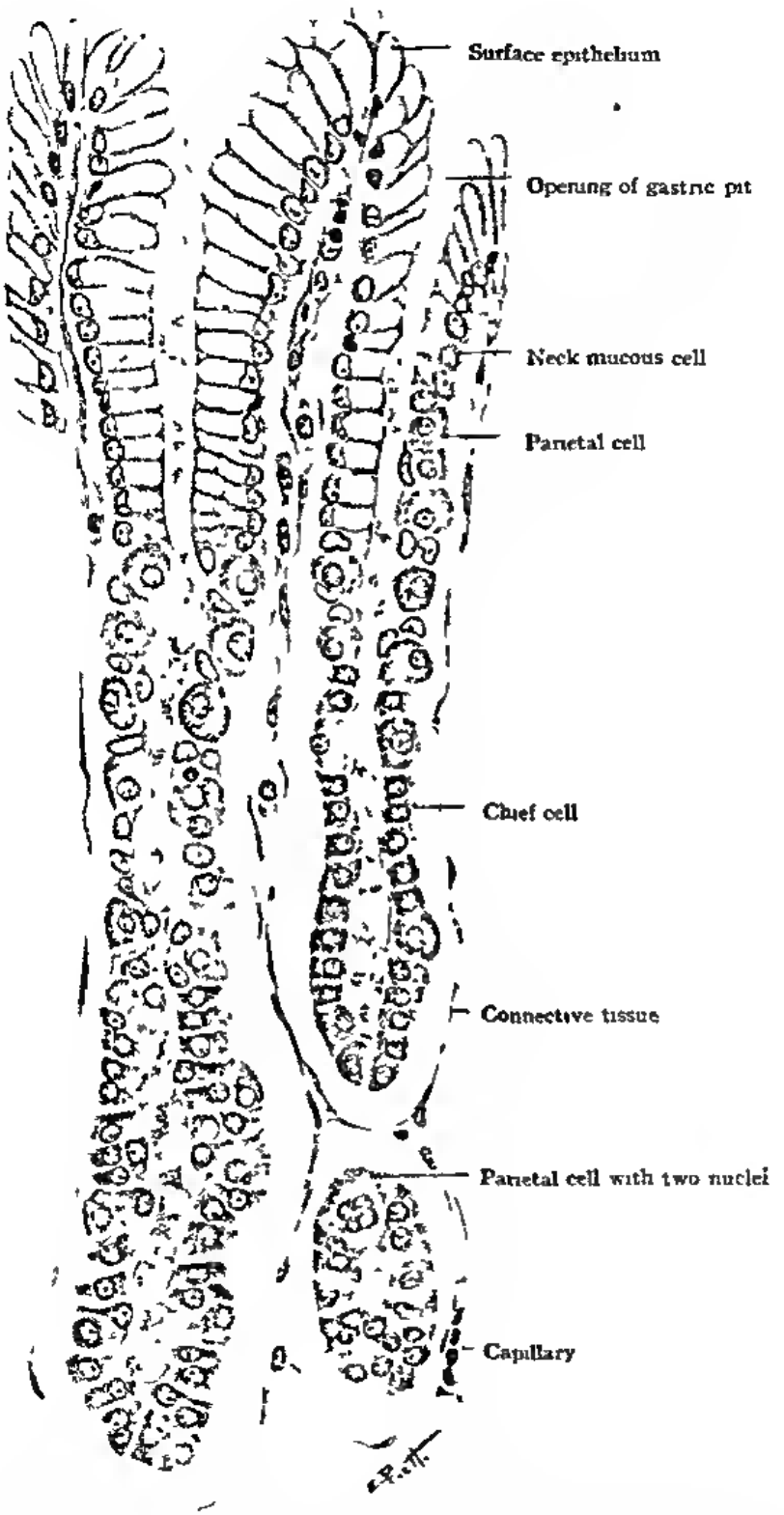


FIG 299.—A VERTICAL SECTION THROUGH THE MUCOSA OF ADULT HUMAN STOMACH

Tissue removed at operation. The chief cells are stained blue, the parietal a deep pink and the neck mucous cells and surface cells a pale pink. Zenker fixation, methylene blue and eosin.

since the adjacent cells likewise contain mucus. This simple layer of mucous cells is continuous at the cardia with the basal layer of the stratified epithelium of the œsophagus, and the transition is abrupt. The outer strata of the œsophageal epithelium may form an overhanging wall, or the number of layers may have become reduced so that such a wall is absent. Sometimes an island of stratified epithelium occurs just beyond the line of transition. The epithelium over the entire cardiac surface dips down into the lamina propria to form shallow pits or crypts (foveolæ gastricæ) into the bottom of which open the glands, which are of three types known as cardiac, gastric and pyloric glands respectively. None of these extends into the submucosa.

The pits are often described as if they were epithelial depressions separate from the glands, since the same sort of epithelium which lines them is found on the free surface. Developmentally, however, they are to be regarded as parts of the glands, comparable with ducts. The epithelial cells of the pits consist of a basal protoplasmic portion containing elongated, round, or sometimes flattened nuclei, and an outer portion containing the centrosome and secretion. The mass of mucus may cause the thin top plate to bulge, and in preserved tissue to rupture, but this may be due to reagents. The mucus first appears in granular form. Terminal bars are present.

Fig. 300.—A Cross Section of a Human Gastric Gland Parietal Cell a Deep Pink
Zenker fixation, methylene blue and eosin

The *cardiac glands* are like the superficial glands at the lower end of the œsophagus, of which they may be regarded as a continuation. The gastric pit serves as a short duct into which open several tortuous branching tubules, lined with mucous cells. They extend only from 5 to 40 mm. into the stomach, and in this narrow zone they present a transition to the gastric glands, shown by the diminished branching and gradual straightening of the tubules, until they become groups of parallel tubes descending from the epithelial pits; and deeply staining eosinophilic cells and granular cells characteristic of the gastric glands become included in their epithelium.

The cells characteristic of the cardiac glands contain a mucus which does not respond readily to mucin stains. Like the superficial glands of the œsophagus, the cardiac glands develop early, and they are found widely distributed among mammals.

The *gastric glands* (sometimes inappropriately called *fundus glands*) occur over the entire surface of the stomach, except near the cardia and pylorus. A small group of them empties into the bottom of each gastric pit. The individual glands are straight or somewhat tortuous, slender structures, with narrow lumens. The portion which joins the pit con-

stitutes the neck of the gland, and the slightly expanded basal end is the fundus; the body is the portion between these two. The cells forming the glands are of four types. Two of these are called chief cells, because they make the main part of the wall of the tubule. In the neck of the gland the chief cells are mucous, in the body and fundus they are serous in type.[1] Less numerous are the parietal cells, and the fourth type, the argentaffin cells, are only occasionally present in the stomach; the latter will be described with the glands of the intestine (p. 348).

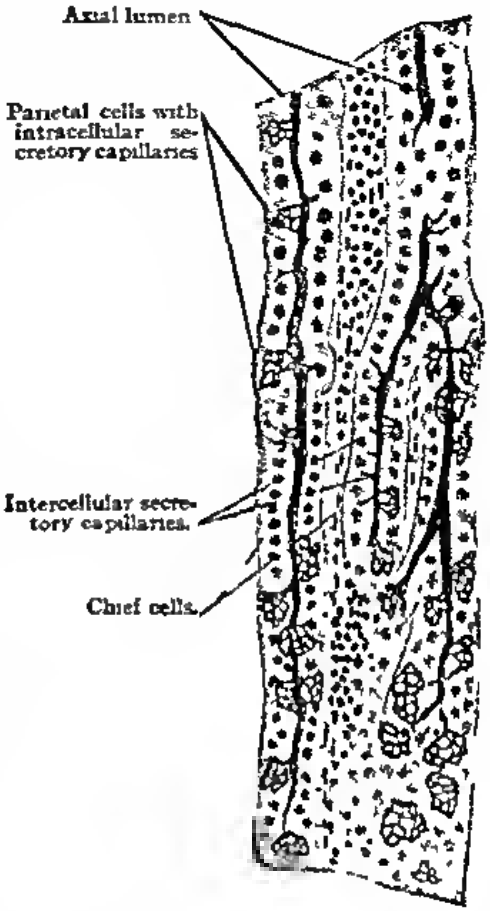


FIG. 301.—GOLGI PREPARATION, SHOWING THE SECRETORY CAPILLARIES IN GASTRIC GLANDS. × 230

The mucous chief cells, or neck chief cells, form a simple columnar epithelium. They are similar to the cells of the crypts, but not so tall; their nuclei may be round or flattened or even concave at the base of the cell, like those in the mucous cells of the salivary glands. The mucin is said to stain differently from that in the cells of the crypts. In sections treated with the usual fixatives and stains the inner part of the cell shows a light network of the protoplasmic strands between the colorless droplets of mucin. In this condition it is hard to differentiate them from the *serous chief cells*, or zymogenic cells, found in the body and fundus of the gland. Unless properly preserved the zymogenic granules are dissolved or colorless, with the result that the inner parts of these cells also present a pale reticular appearance. The nuclei of the chief cells of the body of the glands, however, are round or oval vertically, and in sections in which the zymogen granules have been preserved and stained an abrupt change of cell type can be distinguished, one cell of the layer showing mucous, the next cell serous characteristics. The serous cells form the chief cells of the gland from the neck down to the fundus. They apparently give rise to the pepsin of the gastric juice.

The parietal cells, may be readily distinguished from the chief cells in fresh tissue; the latter are dark and contain refractive granules, whereas the parietal cells are clear. In ordinary preparations they are better preserved than the chief cells, and exhibit a finely granular struc-

[1] BENSLEY, 1932.

ture, being deeply stained with the aniline protoplasmic dyes. They differ so markedly from the chief cells that they have been erroneously believed to develop from the surrounding lamina propria. They occur chiefly along the body of the tubule, being infrequent at its fundus. They are large cells, containing one or occasionally two round nuclei, and are crowded away from the lumen like the cells in the serous crescents. They discharge their secretion through secretory capillaries which produce basket-like networks within the cytoplasm; thus they differ from the chief cells which have only intercellular secretory capillaries. The secretory capillaries of the parietal cells may be demonstrated by the Golgi method, which produces a precipitate wherever secretion is encountered (Fig. 301), and by the use of neutral red in fresh material. After fasting, the parietal cells are small and their intracellular capillaries have disappeared. Following abundant meals, these cells enlarge and may contain vacuoles due to the rapid formation of secretion. They produce the hydrochloric acid which is found in the gastric juice.

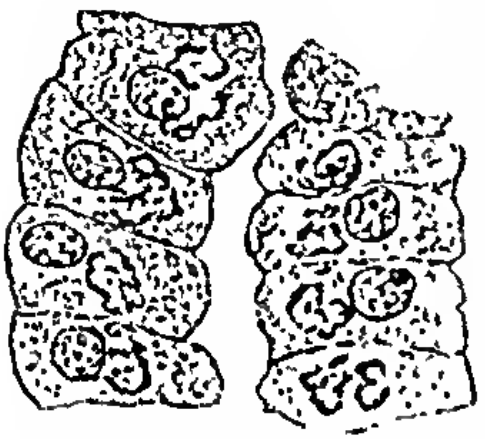


FIG. 302.—THE CHIEF CELLS FROM THE MIDDLE OF A GASTRIC GLAND FROM A 44 YR OLD MAN.

The Golgi apparatus, as a blackened reticulum, lies between the nucleus and the lumen. Chrome-osmium preparation. × 1500. (Kopsch.)

The *pyloric glands* are found near the pylorus, but the area which they occupy is not sharply set off; they pass over into gastric glands through a 'transition zone.' Pyloric glands have very deep pits from which short, winding, branched tubules grow out. Their form in the adult is shown in Fig. 303. The cells in the pits are mucous cells, and those in the tubules are also regarded as mucous cells. The latter are columnar, with rounded nuclei in their basal part, and protoplasm which may closely resemble that of the chief cells. Parietal cells are occasionally found, and such cells have been reported in the duodenal glands and in the superficial glands of the œsophagus. Slender dark cells, apparently due to compression, are found in the pyloric glands of the dog. In certain respects the pyloric glands are transitional between gastric and duodenal glands.

During development, as the lining of the stomach expands greatly, the number both of crypts and of tubules (commonly called glands) becomes greater. The total number of crypts in the entire stomach at birth has been estimated[1] as over 200,000, and of glands as nearly 500,000, while in the adult the numbers have increased to over 3,000,000

[1] SCOTT, 1925.

and 13,000,000 respectively. Others[1] give even higher figures. The ratio of the number of tubules to the number of crypts increases up to middle childhood, when there may be as many as six or seven emptying into each crypt, then decreases until the average number for the adult is about four. The increase in number is accomplished not by the growth of new glands but by the longitudinal splitting from below upward of

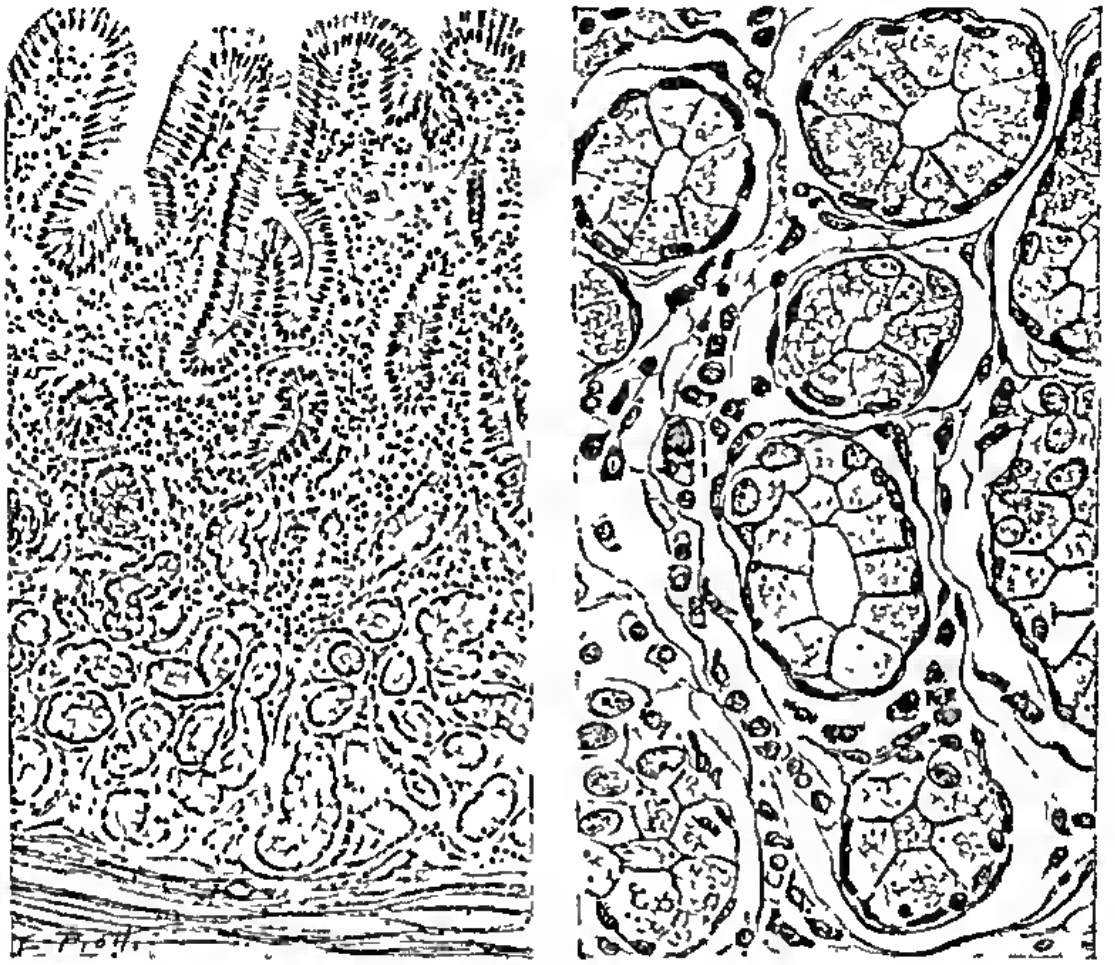


FIG 303.—A VERTICAL SECTION OF HUMAN PYLORIC GLANDS.
On the left under low power magnification, on the right under high power. Formaldehyde fixation; hæmatoxylin and eosin

those already present, and this method is later carried also into the crypts to increase their number and reduce the ratio.

In the glands of the stomach mitotic activity is limited to the region of the bottom of the foveolæ or the neck of the glands. After experimental denudation[2] of a small area of mucosa a sheet of new epithelial cells derived from the neighboring foveolæ grows in from the edges, covers the surface, and then forms new foveolæ and new glands. At first all the cells are of the foveolar type, but later they differentiate into mucous chief cells, serous chief cells, and parietal cells, according to

[1] TOLDT, 1880. [2] FERGUSON, 1928.

location. Replacement of any worn out cells in the normal glands or on the mucosal surface is probably effected from the same region. The cause of the diversity of types of differentiation is unknown. It is interesting to note as a contrast that mitosis occurs near the bottom or fundus of the intestinal glands.

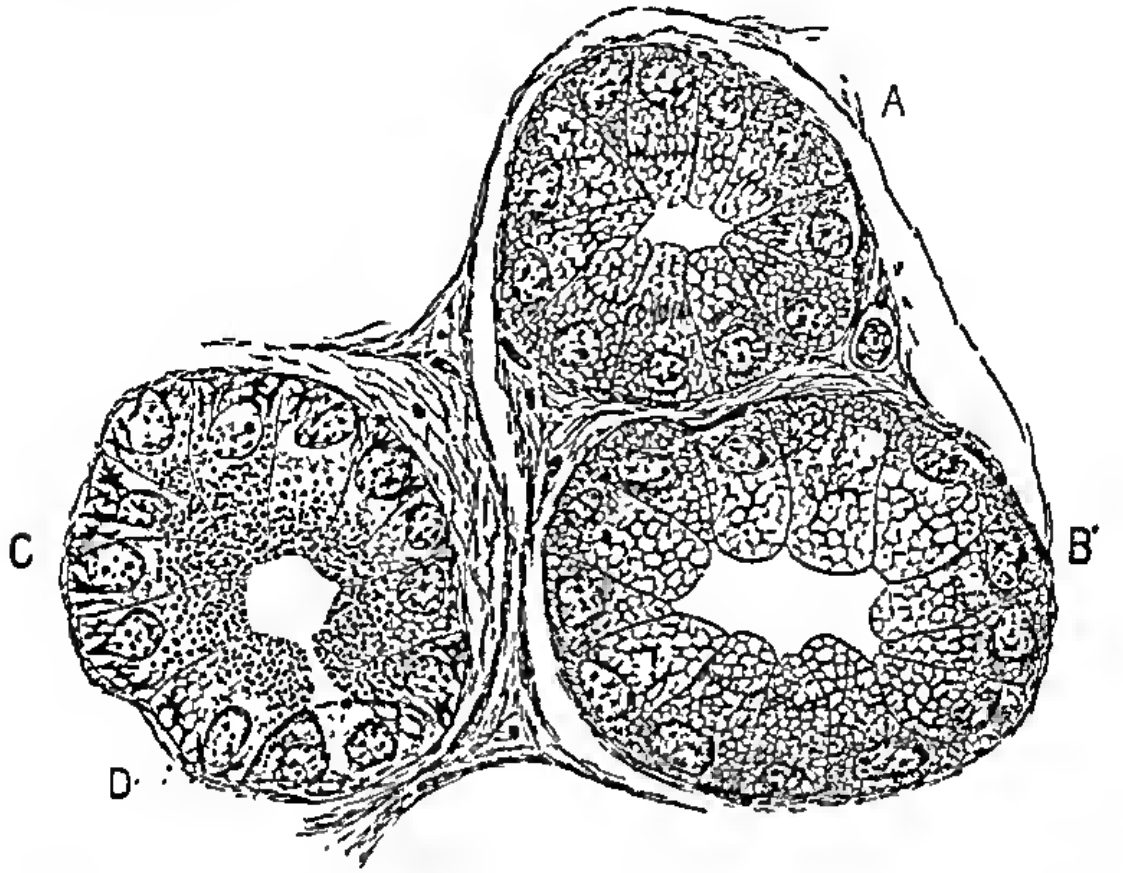


FIG. 304.—THREE TUBULES FROM CARDIAC GLANDS OF MAN

A, mucous tubule with cells in early stage of mucin formation, B, cells show various stages of mucin formation; C, tubule formed of cells like the chief body cells of gastric glands, zymogen granules, D, binucleate parietal cell with intracellular canaliculi. × 1000. (From Benseley, 1902.)

In material which has not been fixed very shortly after death, the epithelium of the stomach and remaining parts of the digestive tube is apt to be badly preserved. The cells are often separated from one another and indistinct in detail. This may be due to 'auto-digestion,' the action of the digestive fluid already present in the lumen on the recently dead cells with which it is in contact. The cells beneath the surface are not affected.

The *lamina propria* consists of the small amount of reticular and connective tissue which is found between the closely packed glands and immediately beneath them. It is sufficient to support the numerous capillaries branching about the glands, the terminal lymphatic vessels and nerves, numerous wandering cells and a few smooth muscle fibers prolonged toward the surface from the muscularis mucosæ. The lymphatic vessels begin blindly near the superficial epithelium and pass between the glands into the submucosa where they spread out and are

easily seen; they continue through the muscularis and pass through the mesentery to join the large lymphatic trunks. Solitary nodules occur in the gastric mucosa, especially in the cardiac and pyloric regions. They originate in the reticular tissue of the lamina propria, and may extend through the muscularis mucosæ into the submucosa, where they may expand to a considerable size. In peripheral sections of such a nodule the expanded submucosal portion may alone appear, and its connection with the lamina propria may not be evident. The muscularis mucosæ may be divided into two or three layers of fibers having different directions. The submucosa contains its plexus of nerves and many vessels, together with groups of fat cells. Its elastic fibers are said to be abundant toward the pylorus.

The muscular coat of the stomach consists of three layers of smooth muscle, an outer longitudinal, middle circular, and inner oblique layer respectively. These layers can be recognized by dissection more readily than by microscopic examination, and were found by Willis[1] in 1674. The middle layer is the one most highly developed. It not only surrounds the body of the stomach, but as the fundus pushes outward, muscle fibers of this layer encircle its apex concentrically. Toward the pylorus, along the antrum, the circular layer gradually thickens, thus forming the *sphincter pylori;* it becomes abruptly thin in the duodenum. There is no sphincter at the cardia, where the circular layer is continuous with that of the œsophagus, but elastic tissue in the muscularis is said to be specially abundant and to 'contribute to the tonus of the cardiac musculature.' The outer longitudinal layer, continuous with the outer layer in the œsophagus and duodenum, is an incomplete layer, being deficient toward the greater curvature. As the body of the stomach bulges outward to form this curvature, the longitudinal fibers apparently become separated into scattered bundles. In the pars pylorica, however, there is a continuous longitudinal layer, and some of its fibers, which become intermingled with those of the sphincter pylori, serve to dilate the pylorus. The innermost layer, composed of oblique fibers, is not represented in the œsophagus and duodenum, and is said to be absent from the pars pylorica. They form a longitudinal strand parallel with the lesser curvature, and they pass from one side of the stomach to the other across the notch between the œsophagus and fundus. These fibers are important in the activity of the gastric canal, but they do not produce the canal as some have supposed. From these longitudinal bundles, fibers curve obliquely toward the greater curvature, where, as transverse fibers, they cross to the opposite side. Thus the musculature of the stomach is so arranged that it is very difficult to determine the plane

[1] Willis, 1674.

of section in a small piece of the gastric wall, which may often be cut obliquely.

The *tunica serosa* consists of connective tissue with well-developed elastic nets, and a covering of peritoneal mesothelium interrupted only along the curvatures, at the mesenteric attachments. It contains the nerves and vessels which supply the stomach. The right and left vagus

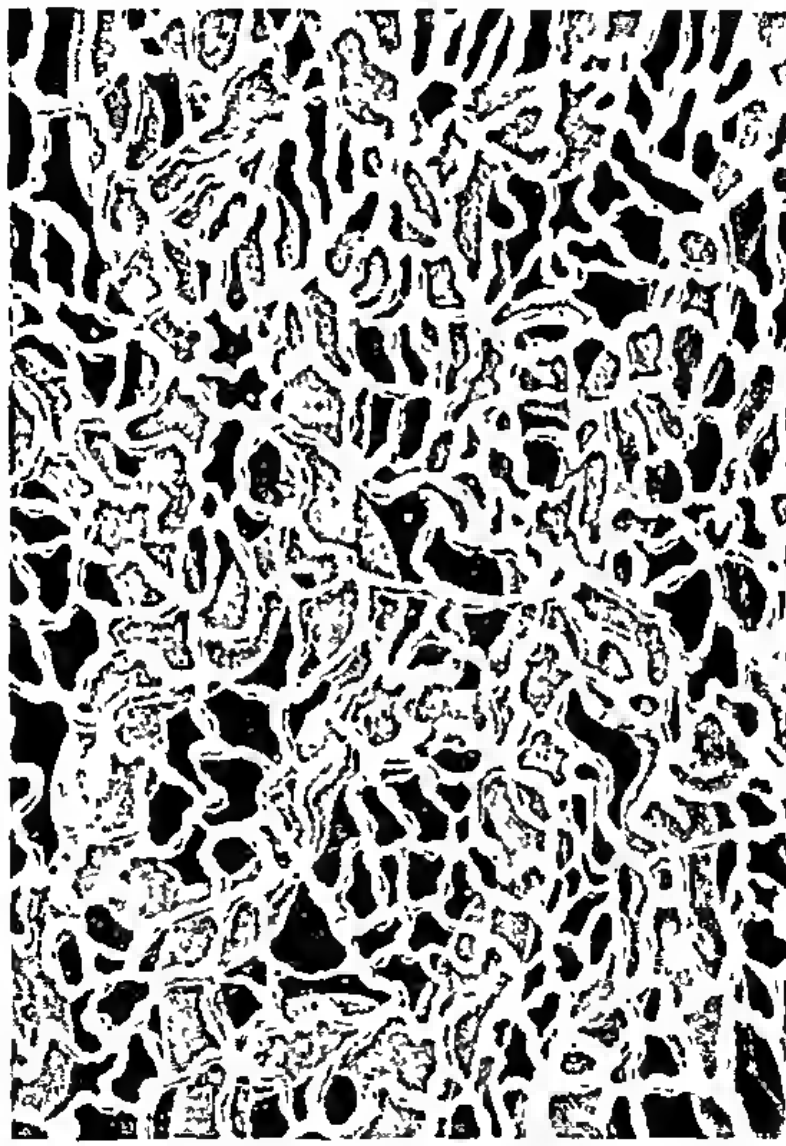


FIG. 305.—SUPERFICIAL AND DEEP LYMPHATIC NETWORKS IN THE WALL OF THE STOMACH OF A DOG. X 30. (Teichmann.)

trunks descend beside the œsophagus as the main stems in a plexiform network, and then come together along the lesser curvature. From there they send plexiform branches over both sides of the stomach, and the main stems continue into the small intestine. Sympathetic nerves from the cœliac plexus pass to the pyloric end of the stomach and join the vagus plexus. The further distribution of the nerves in myenteric and submucous plexuses is similar to that in the small intestine.

SMALL INTESTINE

Duodenum, Jejunum and Ileum

The lining of the small intestine, including the duodenum, jejunum and ileum, has a velvety appearance, due to the presence of innumerable cylindrical, club-shaped or foliate elevations, known as *villi* (hairs or nap). True villi are found in the large intestine of the embryo but they disappear before birth; they are said to occur also in the pyloric end of the stomach, but it is questionable whether these are typical villi or

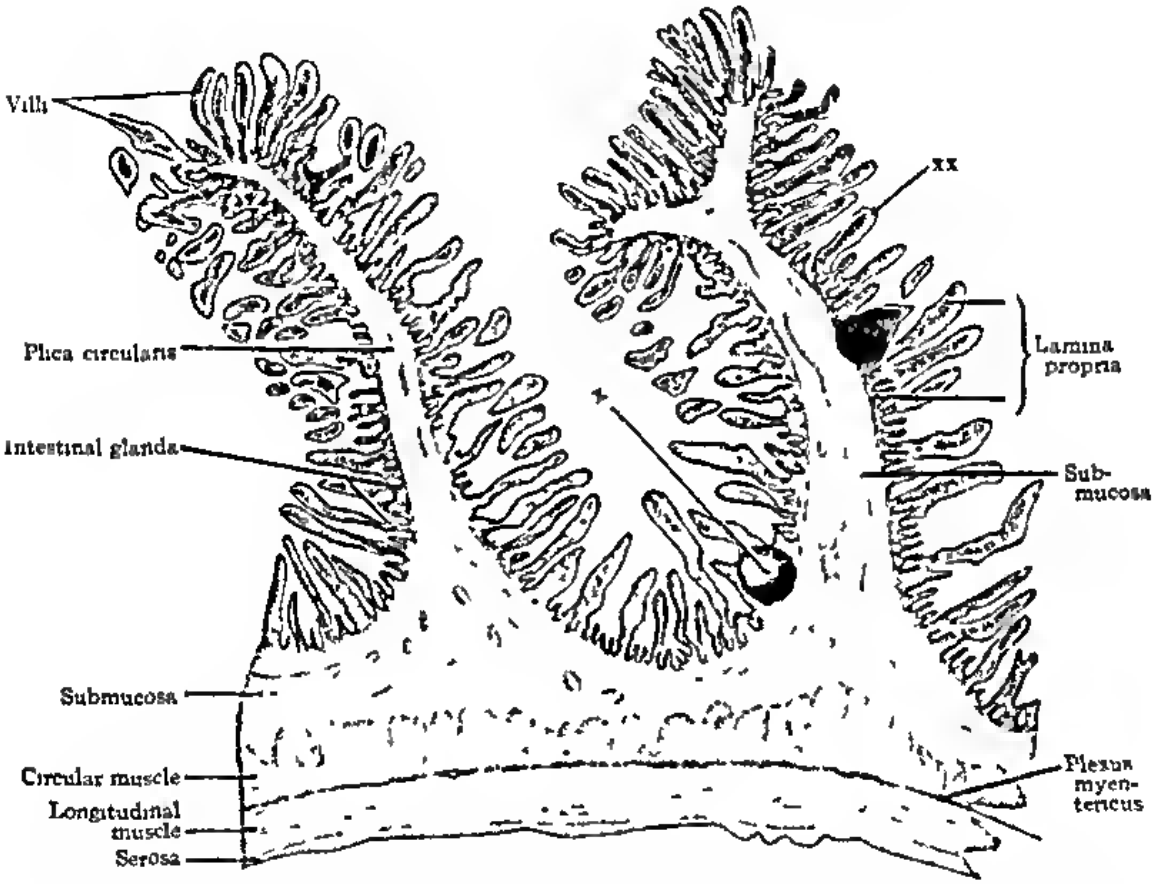


FIG. 306.—A VERTICAL LONGITUDINAL SECTION OF THE JEJUNUM OF AN ADULT MAN × 16.

The plica circularis on the right supports two small solitary nodules, which do not extend into the submucosa, one of them exhibits a germinal center, x. The epithelium is slightly loosened from the connective tissue core of many of the villi, so that a clear space, xx, exists between the two. The isolated bodies lying near the villi (more numerous to the left of the plicæ circulares) are sections of villi that were bent, so that their ends were cut off in sectioning

merely irregular folds. Elsewhere in the digestive tube, villi are absent. At the bases of the villi there are simple tubular pits of glandular epithelium, which extend to the muscularis mucosæ but do not penetrate it; these are the *intestinal glands* (*glandulæ intestinales*, formerly known as crypts of Lieberkühn). A second type of gland found only in the duodenum will be described later. The villi in the jejunum and ileum are rounded, finger-like projections, from 0.2–1.0 mm. in height. Within the duodenum the villi are low, leaf-like folds, 0.2–0.5 mm. high. Their shape cannot be determined from inspecting single sections.

Villi are essentially circumscribed folds, and they have been said to arise through the subdivision of longitudinal ridges,[1] but according to Johnson[2] they develop as low knob-like elevations which increase in height. They may become subdivided, as indicated by bifid villi.

The small intestine contains other elevations of its lining which are much larger than the villi. These are the circular folds (*plicæ circulares*, formerly known as Kerkring's *valvulæ conniventes*), which are seen conspicuously on opening the intestine. They are thin leaf-like membranes, in places very close together, which, as their name implies, tend to encircle the tube. Sometimes they form short spirals, and they may

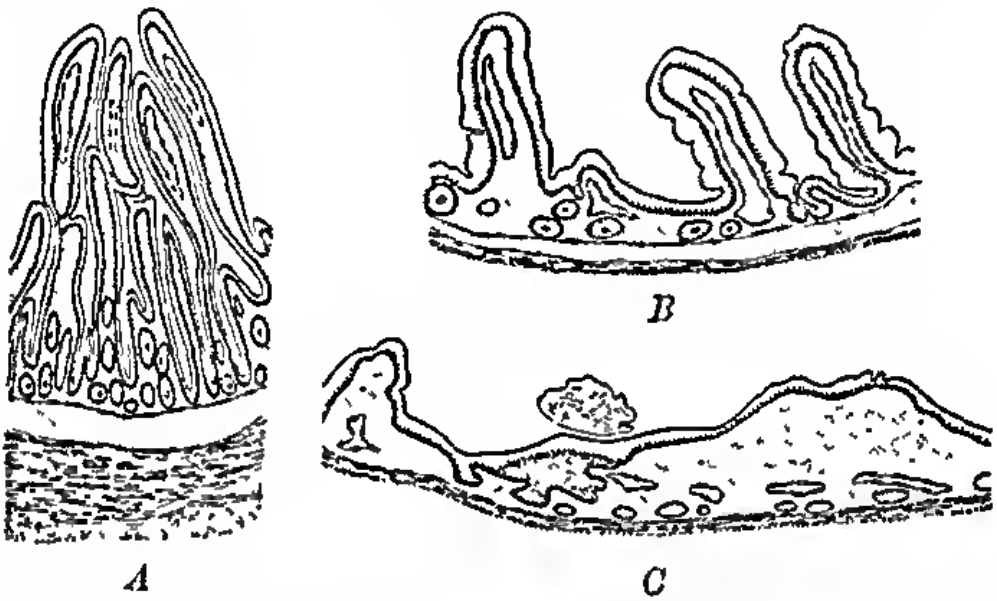


FIG. 307.—EFFECTS OF DISTENTION ON THE SMALL INTESTINE OF THE ADULT GUINEA-PIG. × 5/7 (Johnson.)
A, Strongly contracted, B, normally distended with food, C, distended with a pressure of 150 cm. of water

branch and connect with one another. They begin in the duodenum, and beyond the duodenal papilla they are tall and close together. They are highly developed in the jejunum and form its most characteristic feature. In the ileum they are lower and further apart; and they may come to an end two feet above the colon. The villi correspondingly are taller and more numerous in the jejunum than in the ileum, in the distal part of which they are short and scattered, finally disappearing on the colic surface of the *valve of the colon* (ileo-cæcal valve). Thus few and short villi and scattered plicæ indicate that a section of the intestine is from the ileum. As seen in sections, the *plicæ circulares* are elevations of the submucosa (*Fig. 306*) covered on both sides by the entire mucous membrane —villi, glands and the muscularis mucosæ.

Villi may be regarded as a method of increasing the surface area of the intestine, and thus providing for a greater number of the epithelial cells. For this purpose a projection above the general surface is as effective as a depression (gland). The distribution

[1] BERRY, 1900. [2] JOHNSON, 1910.

of glands and villi in the gastro-intestinal tract corresponds with the processes of digestion and absorption of food. In the stomach the food is mixed with pepsin and hydrochloric acid, the deep branching glands affording surface enough for the necessary active cells, which pour their secretion through the pits. In the duodenum and especially the jejunum absorption takes place. Little secretion is added and the glands are short. For absorption, however, actual contact of the epithelial cells with the partly fluid intestinal contents is necessary, and the cells covering the villi are surface cells capable of such contact. By the time the food reaches the ileum most of the absorption has taken place, and the villi there are low and scattered; in the colon they are absent. The plicæ circulares increase the surface still further, and the whole small intestine is coiled because of its overgrowth in length.

The glands, villi, and plicæ have usually been regarded as permanent structures, serving to increase the secreting and absorbing surfaces of the intestine. In mammals they apparently are not obliterated by the normal distention of the intestine, although the villi may become shorter, the glands shallower, and the plicæ may be partially taken up like the folds of the œsophagus. In the Guinea-pig, and to some extent in the rabbit and cat, Heitzmann found that the villi change their shape with the intestinal contractions and expansions associated with physiological activity. Johnson[1] has shown that in Guinea-pigs the villi and glands of the contracted intestine have the form seen in Fig 307, A; with normal distention due to abundant food, they appear as in B; and with extreme artificial distention, the glands and villi are nearly obliterated as in C. The tube expands to this limit, beyond which additional pressure has no effect until it ruptures. On releasing the pressure, glands and villi return to their normal size. Interesting questions are suggested, as to how the muscle fibers become rearranged in the thin layer when the intestine is distended, and what takes place in the blood and lymphatic vessels.

W. H. Lewis's conception of smooth muscle as held together only by adhesion and returning to its original arrangement, after displacement, by capillary attraction is given on p. 153 (muscle).

Finer Structure of the Glands and Villi. The sides of the glands and surfaces of the villi are covered with simple columnar epithelium. It contains goblet cells separated from one another by cells free from mucus. The cells of the villi are taller than those in the glands, and the goblet cells are somewhat larger, but toward the tip of the villus they become slender and empty. The top plates or cuticulæ become thicker from the fundus of the gland outward to the tips of the villi, and when well developed they exhibit vertical striations which are considered to be protoplasmic processes lodged in pores. The top plate of the goblet cells is thin and apparently ruptures to allow the escape of the mucus.

At the blind lower end or fundus of the glands, there occur certain cells containing many coarse granules in that part of their protoplasm which is toward the lumen (Fig. 308). These cells were first described by Paneth[2] and are known as *Paneth's cells*. They are found in the glands of the duodenum, jejunum and ileum, but not in those of the large intestine. Although they may be observed with ordinary stains, they are

[1] Johnson, 1913*b*. [2] Paneth, 1888.

more strikingly demonstrated in iron-hæmatoxylin preparations. Apparently they produce a special secretion, which enters the lumen of the gland in the form of fine granules when the digestion of fat is taking place, and may perhaps be concerned also with protein digestion but not with that of carbohydrates.[1] They do not contain mucinogen granules, although goblet cells occur in their immediate vicinity.

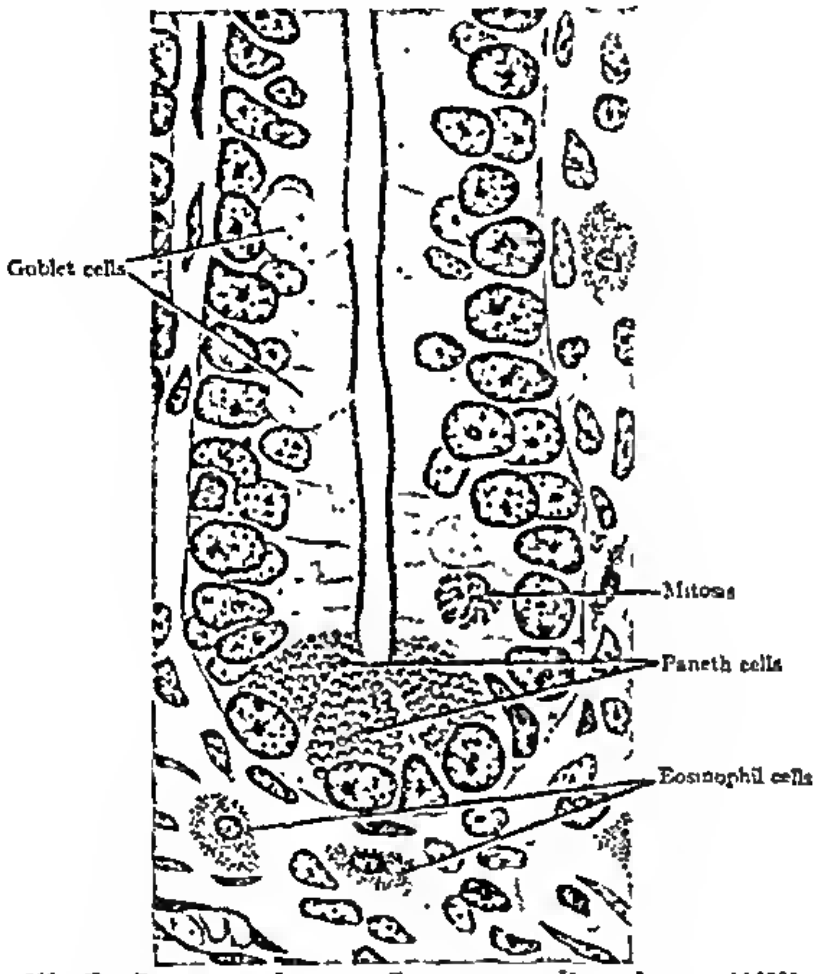


Fig. 308.—The Fundus of an Intestinal Gland from the Human Jejunum. × 1000. Zenker fixation, hæmatoxylin and eosin (von Möllendorff).

Still another type of cell found in the small intestine is the enterochromaffin or argentaffin cell[2] (*Fig. 310*). These are scattered irregularly throughout the intestinal epithelium, usually as isolated units among the epithelial cells. They are present on the villi and in the glands, and while most numerous in the small intestine may also be found throughout the entire tract from the œsophagus to the rectum. In specimens fixed and stained by ordinary methods they are very inconspicuous, appearing as dark cells between the mucous elements. After fixation in potassium dichromate they stand out on account of their dark yellow color, as was

[1] Mirami, 1912. [2] Macklin and Macklin, 1932.

noted by Heidenhain.[1] The granules contained in their cytoplasm are easily impregnated with silver. These two characteristics give the cells their most common names. In shape they may resemble the epithelial cells among which they lie, or be flask-shaped and appear partially extruded from the layer like the parietal cells of the stomach. Their specific granules are minute and located in the basal portion, commonly below the nucleus. They are acidophilic, chromaffin, stain with Heidenhain's iron hæmatoxylin, reduce silver, and turn greenish with basic blues. The basal portion may also contain one or two vacuoles containing fatty material. The nucleus is spherical or oval, rather poor in chromatin. One Golgi apparatus has been reported in the supranuclear region and another in the basal region.

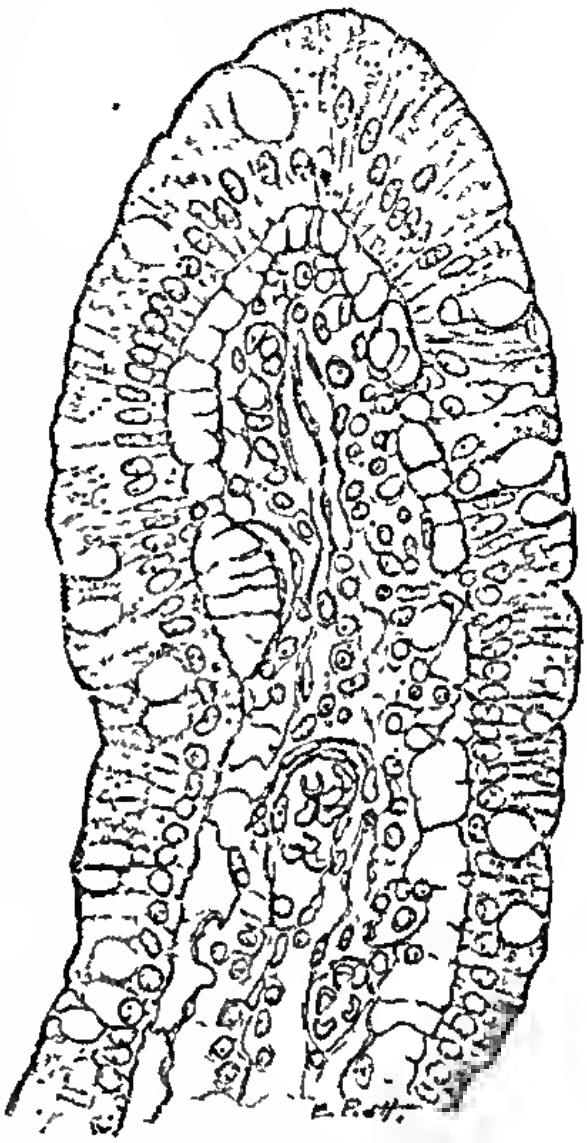


Fig 309.—A Longitudinal Section through the Apex of a Villus of the Human Small Intestine

Note the relationship of the fine reticular fibers to the under surface of the epithelium. Zenker fixation; hæmatoxylin and eosin

Interest in these enterochromaffin cells lies in their possible relation to the cells of the chromaffin organs and the suprarenal cortex. Cells with similar staining reactions have been found in the lamina propria of the intestinal tract, and their possible migration from one position to another has been traced. They also are thought to be in special relation with the nerves, and have been noted migrating along the nerves even to the submucosal ganglia.[2] The migrating cells assume the ability to produce fatty material which is extruded along the nerves. They have been regarded as part of a vast diffuse system, possibly endocrine in function.[3]

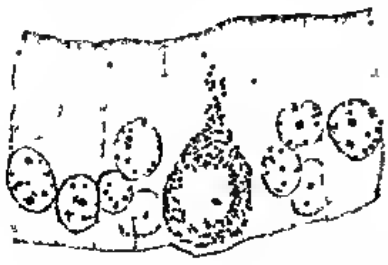


Fig 310.—A Section of Epithelium from the Small Intestine of a Rabbit Showing an Argentaffin Cell. Formaldehyde Fixation. Bodian Impregnation

A short distance above the fundus, the epithelial cells of the glands exhibit mitotic figures. From this it is inferred that the outer cells, including those of the villi, are renewed from below, so that the cells may be said to

[1] Heidenhain, R., 1870. [2] Masson, 1928. [3] Simard, 1934.

creep along the basement membrane until they are cast off. The cells near the bottom of the gland have terminal bars, but they are not so distinct as those of the villi. During division, the cell seems to be drawn up from the basement membrane, as if held in position by the terminal bars. The plane of division is at right angles with the long axis of the cell, and after mitosis the nuclei move back to the basal layer. Lymphocytes which have made their way between the epithelial cells are frequently seen, and when near the lumen and over-stained they may be mistaken for mitotic figures.

FIG. 311.—A RECONSTRUCTION OF A DUODENAL GLAND (BRUNNER'S GLAND) IN THE HUMAN DUODENUM (Maziarski.)

The duodenum contains, in addition to the intestinal glands, branched mucous glands, the bodies of which invade the submucosa. These are called *duodenal glands* (Brunner's glands) and they occur nowhere else in the small intestine (Figs. 311 and 312). Their cells produce a mucus which stains with difficulty, thus contrasting with the mucus of the goblet cells in the tubular glands above them. As in the pyloric glands, occasional parietal cells have been found, and also the dark cells, due to compression. The tubules are provided with a structureless basement membrane. The ducts of the duodenal glands may open on the free surface of the epithelium, or into the lower ends of the *intestinal glands*. The duodenal glands are so numerous toward the stomach that the submucosa may be filled with their tubules. They are also abundant near the *duodenal papilla* where the bile and pancreatic ducts enter the descending portion of the duodenum. Beyond this point they become fewer, and disappear before the end of the duodenum is reached. Except for these glands the duodenum is essentially like the remainder of the small intestine.

Interest in the villi centers chiefly in their relation to the absorption of nutritive material from the intestinal contents (*chyme*). Water, inorganic salts, and the simple sugars pass through the guarding membranes

without change. Within the body they may make new combinations. But other food materials, proteins, fats, and carbohydrates (except the simple sugars) mostly undergo rather profound changes in the digestive tract. They are in general broken down by hydrolysis into simpler prod-

Fig 312.—A Section of Duodenal (Brunner's) Glands Human.
Note the opening of glands into the intestinal crypts above Zenker fixation, hæmatoxylin and eosin

ucts, and then rebuilt in the tissues. The process of passing through the epithelium may be considered as a reversed secretion, the material being taken in by the cells at their upper or free pole, elaborated within the protoplasm, and discharged from the basal surface. Fat, in the form of

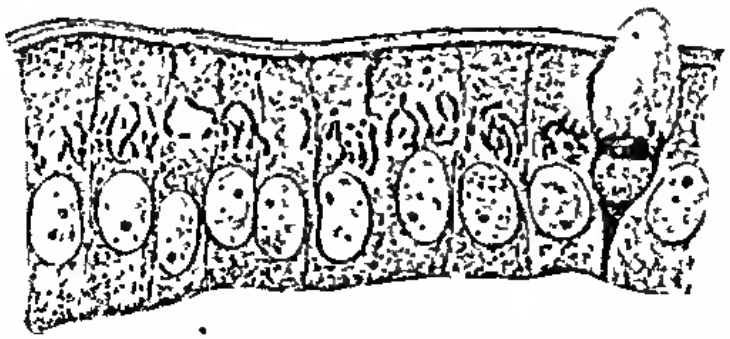


Fig. 313.—Intestinal Epithelial Cells from a 44 Yr Old Man Showing a Golgi Apparatus in the Cytoplasm toward the Lumen Chrome-osmium Preparation ×1500 (Kopsch)

fatty acids which do not blacken with osmic acid, is conveyed through the cuticula and resynthetized within the cell to fat droplets, which are discharged from the base and sides of the cell. Occasionally lymphocytes between the cells ingest these droplets. The major part of the fat, how-

ever, as soon as it passes through the epithelium, appears as very fine fat droplets, which enter the central lymphatic vessel of the villus where they form a fine emulsion. Gage and Fish[1] have named these droplets 'chylomicra.' They are $\frac{1}{2}$ μ to 1.0 μ in diameter, and best seen with the dark-field microscope. Gage traced them in the lacteals and blood vessels, and by means of fat stains (Sudan III) could note the storage of fat after meals in certain organs and in the adipose tissue. Fat is mainly absorbed in the jejunum and upper ileum.

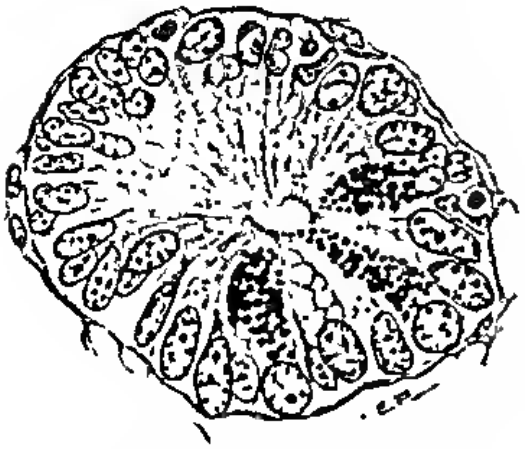


Fig. 314.—A Cross Section of a Gland from a Meckel's Diverticulum of the Human Intestine. On the right there are three Paneth cells. Zenker fixation, hæmatoxylin and eosin.

None of the other food materials can be followed microscopically either through the epithelium or in the vessels, though they may be traced chemically.

Mingazzini thought that the proteins were secreted from the base of the cells in the form of small hyaline spherules, which later broke down and discharged their contents between the epithelium and the reticular lamina propria. In certain fixed specimens a space occurs beneath the epithelium in which pale spherules closely crowded together may be seen. Macklin and Macklin,[2] however, find that the spaces are the result of the shrinkage of the cores of the villi, probably through the contraction of the smooth muscles. This phenomenon takes place during the first few minutes after the cessation of the circulation; it is thus usually found in human material (compare Fig. 309). It may also be caused by improper fixation. The shrinkage expresses from the core the tissue fluid, which may coagulate in the form of spherules. The epithelial layer thus lifted from its basement membrane soon degenerates.

Outer Layers of the Small Intestine. The lamina propria, which forms the cores of the villi and extends between the glands, is a reticular tissue, containing the usual types of free cells and also a large number of plasma cells (see p. 105). Slender strands of smooth muscle extend up and down the villi, being inserted into the reticulum, and by contraction they cause the villi to shorten or to wave about. The *muscularis mucosæ* consists of an inner circular and an outer longitudinal layer, thus duplicating on a small scale the tunica muscularis. The submucosa is a connective tissue layer, such as has been described in the stomach and œsophagus, and the muscularis is divided into a thick inner circular layer of smooth muscle and a thinner outer longitudinal layer, between which is a thin stratum of intermuscular connective tissue. The muscle layers are arranged spirally, a closely wound spiral in the circular layer and a long

[1] Gage and Fish, 1924. [2] Macklin and Macklin, 1926.

spiral in the outer layer. This interesting feature is discussed by F. T. Lewis.[1] The intestine is covered externally by the *tunica serosa*. The distribution of the vessels and nerves in these layers is as follows.

Blood Vessels. The arteries pass from the mesentery into the serosa, in which their main branches tend to encircle the intestine. Smaller

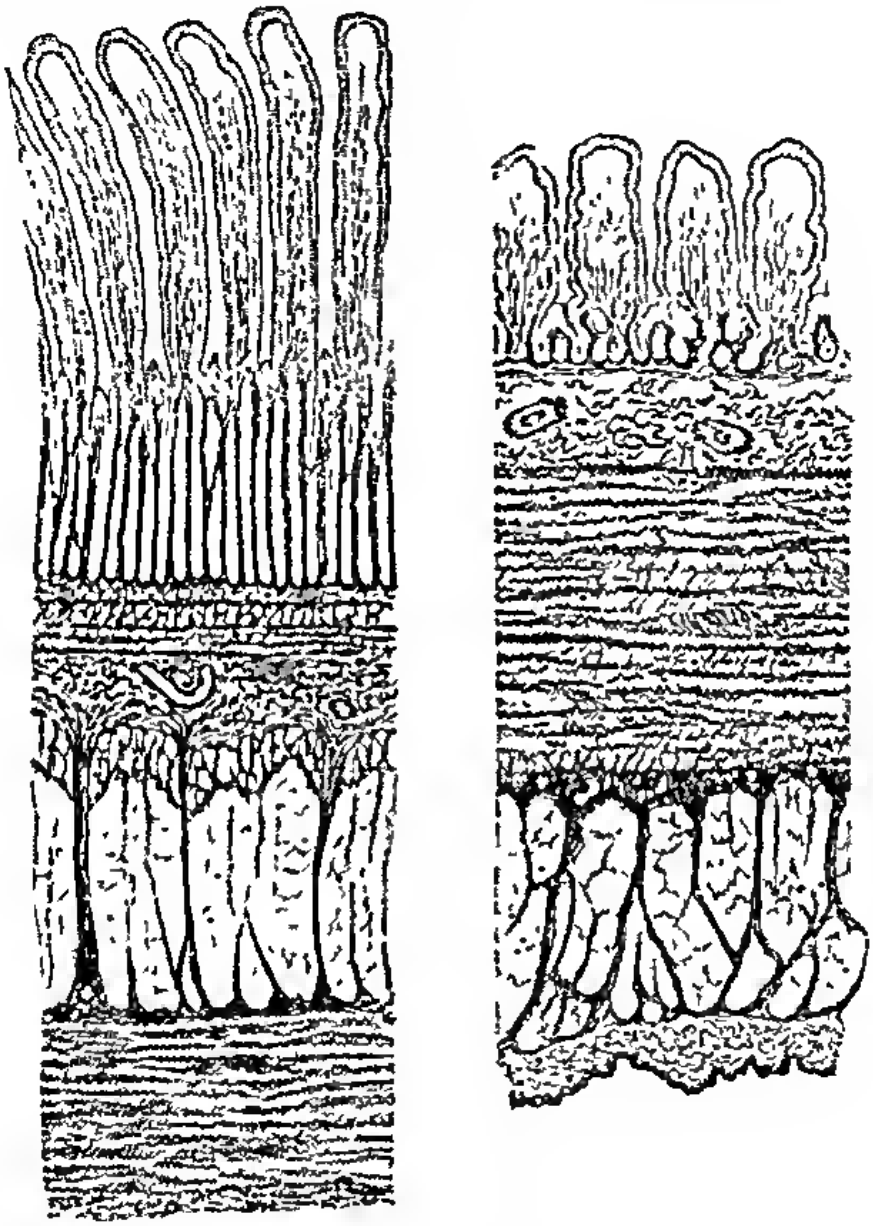


FIG 315.—A SECTION THROUGH A PORTION OF THE WALL OF THE JEJUNUM OF THE DOG, ON THE LEFT AND OF THE PIG ON THE RIGHT. A COMPARISON OF THE DISTRIBUTION OF ELASTIC TISSUE. RESORCIN FUCHSIN. (Trautmann.)

branches from these pass through the muscle layers to the submucosa, in which they subdivide freely (Fig. 316, A). In crossing the muscle layers they send out branches in the intermuscular connective tissue. These and the arteries of the serosa and submucosa supply the capillary networks found among the muscle fibers. The capillaries are mostly parallel with the muscles. From the submucosa the arteries invade the

[1] LEWIS, F. T., 1922.

mucosa, forming an irregular capillary network about the glands, and sending larger terminal branches into the villi. There is usually a single artery for a villus, and it has been described as near the center, with the veins at the periphery, or sometimes on one side of the villus with the vein on the other. The network of blood vessels in the villi is very abundant as shown in Fig. 317. The veins branch freely in the submucosa and pass out of the intestine beside the arteries. The muscularis mucosæ has been described as forming a sphincter for the veins which penetrate

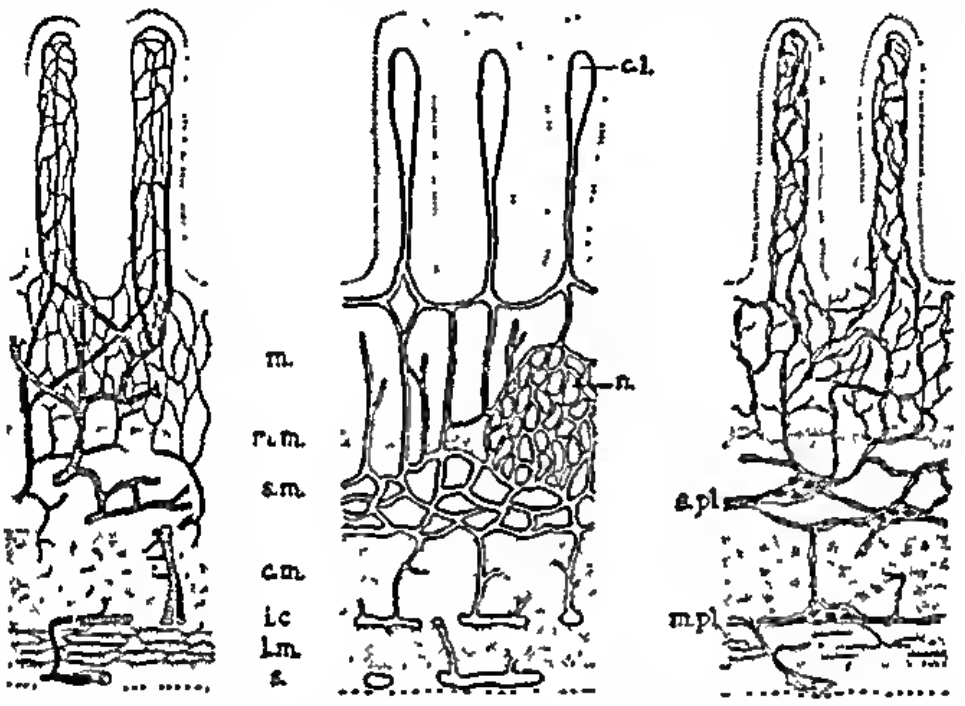


FIG. 316.

A, Diagram of the blood vessels of the small intestine, the arteries appear as coarse black lines, the capillaries as fine ones, and the veins are shaded (after Mall). B, Diagram of the lymphatic vessels (after Mall). C, Diagram of the nerves, based upon Golgi preparations (after Cajal). The layers of the intestine are m, mucosa; m. m., muscularis mucosæ; s. m., submucosa; c. m., circular muscle; i. c., intermuscular connective tissue; L. m., longitudinal muscle; s, serosa; c. l., central lymphatic; n., nodule; s. pl., submucous plexus; m. pl., myenteric plexus.

it; thus it may control the amount of blood within the villi. No valves occur until the veins enter the tunica muscularis; there they appear, and continue into the collecting veins in the mesentery. They are absent from the large branches of the portal veins which receive the blood from the intestines.

Lymphatic Vessels. The intestinal lymphatics (lacteals) appear as central vessels within the villi (Fig. 316, B). Each villus usually contains a single lacteal ending in a blind dilatation; sometimes there are two or three which form terminal loops. In some stages of digestion the distention of these lymphatics is very great and their endothelium is easily seen in sections. When collapsed they are hard to distinguish from the surrounding reticulum. Small lateral branches and a spiral prolongation of the central lymphatic have been found by injection, but these may be

tissue spaces into which the injected fluid has been forced. The lymphatics branch freely in the submucosa and have numerous valves. They cross the muscle layers, spreading in the intermuscular tissue and the serosa, and pass through the mesentery to the thoracic duct.

Lymphoid Tissue. The lymphoid tissue of the intestine occurs primarily in the lamina propria, and in three forms—diffuse lymphoid tissue, solitary nodules, and aggregate nodules. Solitary nodules are seen in Fig. 306. They may break through the muscularis mucosæ and spread in the submucosa, as is shown in Fig. 322. The nodules are surrounded by small vessels, the lymphatics being drawn in Fig. 316, B. Blood vessels may make a similar net, and penetrate the outer portion of the nodule. The germinative centers are similar to those in the lymph glands.

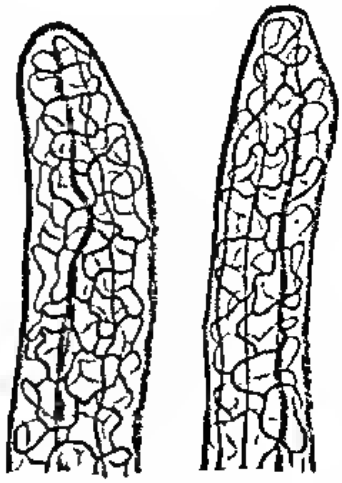


Fig. 317.—Injected Villi, Intestine of Dog.

Aggregate nodules (Peyer's patches) are oval areas, usually from 1 to 4 cm. long but occasionally much larger, composed of from ten to sixty nodules in close contact. The nodules may be distinct or blended in a single mass. They distort and push aside the intestinal glands with which they are in relation, and immediately above the nodules the villi are partly or wholly obliterated. Thus they appear as dull patches in the lining of the fresh-opened intestine, and may be readily seen. When in contact with the lymphoid tissue, the epithelium shows no goblet cells. There are from fifteen to thirty such patches in the human intestine (rarely as many as fifty or sixty), and they occur chiefly in the lower part of the ileum on the side opposite the mesentery. A few occur in the jejunum and the distal part of the duodenum. In the vermiform process, diffuse aggregate nodules are always present, but they do not occur elsewhere in the large intestine.

Nerves. The small intestine is supplied by prolongations of the vagus nerves, which are joined by branches of the superior mesenteric plexus of the sympathetic system. The latter are regarded as the principal supply. This plexus is ventral to the aorta, and sends branches through the mesentery into the serosa. The manner in which they penetrate the other layers, forming the *myenteric plexus* (Auerbach's plexus) between the circular and longitudinal muscle layers, and the *submucous plexus* (Meissner's plexus) in the submucosa, is shown in Fig. 316, C. In surface view, obtained by stripping the layers apart, these plexuses are seen in Figs. 318 and 319. Their branches supply the smooth muscle fibers. From the submucous plexus the nerves extend into the villi, where nerve cells

have been detected by the Golgi method (Fig. 316, C). The nerve fibers probably terminate in contact with epithelial cells and provide for local reflex action, whereby the muscles contract in response to stimulation of the epithelium. Most of the intestinal nerves are non-medullated, but they include a few large medullated fibers said to have free endings in the epithelium. The vagus fibers are said to pass to the intrinsic ganglia, and to excite both secretion and motion; the fibers from the cœliac ganglion run directly to their endings, and inhibit action, but

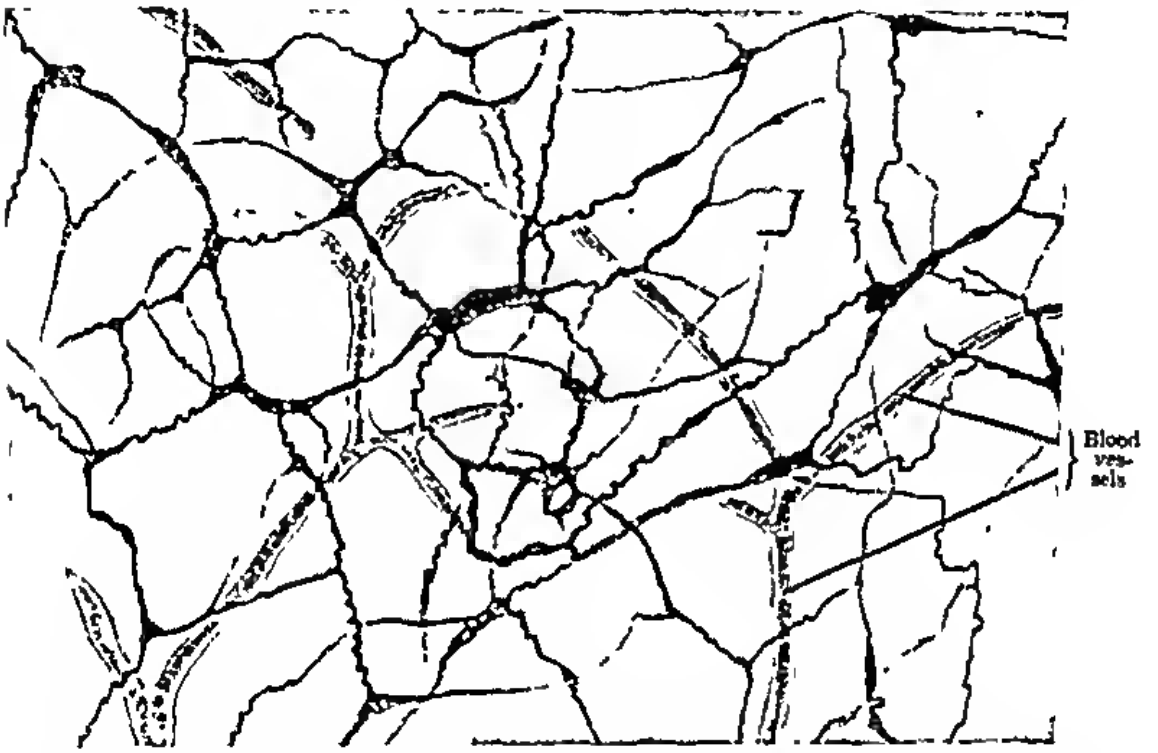


FIG. 318.—THE PLEXUS SUBMUCOSA AS SEEN FROM THE SURFACE. SMALL INTESTINE OF A RHESUS MONKEY. METHYLENE BLUE. (von Möllendorff.)

Florey[1] finds no clear-cut evidence that mucus can be secreted in the gut under direct nervous influences. The plexuses have been carefully studied by Hill.[2]

Mesentery and Peritoneum. The serous membrane which surrounds the intestinal tube and certain other abdominal viscera is a part of the lining of the body cavity. After covering the ventral surface and the sides of the intestinal tube, the two layers of serous membrane come together to form the mesentery and extend to the dorsal body wall; then, separating, they pass laterally as the lining of the abdominal walls and fuse in the mid-ventral line. This serous membrane, or peritoneum, consequently forms a closed sac. It is divisible into the visceral peritoneum which covers the viscera, and the parietal peritoneum which lines the body walls. In all cases its free surface is covered with a single layer of

[1] FLOREY, 1930. [2] HILL, C. J., 1927.

flat polygonal cells, resembling endothelium. Although quite flat, the cells have a thin cuticular border which is said to be striated, and the cuticulæ of adjacent cells fit together closely. The lateral walls of these flat cells are connected with one another by protoplasmic bridges. The cells are markedly phagocytic. Beneath this simple epithelium is loose connective tissue, with elastic fibers and many cells, both fixed and wandering, of all the types usually found in connective tissue. In the

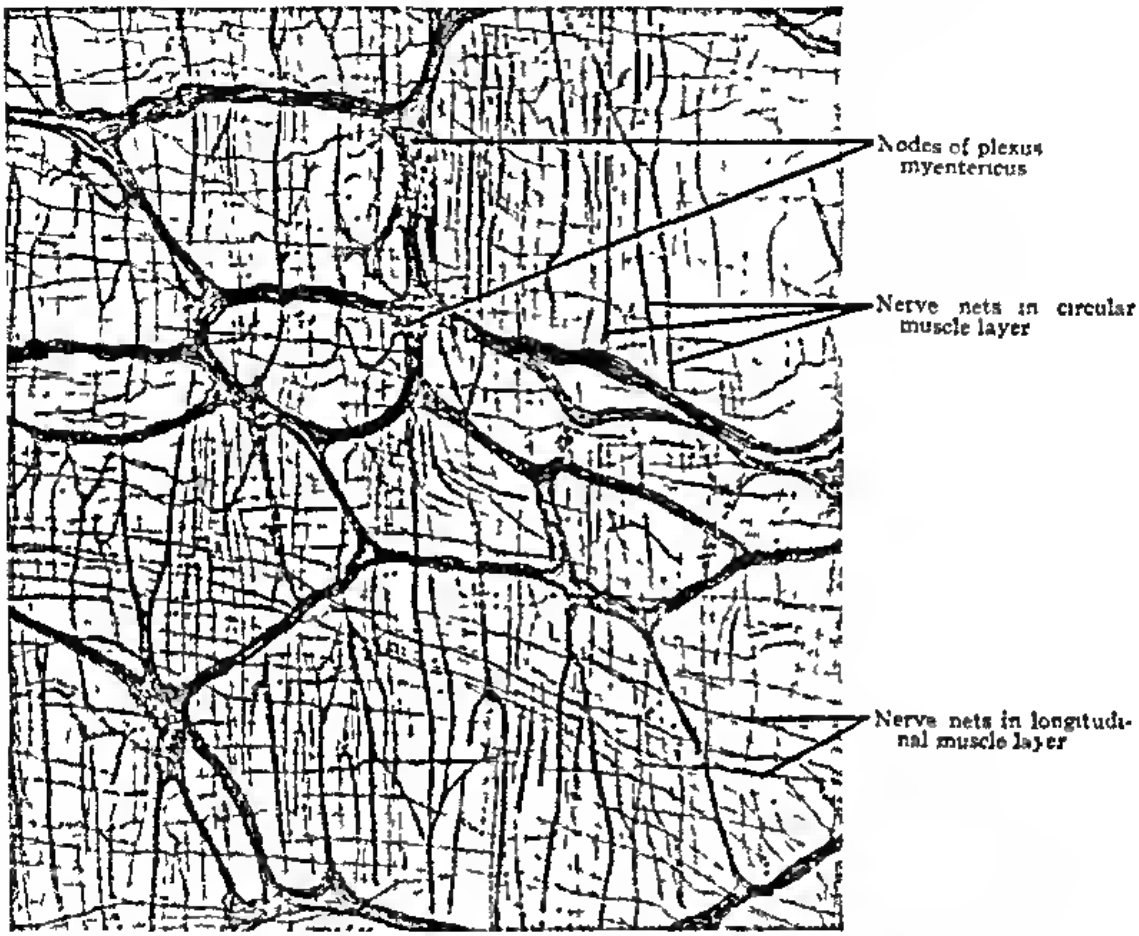


FIG 319.—A PLEXUS MYENTERICUS AND THE FINER DISTRIBUTION OF NERVES IN THE CIRCULAR AND LONGITUDINAL MUSCLE STRATA AS SEEN FROM THE SURFACE JEJUNUM OF A RHESUS MONKEY METHYLENE BLUE (von Möllendorff)

omentum especially there are large numbers of macrophages, and their presence may account for the great immunity from infection that the peritoneum shows.

In the mesentery, a thin layer of connective tissue with elastic networks and interwoven bundles of white fibers fills the interval between the two epithelial layers. In this connective tissue there are many lymphatic and blood vessels, and nerves to the various organs. Mast cells may be found along the vessels, especially in young animals (Fig. 76 p. 106), and various other forms of wandering cells occur. The connective tissue layer is denser in the parietal than in the visceral peritoneum. In places where the peritoneum is freely movable there is a *subserous layer* of loose fatty tissue, but there is no subserous layer in the intestine.

Scattered throughout the peritoneum, but chiefly near the diaphragm, occur 'milk spots' or *taches laiteuses*,[1] small rounded or oval areas where the mesothelium is thickened and the underlying cells seem to be producing lymphocytes and histiocytes, perhaps also red blood corpuscles.[2] They may be inconstant formations, vanishing and recurring. Free cells of the various types mentioned may be found in the peritoneal fluid.

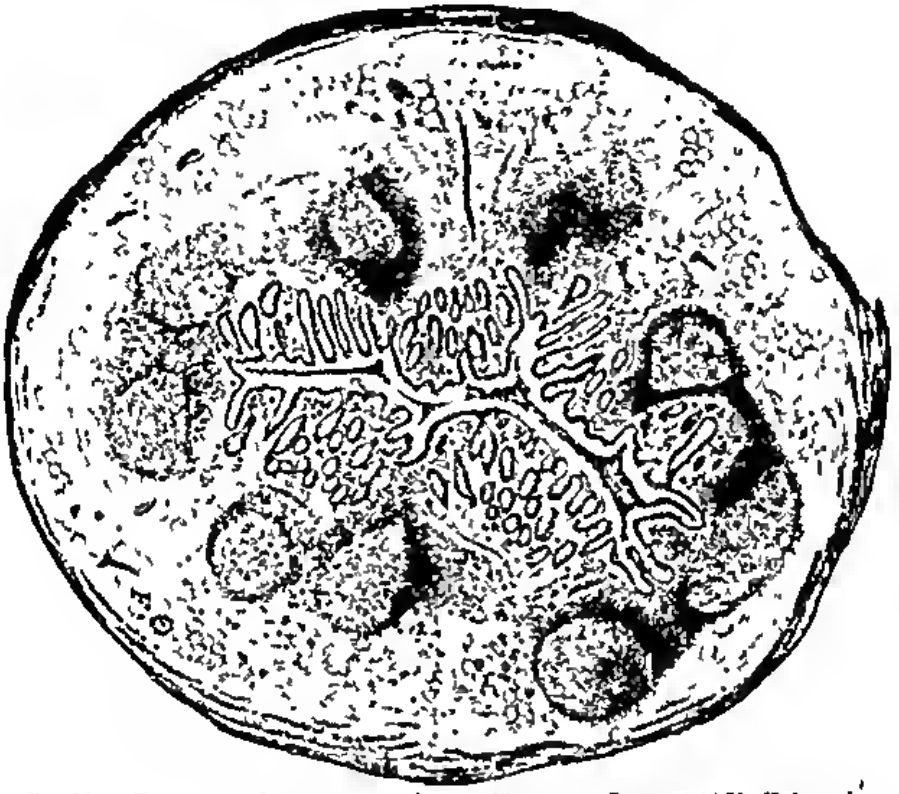


FIG. 320.—TRANSVERSE SECTION OF THE HUMAN VERMIFORM PROCESS. × 20. (Sobotta.)
Note the absence of villi and the abundance of nodules. Clear spaces in the submucosa are fat cells. Only a part of the circular layer of the muscularis has been drawn.

LARGE INTESTINE

Vermiform Process. The vermiform process, or appendix, is a 'worm-like' prolongation of the cæcum. Although small in size, in structure it more closely resembles the large intestine, of which it is a part, than the small intestine. In embryos of three and one-half to five months it is lined with villi, but with further development the villi flatten out and disappear. Meanwhile the glands, which are of the same type in both small and large intestines, have developed and are increasing in number and in length. Sometimes they penetrate the muscularis mucosæ. In the adult (Fig. 320) they are simple tubes, occasionally forked, thus indicating the way in which they multiply in the embryo. As early as the fourth month, lymphoid tissue has been found in the vermiform process,

[1] RANVIER, 1874. [2] MARCHAND, 1924.

and at birth the lymphoid nodules in the lamina propria are abundant and more or less confluent. The great development of lymphoid tissue is the most important histological feature of the vermiform process in the adult. It may invade and partly break up the muscularis mucosæ, and extend into the submucosa. The latter, together with the inner circular and outer longitudinal muscle layers, and the serosa, is similar to the corresponding layers of the small intestine already described.

During the fifth month of embryonic life, Stöhr has found an interesting normal form of degeneration in the glands of the vermiform process.[1] The lamina propria around

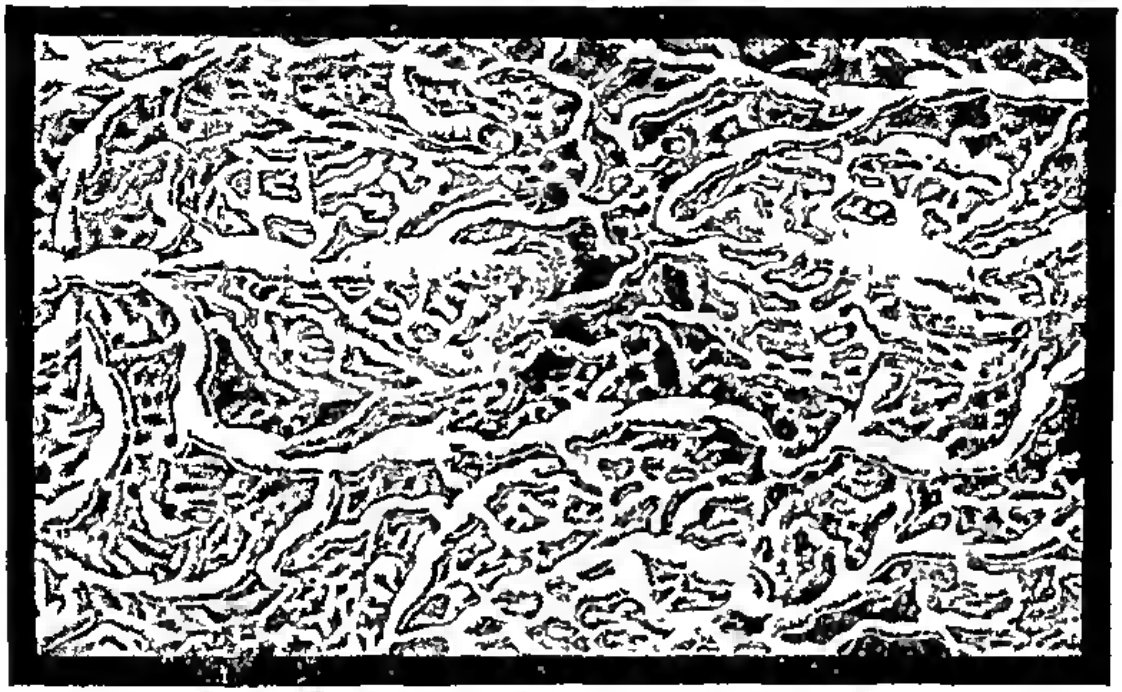


FIG 321.—A LYMPHATIC NETWORK IN THE PROCESSUS VERMIFORMIS OR APPENDIX OF MAN
Note the enlargement of the vessels over the lymph follicles and the position of valves in the larger vessels. × 40 (Teichmann)

them appears to thicken, and the goblet cells in the neck of the degenerating gland, after becoming flattened, produce a solid strand. The strand then ruptures and the detached fundus becomes cystic. Subsequently it shrinks to a small nodule surrounded by dense connective tissue, and ultimately disappears. This degeneration is said to be limited to the fifth and sixth months.

The lumen of the normal vermiform process in the adult, when empty, is thrown into folds, between which are deep pockets; but the normal condition is found in scarcely 50% of individuals over forty years of age. Often the lumen is narrowed or even obliterated. The epithelium with its glands and the lymphoid nodules then disappear, and are replaced by an axial mass of fibrous tissue. This is surrounded by the unaltered submucosa and muscularis; the serosa may show the results of inflammatory conditions.

[1] STÖHR, 1898.

Cæcum and Colon. In the human fetus the cæcum and colon somewhat resemble the small intestine, with villi and glands containing Paneth cells; but the production of new cells does not keep pace with the expansion of the epithelial tube, and the villi therefore gradually flatten and disappear at about the sixth month. In the parts of the fetal intestine distended by secretions and desquamated cells (constituting the *meconium*), they disappear earlier than in the contracted portions.[1] The Paneth cells are said to be present in small numbers in the upper two-thirds of the colon until the second year.

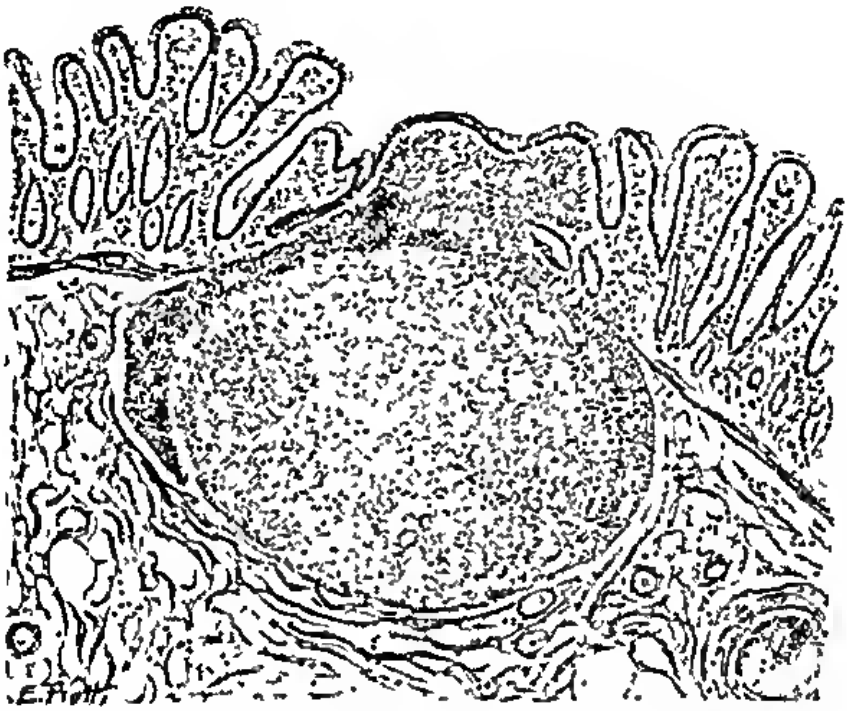


FIG. 322.—SECTION THROUGH THE WALL OF THE LARGE INTESTINE OF THE HUMAN ADULT SHOWING IN THE CENTER A LARGE LYMPH NODULE IN THE SUBMUCOSA WHICH PENETRATES INTO THE MUCOSA. Formaldehyde fixation, hæmatoxylin and eosin.

After the villi have gone, the mucosa contains only tubular pits or glands, lined with simple columnar epithelium (Fig. 323). These glands are similar to those in the small intestine but are longer—sometimes twice as long (0.4–0.6 mm.). They contain more goblet cells, but cells of Paneth are absent. Striated cuticular borders appear near the outlets of the glands, and are well developed upon the columnar cells lining the intestinal lumen. Solitary nodules are numerous, especially in the cæcum.

The *tunica muscularis* of the colon and cæcum has a characteristic arrangement not found in the vermiform process. The longitudinal smooth muscle fibers of the outer layer become gathered into three equidistant longitudinal bands or *tæniæ;* between them the longitudinal

[1] JOHNSON, 1913*a*.

fibers form a thin layer which may be interrupted. The tæniæ, one at the mesenteric attachment, the other two lateral, come together at the root of the vermiform process and are continuous with its outer muscle

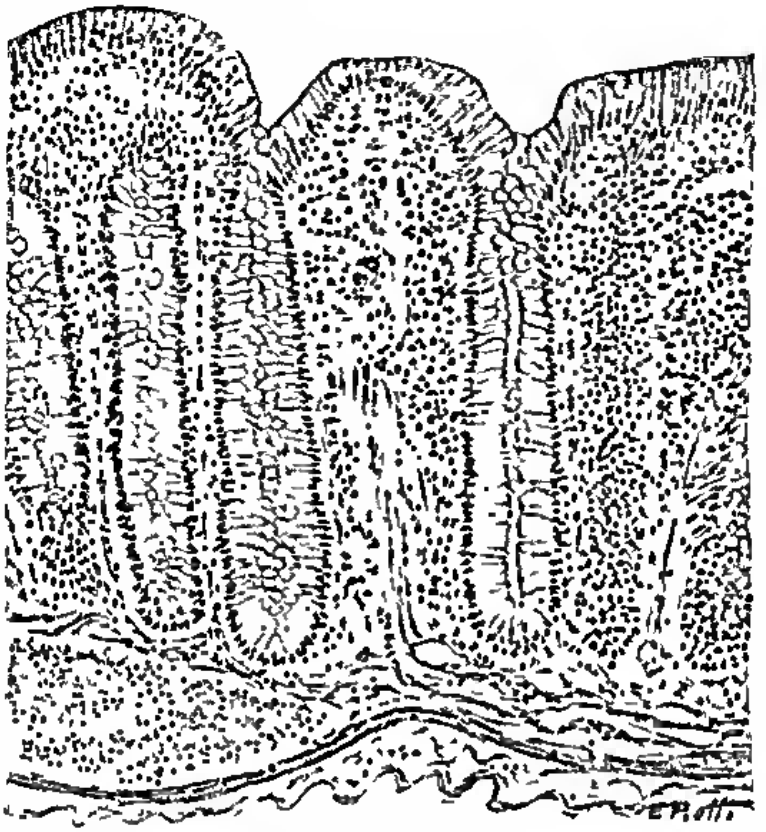


FIG 323.—A SECTION THROUGH THE MUCOSA OF THE ADULT HUMAN LARGE INTESTINE. Formaldehyde fixation, hæmatoxylin and eosin.

layer. Between the tæniæ the wall of the colon is thrown into a succession of folds, the *plicæ semilunares*, due to the local contraction of bundles of the circular fibers; between the folds the walls bulge to form the *haustra* (Lat., buckets). The valve of the colon (*valvula coli*) is a pair of folds or *labia*, which resemble the semilunar folds; that is, they include fibers of the circular muscle layer, but the layer of longitudinal fibers passes directly from the ileum to the colon without entering the valves. A peculiarity of the colon is that fascicles of the longitudinal fibers in the tæniæ frequently turn laterally to join the circular layer. This arrangement interrupts the continuity of the circular layer so that the different intertænial areas may contract independently.[1]

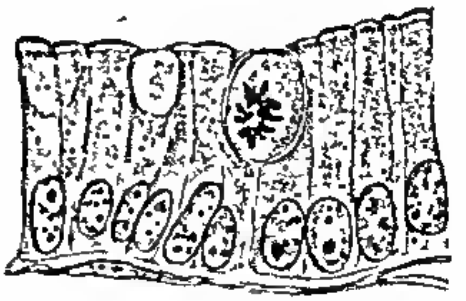


FIG 324.—INTESTINAL EPITHELIUM FROM A MONKEY ONE CELL IN MITOSIS. Susa fixation; hæmatoxylin and eosin

The serosa contains lobules of fat which form pendulous projections known as *appendices epiploicæ*. The vessels and nerves either enter the

[1] LINEBACK, 1925.

colon at the mesenteric tænia, or course around the wall just beneath the serosa in the interhaustral folds to the lateral tæniæ, where the vessels may form anastomotic arches before penetrating the muscular wall.

Rectum. The rectum is divided into two parts, an upper which extends from the third sacral vertebra to the pelvic diaphragm, and a lower which continues downward to the anus. The lining of the first part is thrown into several folds, the *plicæ transversales recti* (valves of Houston). These are large semilunar folds which usually extend only part way around the rectum, but they have been described in some cases as having a spiral arrangement. The second part of the rectum, the *pars analis recti* (anal canal), presents on its inner wall a number of longitudinal folds, known as *rectal columns* (columns of Glisson or Morgagni). At their lower extremities the columns unite with one another, thus forming small transverse plicæ or *anal valves*. The grooves between the columns extend downward behind the valves, forming a series of blind pockets, the *sinus rectales*.

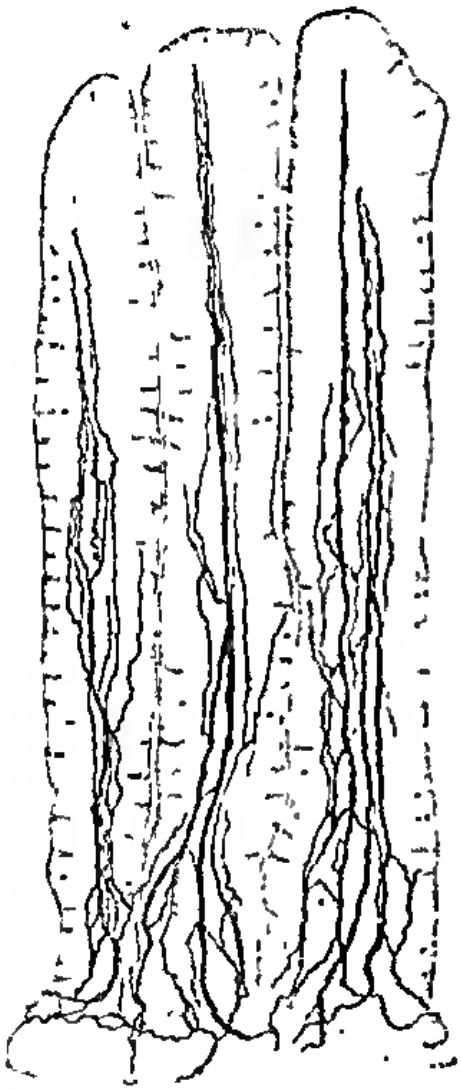


Fig. 325.—Nerve Fibers in the Mucous Membrane of the Intestine of a Rabbit. The Section Includes Two Glands. Golgi Method (E. Müller.)

The mucous membrane of the first part of the rectum is similar to that of the colon, but its glands are somewhat longer (0.7 mm.). Solitary nodules are present. The muscularis mucosæ, submucosa, and circular layer of smooth muscle also resemble those of the colon, but the three tæniæ spread out and unite so as to form a continuous layer of longitudinal muscle. In the upper part of the rectum this layer is specially thickened dorsally and ventrally. As the rectum loses its mesentery, the tunica serosa is replaced by adventitious connective tissue.

The pars analis recti is the region of transition from mucous membrane to skin. This transition is not gradual but takes place in three steps, thus forming three distinct superimposed zones. From above downward these are the *zona columnaris*, *zona intermedia*, and *zona cutanea*

(Fig. 326). The last, however, does not belong to the *pars analis*, properly speaking, but to the outside skin.

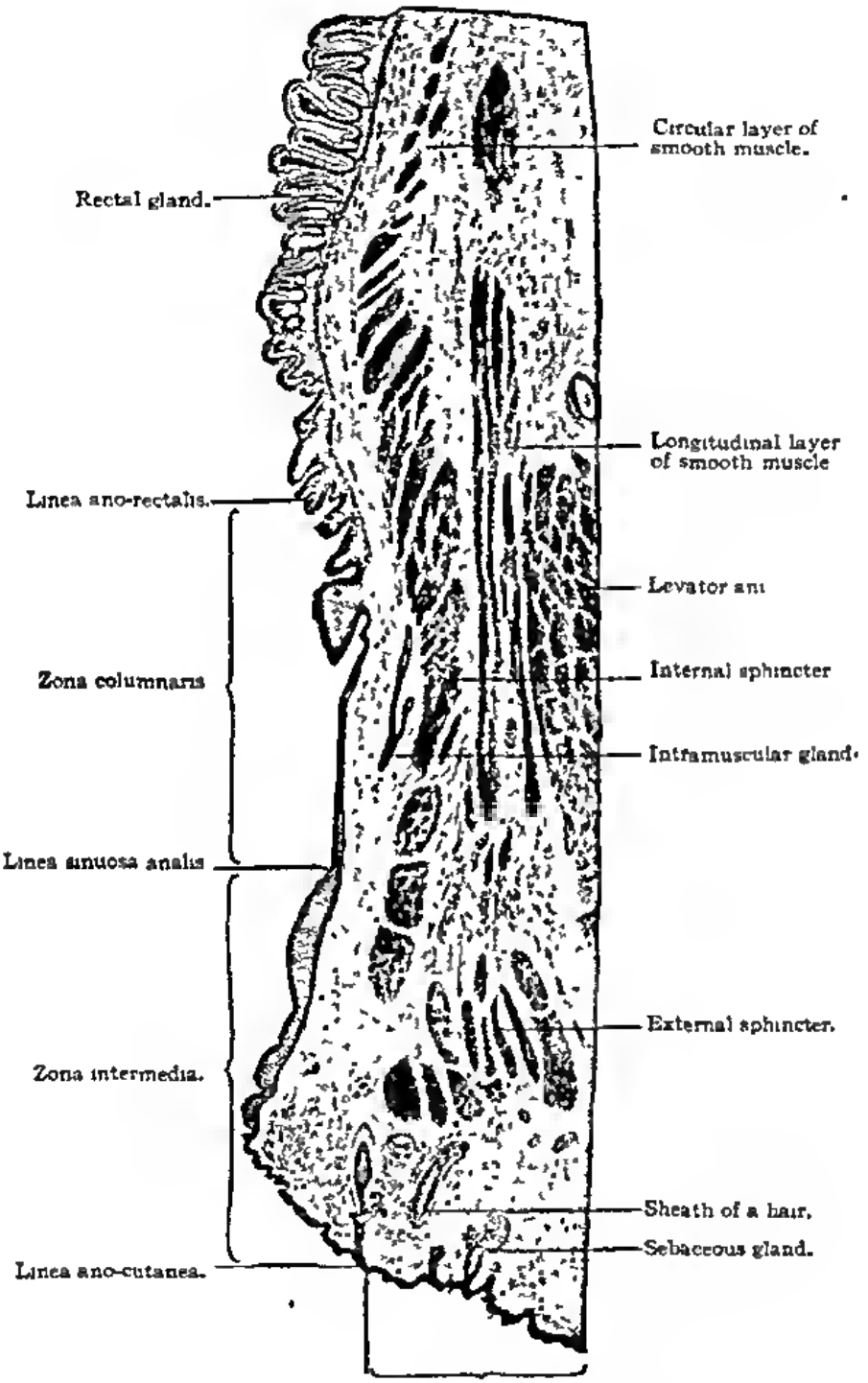


FIG 326.—LONGITUDINAL SECTION THROUGH THE PARS ANALIS RECTI
From a human embryo of 187 mm (about four months) (T P Johnson)

The zona columnaris is the region of the rectal columns, but these are not always limited to this zone. They may extend upward into the first part of the rectum for a short distance, and they may also be continuous downward with the so-called *anal skin folds.* In the upper part of the

zona columnaris the simple columnar epithelium of the superior portion of the rectum becomes two- or three-layered. Its outer cells are columnar, with finely granular protoplasm. The transition takes place gradually at the *linea ano-rectalis*. In the upper part of the zone there are usually a few intestinal glands containing numerous goblet cells, and a few goblet cells are found also in the surface epithelium. In the lower part of the zona columnaris, arising from the rectal sinuses, there are a few branched tubular gland-like structures, the *intramuscular glands*. There are seldom more than six or eight in any one rectum. The main ducts of these glands extend outward, and usually downward, and penetrate the internal circular muscle (internal sphincter). Here a flask-shaped swelling is usually met with. Extending beyond this ampulla there are several tubular branches which continue through the internal sphincter and end blindly in the intramuscular connective tissue. Occasionally a tubule is seen piercing the longitudinal muscle layer. Around the terminations of the tubules, which are sometimes swollen, there is a small amount of lymphoid tissue. The epithelium lining the main ducts of these glands consists of several layers of polygonal cells, but the ampullæ and branches are lined with one or two layers of cuboidal cells. Secretory cells are present in the embryo and at birth, but are apparently wanting in the adult.

The transition between the zona columnaris and zona intermedia is marked by a rather abrupt change in the epithelium, which becomes many-layered and squamous. This transition takes place at the level of the anal valves, but between the valves it extends upward on the rectal columns. Thus it follows a zig-zag line, the *linea sinuosa analis* (ano-cutaneous line of Hermann). Within the zona intermedia the epithelium, composed of several layers of polygonal cells, is thicker than the epidermis. Dermal papillæ are present, but hairs and sweat glands are absent. In the lower part of this zone there are a few isolated sebaceous glands without hairs, and the epithelium is slightly cornified. Thus it gradually goes over into skin, forming a true *linea ano-cutanea*, but this line is not well marked. It has been defined as the place where the first sheaths of the hairs appear.

The skin immediately surrounding the anus forms the zona cutanea. Sweat glands are absent from the region bordering on the anus, but at a distance of 1.0–1.5 cm. there is an elliptical zone, 1.25–1.5 cm. wide, containing simple tubular coiled glands, the *circumanal glands* of Gay. These are very similar to sweat glands but are considerably larger.

The outer layers of the pars analis recti include a very vascular tela submucosa, which contains numerous nerves and lamellar corpuscles. The muscularis mucosæ terminates in slender longitudinal bundles

which extend for varying distances into the rectal columns (forming the *M. dilatator ani internus* of Rüdinger). The circular layer of the tunica muscularis becomes thickened at its termination, forming the *M. sphincter ani internus;* it extends a little below the linea sinuosa analis. Beyond the internal sphincter, which is composed of smooth muscle, striated muscle fibers surround the anus forming the *M. sphincter ani externus.* The outer longitudinal layer of the tunica muscularis ends in relation with connective tissue strands which diverge as they pass downward through the external sphincter, to terminate in the subepithelial tissue of the zona cutanea.

LIVER

The liver is a large reddish-brown gland situated mostly in the upper right quadrant of the abdominal cavity. Its texture is firm, yet plastic enough to mould itself to neighboring structures. The gland is almost completely covered by peritoneum, except where this layer is reflected as ligaments, behind the gall bladder at the place in which the two organs are in contact and at the porta hepatis where the branches of the portal vein and the hepatic artery enter and the branches of the hepatic duct leave. The surface of the liver is smooth, with a fine mottled appearance caused by the outlines of the tiny hepatic lobules shining through the thin covering. It is an organ essential for the maintenance of life, with both exocrine and endocrine secretions although the glandular cells are all of one kind. Among its many functions are the secretion of bile, the metabolism and the storage of carbohydrates, fats, proteins and their derivatives, also the storage of vitamin A. Besides producing bile salts, urea, fibrinogen and heparin are formed in the liver.

Development and General Structure. The liver first appears in human embryos of about 2.5 mm. as a diverticulum of the ventral wall of the fore-gut, near its junction with the yolk-sac. If the embryo is placed in an upright position (Fig. 327, A), the liver is seen to be below the heart and between the vitelline veins as they pass from the yolk-sac to their cardiac termination. The diverticulum projects into a mass of mesoderm, to which His gave the old anatomical term for diaphragm, namely *septum transversum.* The diaphragm develops in the anterior or upper part of this septum; the lower or posterior part constitutes the ventral mesentery, which extends from the fore-gut to the ventral body wall. The hepatic diverticulum is in the mesenteric part of the septum, although it is always connected with the overlying diaphragmatic shelf.

In a 4 mm. embryo (Bremer) the diverticulum is still median, the cavity from the fore-gut extending but a short distance into it. Irregular masses and anastomosing cords of cells have begun to form through

extensive proliferations in the anterior and ventral walls of the diverticulum and some of these are seen in contact with vessels of a plexus of vitelline veins.[1]

Very early the liver becomes divided into two parts, (1) the somewhat rounded diverticulum proper, lined with columnar cells with pale cytoplasm, and (2) a mass of anastomosing cords or trabeculæ, composed of deeply-staining cells with round nuclei and abundant granular cytoplasm. These two parts are so unlike in appearance that they have been thought to proceed from different germ layers, the trabeculæ being described as formed from mesenchyma in the septum transversum. This opinion is erroneous; the entire structure is entodermal, and the tra-

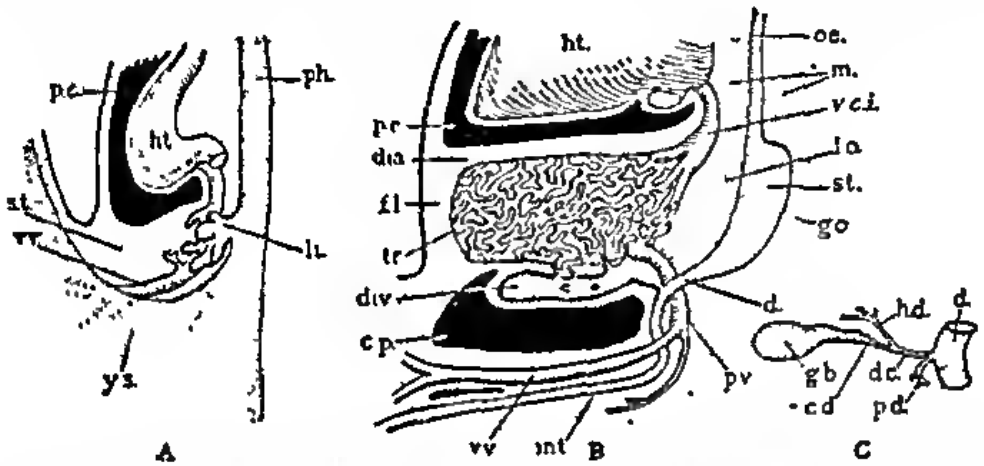


FIG. 327.—DIAGRAMS OF THE DEVELOPMENT OF THE LIVER

A, From a 4·0-mm. human embryo. B, From a 12 mm. pig. C, The ducts in the human adult. c. d., Cystic duct; c. p., peritoneal cavity, d., duodenum, d. c., ductus choledochus, dia., diaphragm, div., distal end of the diverticulum, f. l., falciform ligament; g. b., gall bladder, g. o., greater omentum, h. d., hepatic duct, ht., heart; int., intestine, li., liver, l. o., lesser omentum, m., mediastinum, oe., œsophagus; p. c., pericardial cavity; p. d., pancreatic duct, ph., pharynx, p. v., portal vein, s. t., septum transversum, st., stomach, tr., trabecula, v. c. i., vena cava inferior, v. v., vitelline vein, y. s., yolk-sac

beculæ grow out from the diverticulum. They encounter the vitelline veins, which ramify around them, producing the lacunar vessels or sinusoids already described (*Fig. 191, p. 219*).

In an embryo of 10–12 mm. (Fig. 327, B), the hepatic diverticulum has elongated and is connected with the mass of anastomosing trabeculæ at several points. It shows also some detached ducts and round knob-like swellings. The vitelline veins have been subdivided by the trabeculæ to form the sinusoids. The part of the vein below the liver becomes modified to form the portal vein, which thus brings blood to the sinusoids. The upper part, collecting the blood from the sinusoids after its passage through the liver, is known, near to the liver, as the hepatic vein, while its continuation to the heart becomes the upper part of the inferior vena cava. In the 10-mm. embryo the circulation of the liver is wholly venous. The trabeculæ consist of cells which are doubtless very active, taking up and transforming material received from the blood, but bile is not

[1] BREMER, 1906

secreted at this stage, since no complete system of ducts has been demonstrated. In later stages the mass of anastomosing trabeculæ is converted into peculiar tubules of liver cells, drained by a system of ducts lined by clear cuboidal or columnar epithelial cells. These latter all empty into a single *hepatic duct*, which represents one of the original connections between the trabeculæ and the diverticulum. (In the otter there are said to be as many as seven persistent ducts.) The hepatic duct (Fig. 327, C) is joined by the cystic duct which comes from the tapering pyriform *gall bladder* (*vesica fellea*). After the hepatic duct has joined the cystic duct, the common bile duct (*ductus choledochus*) thus formed proceeds to the duodenum into which it opens, together with the pancreatic duct, at the *duodenal papilla*. The common bile duct is an elongated portion of the original hepatic diverticulum.

Development of the Veins of the Liver. The hepatic trabeculæ are always in close relation with the veins which are conveying nutriment to the heart. These are (1) the vitelline veins conveying nutriment from the yolk-sac, (2) the umbilical veins conveying nutriment from the placenta, and (3) the portal vein conveying absorbed food from the intestine. The liver also has important relations with the vena cava inferior.

The portal vein, which is the principal afferent vessel of the adult liver, is derived from the vitelline veins The latter, as they pass from the yolk-sac into the abdominal cavity, fuse with one another so as to form a single trunk (Fig. 327, B, *v.v.*). On reaching the duodenum, the trunk separates into its components, and they pass into the liver as the right and left vitelline veins. Before entering the liver they anastomose with one another dorsal to the duodenum, as shown in the figure. Thus, with the connections between the right and left veins within the liver, two complete venous rings are formed around the intestine. Because of the rotation of the stomach and duodenum certain portions of these rings offer a straighter pathway, and the rest degenerate. From the persistent portion branches grow out which are to be the superior mesenteric vein, receiving blood from the primary loop of the intestine, and the splenic vein, which not only drains the spleen but receives the inferior mesenteric vein and pancreatic and gastric branches. As the yolk-sac degenerates the main vitelline trunk disappears. From the junction of the splenic with the superior mesenteric veins as far as the liver the persistent vitelline channel is called the portal vein; its blood flows through the liver in the vitelline sinusoids.

The formation of the rings as above described takes place with great constancy, and apparently the only variations observed in their atrophy are the two cases described by Begg.[1]

The umbilical veins are at first a pair of vessels, but they early unite in the umbilical cord. The single vein thus formed brings the embryonic blood back to the body after its excursion to the placenta. On reaching the body, the vein divides into right and left vessels, which are contained in the ventral body wall, and at first pass through the lateral body walls to join the cardinal veins, by which their blood is carried to the heart. When the expansion of the liver has reached the ventral wall, its sinusoids anastomose

[1] Begg, 1912.

with branches of the umbilical veins through the ventral mesentery, and the umbilical blood uses and enlarges this new channel as a short-cut through the liver to the heart. The right umbilical vein then atrophies; in Fig. 328 it is smaller than the left. Gradually the left vein loses its connection with the cardinal and shifts to the median line. It then passes from the umbilical cord to the under surface of the liver along the free edge of the falciform ligament, where, after the umbilical cord has been severed, it degenerates to form the *round ligament of the liver*. This extends to the *porta* or entrance to the liver, where the portal vein goes in and the hepatic duct comes out. Beyond this point the umbilical vein may be followed as the *ductus venosus* in the embryo, or the ligament of the ductus venosus in the adult, to the vena cava inferior. The ductus venosus may be defined as the channel made by the umbilical vein in passing to the vena cava inferior across the under surface of the liver. It is sometimes completely enfolded by the hepatic trabeculæ, and it communicates with the hepatic sinusoids. It follows the line of attachment of the lesser omentum, and empties into the vena cava inferior.

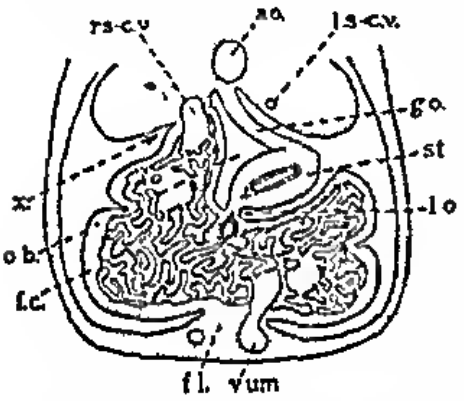


FIG. 328.—CROSS SECTION OF A MAMMALIAN EMBRYO, TO SHOW THE ADHESION, x, BETWEEN THE RIGHT LOBE OF THE LIVER AND THE DORSAL ABDOMINAL WALL.

ao, Aorta, f. c, fibrous capsule and serosa, f. l, falciform ligament, g o, greater omentum, l o, lesser omentum, l. s-c. v, left subcardinal vein, o. b, omental bursa, r. s-c. v., right subcardinal vein, st., stomach, v. um, left umbilical vein

The vena cava inferior apparently does not send much blood into the liver but passes along its dorsal surface. An essential part of this great vein, however, is formed from the hepatic sinusoids. Before the vena cava inferior has developed, the blood in the dorsal body wall flows to the heart through the posterior cardinal veins, one on each side of the aorta. Each posterior cardinal vein shows a ventral subdivision, the right and left subcardinal veins respectively, which are seen in section in Fig. 328. These two anastomose lower down. As shown in the figure, the stomach prevents the liver from approaching the dorsal body wall (at the root of the mesentery) on the left, but on the *right* there *is no such obstruction*, and the liver approaches and fuses with the body wall immediately in front of the right subcardinal vein, at 'x.' This fusion constitutes the coronary ligament; and, across it, the right subcardinal vein anastomoses with the hepatic sinusoids. By a rapid enlargement of this anastomosis, the trunk of the vena cava inferior is formed. It drains the posterior cardinal system of veins, and the outlet of the vitelline veins into the heart becomes the terminal portion of the inferior vena cava; the main vessel from the liver, the hepatic vein, is thereafter described as a branch of the vena cava inferior. The development of the posterior part of the vena cava inferior is described in connection with the Wolffian body (p. 454); a fuller account is given by F. T. Lewis,[1] McClure and Butler,[2] and Butler.[3] Occasionally the trunk of the vena cava is entirely surrounded by a band of hepatic tissue.

The Hepatic Artery. The liver in an embryo of 10 mm. has no arteries, but at that stage the hepatic artery can be followed to the porta. Later it extends through the connective tissue around the gall bladder, so that the cystic branch of the adult appears to be the main vessel in the young embryo. Still later, as the connective tissue which surrounds the struc-

[1] LEWIS, F. T., 1902. [2] MCCLURE AND BUTLER, 1925. [3] BUTLER, 1927.

tures at the porta gradually extends into the liver around the branches of the hepatic duct and portal vein, the hepatic artery sends branches in with it, and they form capillaries which empty into the adjacent portal sinusoids. Branches of the artery ramify also in the connective tissue capsule around the entire liver. The quantity of blood supplied to the liver by the artery always remains much smaller than that brought in by the portal vein, though the current in the small artery is much swifter than that in the vein. Pfuhl[1] describes thin-walled capillaries between end arterioles and sinusoids (not always present) to equalize the pressure from the two sources. There are no vessels between the hepatic cells other than the 'capilliform sinusoids' derived directly from the embryonic lacunæ of the vitelline veins.

Bile Canaliculi. Bile canaliculi are seen between the cells of the anastomosing liver cords in embryos of 10 mm. although they may occur earlier. Hendrickson,[2] using the Golgi method of impregnation saw the canaliculi richly developed in an embryo of 50 mm. and in one of 100 mm. they formed numerous polygonal meshes. Two views are commonly acknowledged as to the development of bile canaliculi, (1) that they arise independently through a confluence of globules between the cells, and (2) that they represent minute continuations of the lumen of the hepatic diverticulum.[3] Secretion of bile begins in the human embryo toward the end of the third month.

Microscopic Structure. A section of the embryonic liver, or of the human liver at birth, shows great areas of anastomosing trabeculæ, with intervening sinusoids, and occasionally a large vein. The trabeculæ are in reality the active end pieces of the gland, and are provided with a tiny lumen draining into the hepatic ducts. They seem passively floating in the blood stream, and the gradual assumption of adult characteristics in the liver is chiefly due to rearrangements of the blood channels according to well-known 'laws.' The sinusoidal net is altered by the ingrowth of connective tissue along the portal vein branches, coating the most advantageously placed sinusoids and turning them into veins, and cutting off their side branches. The same connective tissue encloses some of the neighboring cords, which then become ducts and lose their side branches.

Because of the sponge-like arrangement of the sinusoids and trabeculæ, this process does not isolate the side branches thus cut off, but merely changes their attachment and alters the direction of flow of their contents. A similar, but less massive, ingrowth of connective tissue gradually coats the hepatic veins and their branches. Between the terminal twigs of the portal vein and those of the hepatic vein run the sinusoids, just

[1] Pfuhl, 1932. [2] Hendrickson, 1898. [3] Bloom, 1926b.

as the capillary net connects the arterioles and venules in the ordinary circulation. One remarkable feature is that in all parts, even of the adult

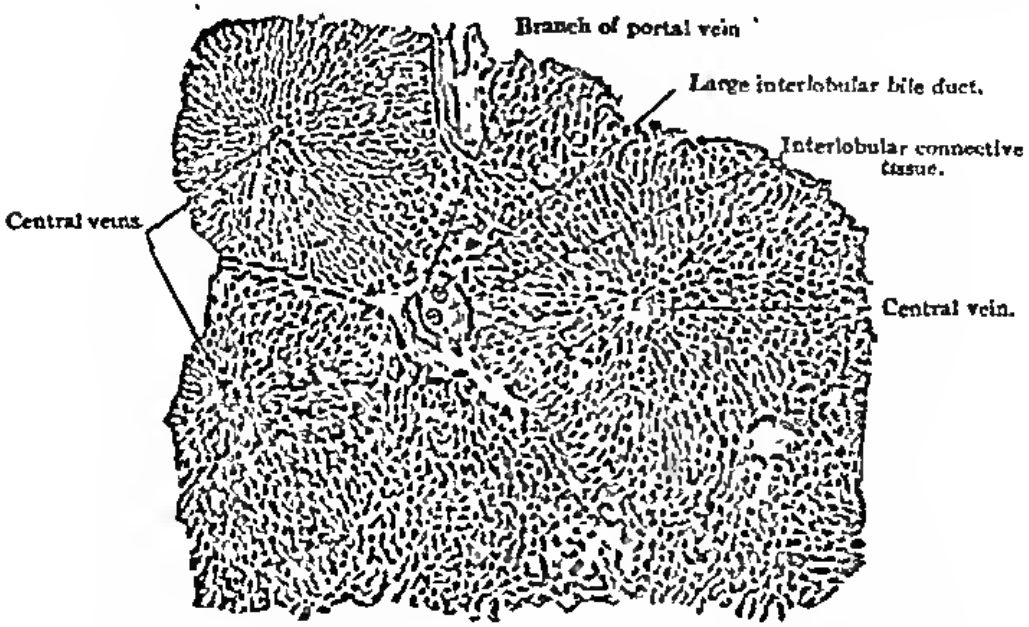


FIG. 329.—FROM A TANGENTIAL SECTION OF THE HUMAN LIVER. × 40.

The three central veins in cross section mark the centers of three lobules, which are not sharply separated, at the periphery, from their neighbors. Below and at the right the lobules are cut obliquely and their boundaries are not seen.

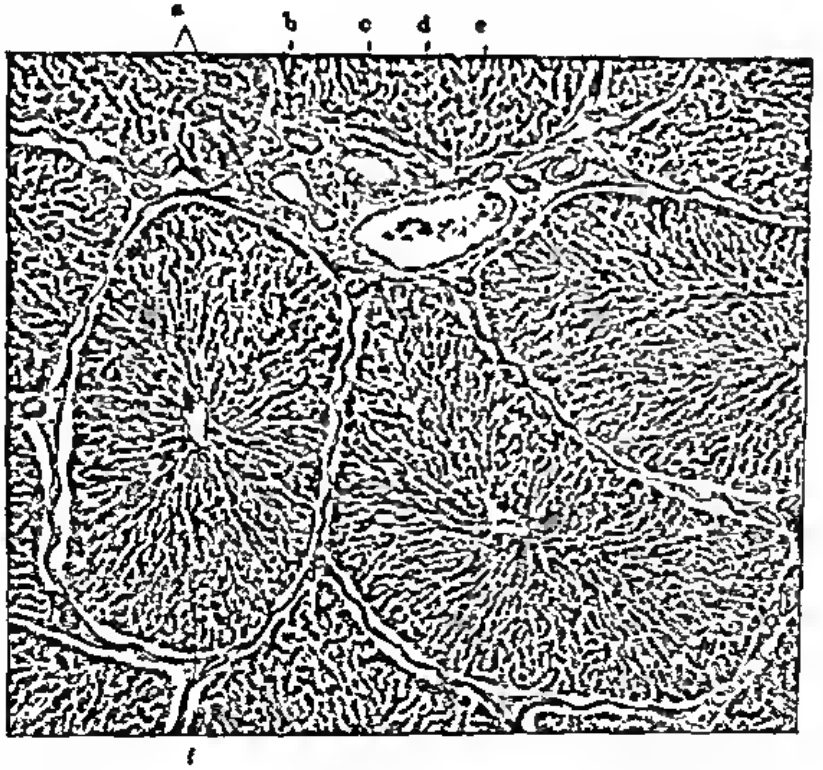


FIG. 330.—LIVER OF A PIG. (Radasch.)

The lobules have artificially shrunken from the interlobular tissue, a; b, bile duct; c, hepatic artery; d, interlobular vein (a branch of the portal), e, trabeculæ, f, central vein.

liver, the sinusoids are equidistant from the entrance of the portal vein and the exit of the hepatic vein, as measured along the blood channels. Two injection masses, forced simultaneously into the two veins, portal

and hepatic, will meet in all the sinusoids. Just as the terminal arteries and veins in the ordinary circulation interdigitate with each other, so in the liver the terminal branches of portal and hepatic veins interdigitate. As is the case with capillaries, the length of the sinusoids is governed by the rate of blood flow and the time necessary for the exchanges between blood and tissues. In the adult liver this averages about half a millimeter, though there is wide variation.

Hepatic Lobules. The structural or anatomical unit of the mammalian liver is called the *hepatic lobule.* These subdivisions were first recognized in the liver of the pig (Wepfer, 1664) and in 1666 Malpighi made the general statement that the entire liver is composed of a multiplicity of lobules. When a section of liver is observed under a low-power microscope, the cut lobules are marked out into irregular, polygonal areas by peripheral islands of connective tissue which are at quite uniform distances from one another. These islands were named *portal canals* by Kiernan (Trans. Roy. Soc. London, 1833, pp. 711–770) and they represent in sections the cut ends of the connective tissue sheath—the *capsula fibrosa* or Glisson's capsule—which has grown in from the hepatic portal around branches of the portal vein, hepatic artery and bile duct. In the normal human liver the lobules are indistinctly defined, but under pathological conditions they may become circumscribed by extensions of the capsule spreading from one portal canal to another. This separation of lobules by a seam of connective tissue is present as a normal feature in the liver of the pig (bear, camel and several other mammals[1]). A variety of shapes are displayed by isolated lobules (acid maceration) and by wax-plate reconstructions of them; while single lobules are found most frequently, double and multiple ones occur forming lobular complexes. The widths of the individual lobules in the adult human liver generally measure between one and two millimeters (Pfuhl gave the average as 1180 μ).

FIG 331.—ISOLATED LOBULES FROM THE LIVER OF A PIG

Formaldehyde fixation, maceration in equal parts concentrated hydrochloric acid and water. Drawn under a hand lens.

Blood Vessels of the Liver. The portal vein conveys the blood from the stomach, intestines (except the lower part of the rectum), spleen and pancreas into the liver at the porta hepatis. In its course within the organ the vein becomes divided through numerous and successive branchings into small vessels seen lying in the portal canals. These small branches in turn give rise to fine interlobular veins (*rami septales*) and precapillary venules which enter into the sinusoids of the lobules. The

[1] PFUHL, 1922.

sinusoids are small (9 to 12 μ wide) tortuous vessels which converge radially between the hepatic *trabeculæ* toward the center of each lobule, where by confluence they form a central or intralobular vein. Occasionally there are two central veins side by side. Each central vein empties at right angles into a *collecting* vein (sublobular vein of Kiernan) which joins other and similar veins and finally makes up two or more hepatic veins opening into the vena cava inferior.

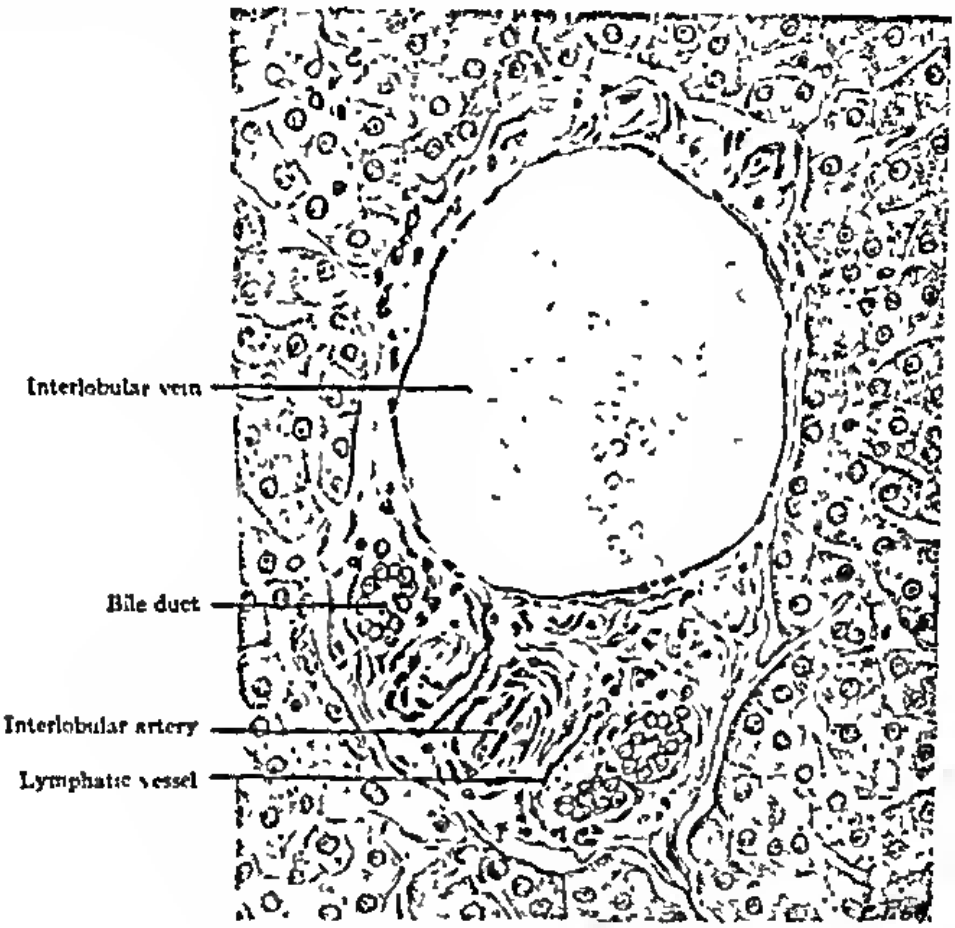


FIG. 332.—A SECTION THROUGH A PORTAL CANAL OF THE HUMAN LIVER. Zenker fixation, hæmatoxylin and eosin.

The portal vein in its course through the liver is accompanied by the hepatic artery, but the union of the smallest branches of the artery with the sinusoids cannot be made out as clearly as with the vein. The artery is said to empty in three ways: (1) directly into the sinusoids at the borders of the lobules, (2) through capillary connections between the interlobular arterioles and venules and (3) by arterio-venous anastomoses between vessels of the same type. Arterio-venous anastomoses have been stated to occur less frequently in the mammalian than in the frog's liver.

Approximately 75 per cent. of the blood to the liver enters through the portal vein under relatively low pressure and the rest through the hepatic artery under much higher pressure. A change in pressure alters the flow, with some sinusoids open and others closed. Wakim and Mann

(1942) have called this the 'storage and non-storage phase' of the sinusoids. When the blood flows into the liver faster than it leaves, the sinusoids become engorged and the capacity of the organ exceeds greatly the normal—it has been estimated that as much as 61 per cent. of the total blood of the body may be stored in the liver of a dog in anaphylactic shock. In the frog sphincters have been described controlling the inflow and outflow of blood of the sinusoids permitting storage and release.

The wall of the portal vein contains two layers of smooth muscle, an inner circular and an outer longitudinal. In the middle-size and smaller branches the longitudinal layer disappears first and the circular layer becomes gradually reduced in thickness. When the smallest branches, rami septales, are reached the veins are no more than endothelial tubes surrounded by thin strands of muscle, which disappear in the precapillary venules. The adventitia of the portal vein and its branchings is composed of loose collagenous connective tissue intermingled with elastic tissue—these become thinner in the smaller branches. There are marked species differences in the development of the musculature in the portal vein; in large animals as horses, cattle and elephants, besides in smaller animals like the dog and some rodents the amount of muscle is relatively richer than in man. A very weak musculature is present in the portal vein of the bear.

The central veins vary in diameter (27 to 70 μ, v. Ebner[1]) and according to Pfuhl those of 40 to 50 μ are met with most frequently. They resemble sinusoids more than veins, having in man an endothelial lining with no more than spirally arranged connective tissue fibers around it. Elastic tissue fibers are found only in the larger central veins and in these they occur sparsely. No smooth muscle is present in the central veins of man, but scattered muscle cells have been described in the larger veins of the dog. The smaller collecting veins are not only larger (90 to 200 μ in diameter) but have thicker walls than the central veins—subendothelial connective tissue tends to form two zones with circular and longitudinal fibers. Elastic elements are delicate, numerous and irregularly arranged in nets throughout the walls. Only in the larger collecting veins, however, are the two zones clearly delimited into inner circular and a less orderly arranged outer longitudinal layer. Smooth muscle is subject to great individual variability in man, seldom being seen in veins of less than 0.5 mm. in diameter. Nutrient arteries—*vasa vasorum*—are observed only in the thick walls of the largest tributaries of the hepatic veins.

The efferent veins of the liver in the dog are peculiar, in that smooth muscle cells are present throughout, being scattered in the walls of the

[1] v. Ebner, 1902.

central veins and forming highly developed spirals, which cause the walls to project into the lumen in the larger collecting veins. These formations have been named 'spiral valves' or 'throttle veins.' (Arey 1941, after examining the livers of more than thirty species of mammals found throttle veins only in the dog, raccoon and seal. They were stated absent in wild dogs, *e.g.* the bush dog and the Cape-hunting dog.) The spasmodic closure of these atypical veins has been given as an attributing factor producing the passive congestion in the liver of the dog in anaphylactic and peptone shocks.

The hepatic artery has in its wall circularly disposed smooth muscles with a layer of longitudinal muscle between them and the endothelium.

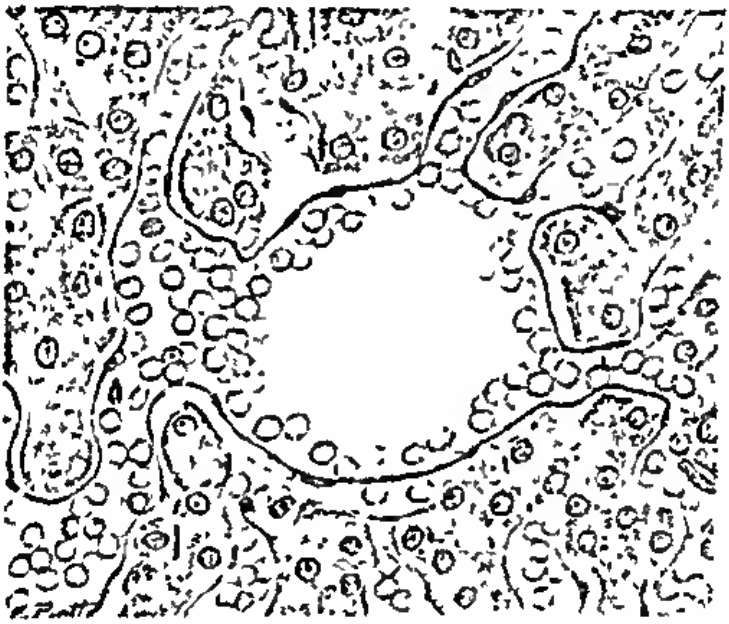


FIG. 333.—A CENTRAL VEIN WITH SINUSOIDS OPENING INTO IT. HUMAN LIVER. Zenker fixation. haematoxylin and eosin.

As the artery becomes smaller these muscle layers become thinner, until in the precapillary branches the vessel consists of only endothelium surrounded by a sparse connective tissue adventitia.

Hepatic Sinusoids. Charles S. Minot (1900) introduced the name '*sinusoids*' for the capilliform spaces within the lobules of the liver and in several other organs. They differ from capillaries in having a different developmental history and in the structure and relations of their lining. The development of sinusoids has been discussed in the section on blood vessels on pp. 218–219. The lining lies close to the hepatic parenchyma with little or no connective tissue between them. This is an essential characteristic and the small amount of connective tissue when present arises secondarily and forms a latticework of reticular fibers. The lining cells, often termed endothelial are unlike those of the ordinary blood capillaries in that the outlines of the individual cells cannot be made out.

Some histologists have regarded the lining as a syncytium, from which it would be inferred that spaces exist between the nucleated protoplasmic areas. Others think of it more as a plasmodium or nucleated cell territory not marked out into cells. Probably the true structural nature of the lining will not be known until parts of it have been stripped off as by micro-dissection and studied as a sheet.

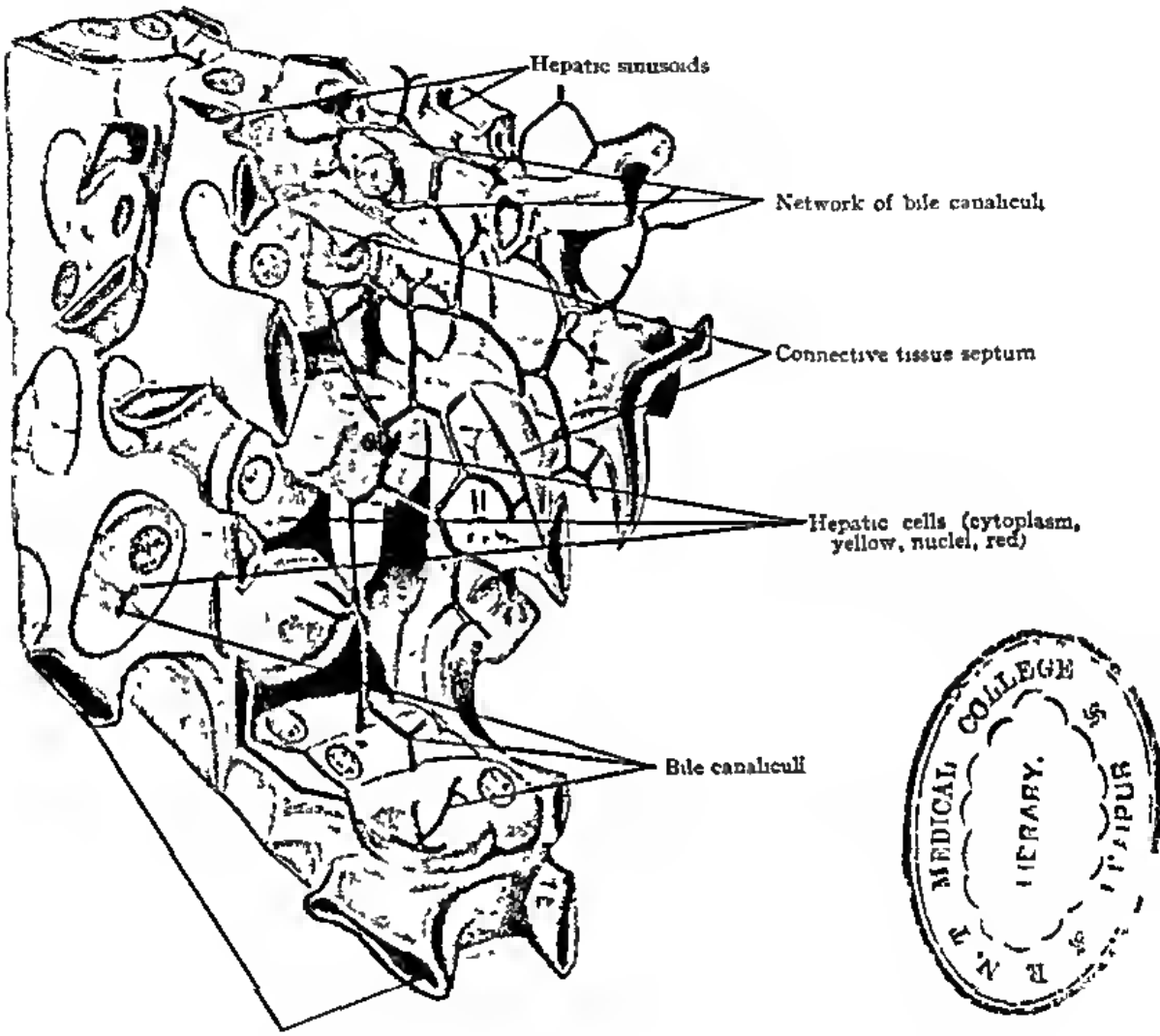


FIG. 334.—A RECONSTRUCTION OF SINUSOIDS IN THE LIVER.
These are represented in blue, the bile canaliculi in green and the hepatic cells in yellow with red nuclei (Braus)

The lining cells are extremely thin with dark-staining elongated oval nuclei. Various transitions occur between them and the larger phagocytic *stellate* or Kupffer[1] cells, which hang into the sinusoidal lumens. These stellate cells are included in the system of macrophages or histiocytes and on becoming loosened float free in the blood stream. In sections of liver, the Kupffer cells often contain remnants of phagocytosed red blood corpuscles and particulate matter. When suspensions of colloidal

[1] KUPFFER, 1876 AND 1899.

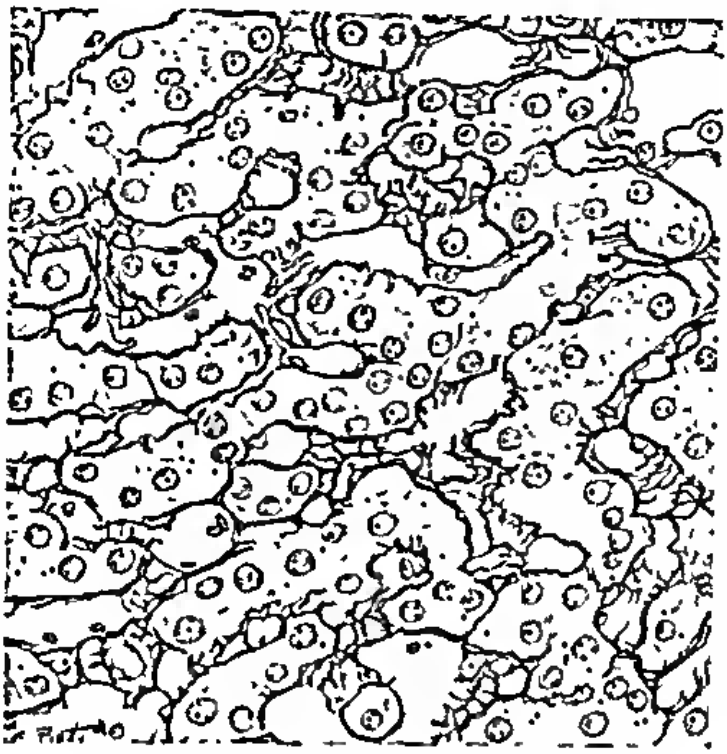


Fig. 335.—Argyrophil Reticulum in the Human Liver. Alcohol fixation, Pap's method and hæmatoxylin.

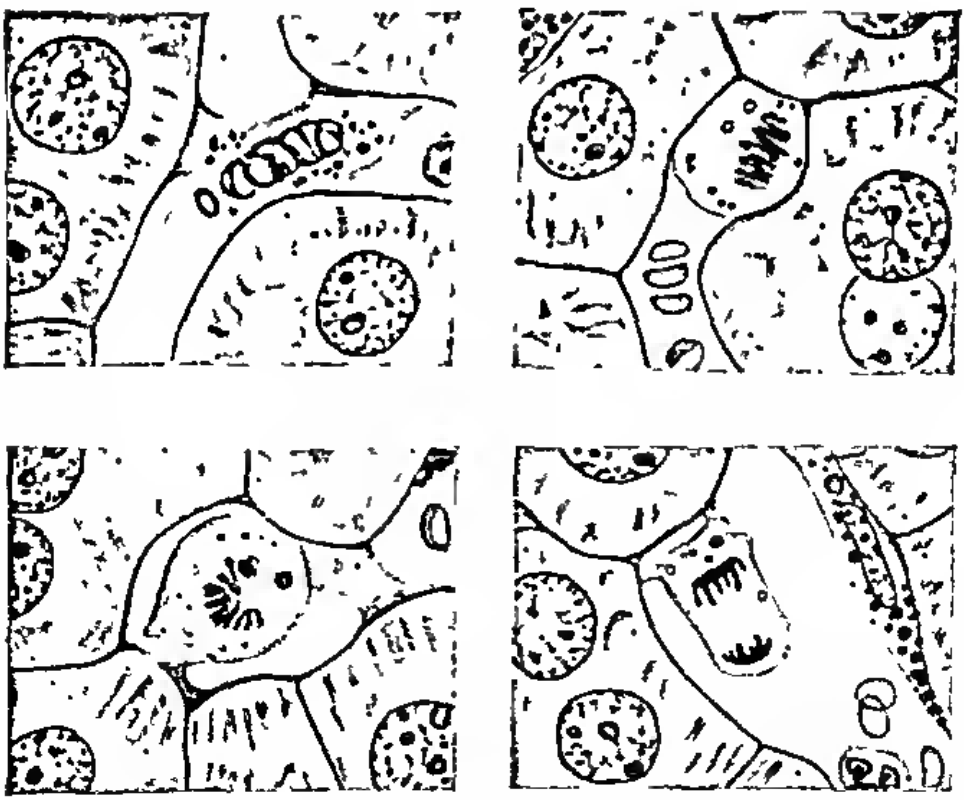


Fig. 336.—Trypan Blue Storage and Mitotic Division of Stellate Cells in the Liver of a Mouse. (Ortiz Picon.)

metals and dyes, or particles, as carmine, carbon (India ink) and saccharated oxide of iron are injected into an animal, the Kupffer cells take up and store the particles. The behavior of the Kupffer cells is not the same toward all particles—a selection is exhibited, in that some particles are taken up more readily than others. The phagocytic activity is also quite variable among different species of vertebrates. Particle

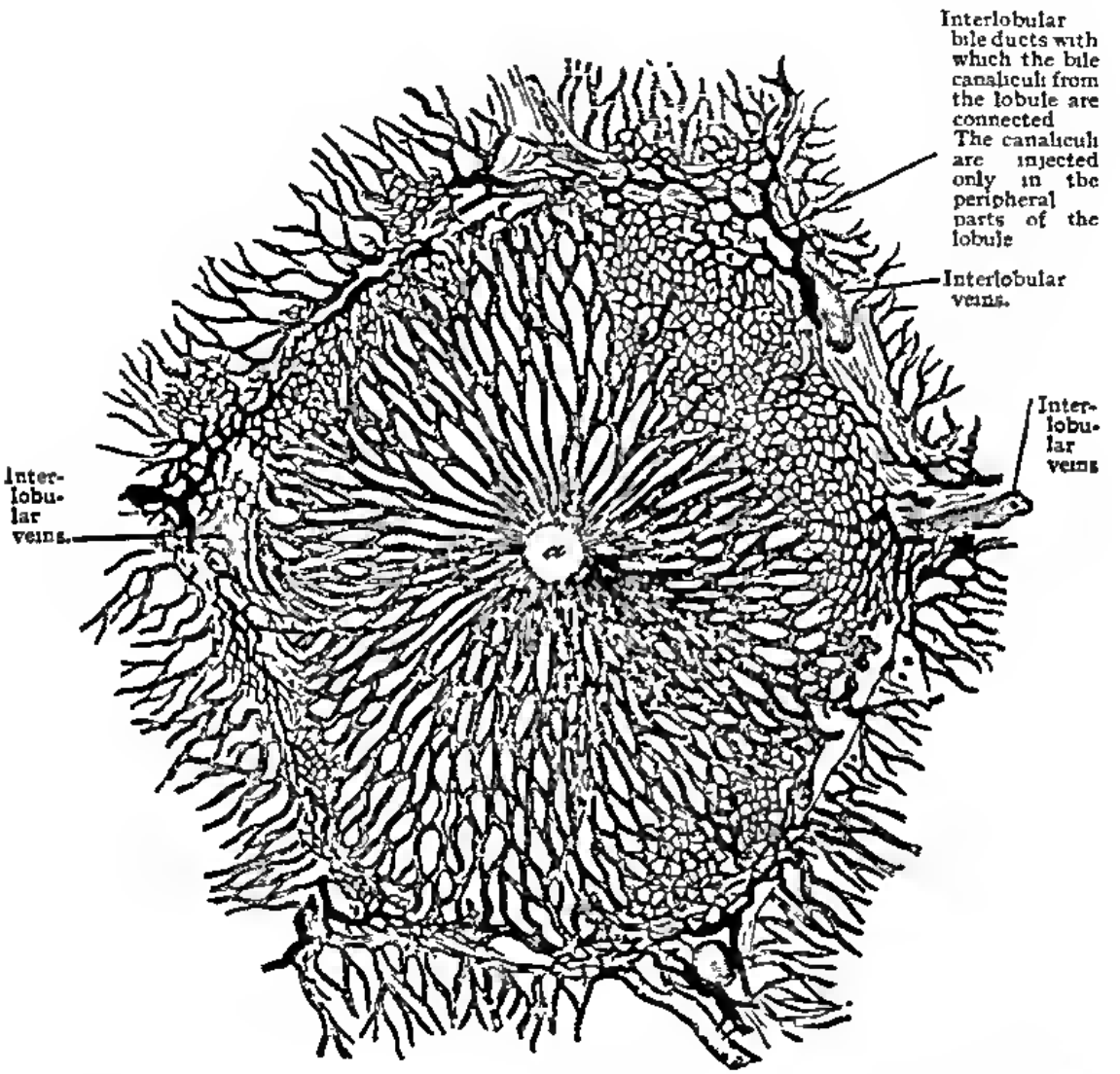


FIG. 337.—A SECTION OF A LIVER LOBULE WITH THE BLOOD VESSELS AND BILE DUCTS INJECTED. (Schafer, after Cadiat.) *a*, central vein.

laden Kupffer cells either become free macrophages or the phagocytosed particles are transferred to the cells of the hepatic parenchyma and eliminated into the bile. When excessive amounts of dyes and particulate matter are injected, the lining cells are stimulated to produce more and more stellate cells, which on becoming free appear in the blood stream in 'showers of macrophages.' The most highly developed transitional cells take their place, while the regenerating lining cells fill up the deficiencies. The Kupffer cells divide by mitosis. Free stellate cells are

typical histiocytes, showing various forms and sizes in the same and in different animals.

The lobule of the liver differs from the vascular unit of other glands, as described on p. 290, in that the flow of blood is centripetal and of secretion of bile centrifugal. Attempts have been made to recognize a structural unit in the liver, of which a terminal branch of the portal canal should be considered the center and 'central' veins the boundaries. Both blood and secretion would then flow 'correctly.'

In comparing the lobule of the liver with the structural unit of other glands one must keep in mind two characteristics of the liver in its development. In other compound secreting glands the original diverticulum divides repeatedly, the smaller branches spreading in all directions and remaining distinct from each other, even to the final end pieces, which end blindly. The liver diverticulum sends many branches from its upper or oral side (Fig. 327, B), and these branches anastomose with each other to form the network of hepatic cords. The anastomoses allow frequent rearrangements in the connections of the cords (which are really elongated secreting tubules) as has been explained. Since

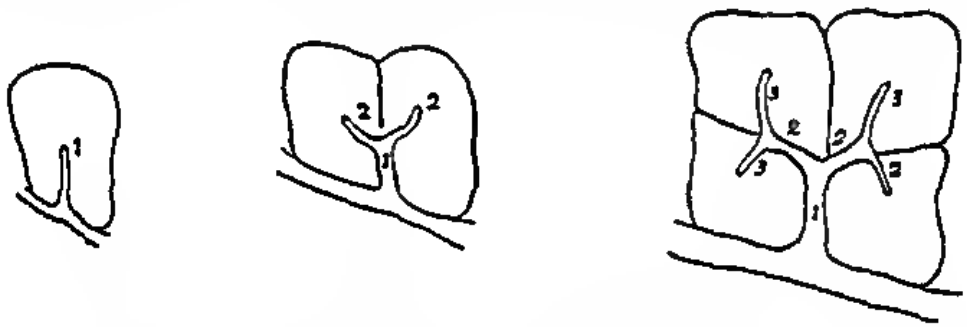


Fig. 338.—Diagram Showing Method of Increase in Number of Lobules of Liver. (Johnson.)

they are enclosed in the same connective tissue, two or more terminal ducts accompany one terminal portal branch. Each duct has branches, the liver cords, from only one side.

Branching glands tend to spread out in fanshape, but an anastomosing gland (if we consider only the connection between the branches of a single terminal duct) tends to come to a point. The flow of the blood from the portal vein would elongate the meshes of the network of trabeculæ. Four or more such groups of trabeculæ as are shown, arising from separate terminal ducts, would form a lobule. Anastomoses between adjacent trabeculæ would occur, and the only terminal ends would be at the center. Frequently the cords form loops instead of ending blindly. The terminal branch of the hepatic vein now seems central, since all the trabeculæ radiate from it. Such structures form the *lobules of the liver*. They are not vascular units, and so differ from those of most other glands.

The development of the liver lobules occurs only after birth, and is said[1] to be due to the suction in the hepatic vein caused by the expansion of the chest wall. The current in this vein is thus intermittently more rapid than that in the portal vein and hepatic artery combined, and the same forces which mould ordinary vascular units radially around the swiftly flowing arterial stream act in the reverse manner radially toward the more active venous outflow. The lobules when first recognizable in infants are smaller than those of the adult.

With the growth of the liver the lobules are continually rearranged. When the cords elongate to such an extent that the sinusoids between them become longer than is

[1] Pfuhl, 1932

necessary for proper function, the central vein divides, or rather two of its branch sinusoids become central veins, making two daughter lobules. At the periphery new terminal portal branches and ducts are formed by the encroachment of the connective tissue. The process has been described by Mall[1] and by Johnson[2] and is shown in Fig. 338.

In some animals, *e.g.* pig, the connective tissue which accompanies the portal vein and branches of the bile duct increases normally until each lobule is surrounded by a definite capsule. The lobules of the pig can therefore be isolated.[3] They are short cylinders of varying shapes and sizes, ranging from 0.5 mm. to 2.0 mm. in diameter, and averaging 702,000 in number in the pig's liver.

Parenchyma. The parenchyma or essential tissue of the liver is found in the anastomosing trabeculæ of the lobules. In presenting characteristics exhibited by most glands, the cells are arranged with at least one surface in contact with a lumen, a bile canaliculus and another to a blood vessel, a hepatic sinusoid.

Hepatic cells isolated from pieces of fresh liver tend to be more or less rounded, but after fixation, in sections they display four, five, six or more surfaces. Each cell contains one, sometimes two and occasionally more rounded nuclei. Cell sizes vary in the same and in different species—in man the diameters average between 18 and 26 μ (the extremes 13 to 35 μ).

The nuclei, like the cells vary in size, normally and after various technical procedures. Measurements of the diameters of nuclei made on sections of human liver are given in the table below.

Celloidin embedding.....	6–9 μ, average 7.45 μ. Clara, 1930a.
Paraffin embedding	4.4–11.25 μ, average 6.25 μ. Jakobj, 1935.
Frozen unembedded.....	5.4–12.5 μ, average 7.32 μ. Michaelis, 1938.

It is not uncommon to find nuclei of different sizes in binucleate and multinucleate cells, also a large nucleus in a large hepatic cell. Binucleate and multinucleate cells occur with very different frequencies in the same and in different animals. They are found but seldom in the livers of animals with cold blood. Among mammals they are seen most often in rodents—fully one-half or more of the hepatic cells of rabbits contain more than one nucleus. Other animals, as the ungulates, the horse and cat have fewer hepatic cells with two nuclei. This binucleate and multinucleate condition in the liver has been variously interpreted—functional changes, delayed completion of mitosis, and amitosis.

The nuclear membrane is thin, yet distinct and often through shrinkage of a nucleus it becomes wrinkled or indented. It is capable of considerable stretching, as seen in the livers of dogs and other canidæ containing intranuclear crystals, the lengths of which may exceed the

[1] Mall, 1906. [2] Johnson, 1919. [3] Johnson, 1918.

normal diameters of the nuclei several times[1]. The nuclear reticulum is delicate and the scant chromatic substance is condensed in block-like masses on the inner surface of the nuclear membrane and at nodal points on the reticulum. Each nucleus contains one or two oxyphilic nucleoli, which average about 1 μ in diameter.

In counts of 4000 nuclei, 1000 in sections of liver from each of four dogs ranging in age from 1 day to 10 years and 3 months old, 3658 or 91.45% contain but 1 nucleolus; 283–2; 52–3; and only 7 have 4 nucleoli No deduction is made as to relationship between age and number of nucleoli, because of the unappreciable differences in the individual counts. These true nucleoli vary in diameter from 1 to 2.5 μ and generally the largest are seen in nuclei containing a single nucleolus.[2]

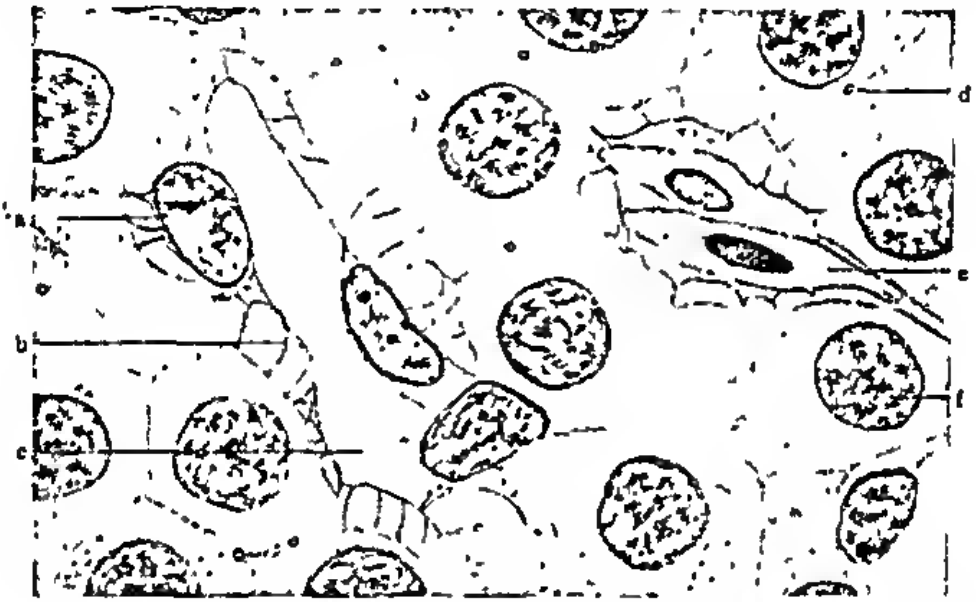


Fig 339.—Section of the Liver of a Salamander (Necturus). × 380.
a, Endothelial cell, b, endothelial reticulum c, blood vessel, d, bile capillary between liver cells, e, red corpuscle, f, nucleus of hepatic cell

Besides the nucleoli, the nuclei in the hepatic cells of some animals (with cold blood and mammals) often contain a larger oxyphil body surrounded by a clear area; at times this inclusion is so large as to crowd the other nuclear structures into a narrow seam next to the nuclear membrane. The significance of these bodies is debatable, as they occur both in the livers of animals with and without evidences of infection. Other inclusions, as neutral fat droplets and pigment granules giving an iron reaction have been observed in normal nuclei.

The cytoplasm of the hepatic cell, in the fresh unfixed state has a yellowish color and contains strongly refractive granules. Fixation may cause various appearances to be displayed, a fine net or foam-like structure enclosing fat droplets, granules of glycogen, secretion and pigment, irregular masses (derived from precipitated protein) and the mito-

[1] Weatherford, 1938 and 1939. [2] Weatherford and Tribble, 1940.

chondria. Near the periphery of a lobule the cells may contain fat droplets of varying size, found normally in well-nourished individuals. Pathologically the droplets may be large and widely distributed. In some animals as the cat, the infiltration of fat may be so great after a heavy meal that the hepatic cells bear a superficial resemblance to adipose tissue cells. Glycogen occurs in granules, larger angular masses and droplets, especially after abundant meals. In ordinary histological specimens both fat and glycogen have been dissolved, leaving vacuoles; those

FIG 340.—HEPATIC CELLS

a, human liver showing bile pigment (yellow) Formaldehyde fixation, hæmatoxylin and eosin; b, rabbit liver showing the storage of cholesterol-esters (animal fed cholesterol) Formaldehyde fixation; hæmatoxylin and Sudan III; c, human liver showing glycogen storage Absolute alcohol fixation, hæmatoxylin and Best's carmine

caused by glycogen may sometimes be distinguished by their irregular shape. The hepatic cells are subject to marked changes corresponding to the different functional states of the organ. During the assimilation of food, the cells are swollen and contain large droplets of fat and stores of glycogen and of protein. The intercellular bile canaliculi are small and inconspicuous. In the fasting condition, the cells appear proportionally smaller with little fat and glycogen, but contain dark-staining granules of the antecedents of bile. The bile canaliculi are now distended with secretion. In man it is unusual to find the extreme of either of

these stages, but in the rabbit Forsgren[1] observes rhythmic phases during which assimilation and secretion alternate. At the height of the assimilatory phase, the liver of a 2 kg., rabbit weighs 144 grams and contains 13 per cent. glycogen, while at the height of the *secretory* phase, the weight is but 50 grams and less than 1 per cent. is extracted as glycogen. Transitional phases are seen with storage at the center of the lobule and secretion at the periphery.

Hepatic cells are rich in mitochondria occurring as filaments, rods, and spherical granules. Because of the number of functions of the liver, the associated increase and decrease in water content and the ease with which liver cells are injured there is a constant change from one form of

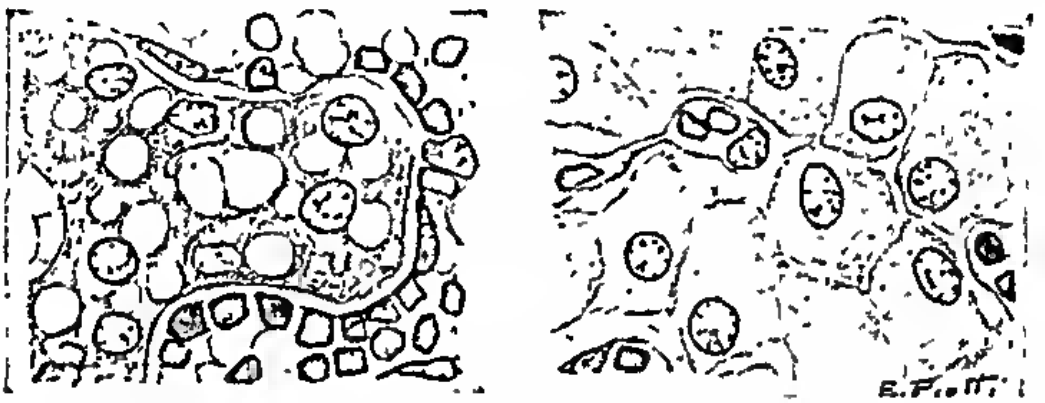


FIG. 341.—CELLS FROM TWO HUMAN LIVERS, IN STORING AND SECRETING PHASES. FAT AND GLYCOGEN FILL THE CELLS IN ONE CASE, IN THE OTHER, SECRETORY GRANULES AND BILE CANALICULI ARE EVIDENT.

mitochondria to another. These changes may be produced experimentally (Fig. 8). At the periphery of the lobules there is often seen a row or two of hepatic cells darker than the majority of cells further in the lobules.[2] These so called 'dark cells' contain an abundance of long filamentous mitochondria and have been spoken of as 'mitochondria rich' cells. They are probably no different from other hepatic cells except for being better nourished because of their position in the lobules.

A Golgi apparatus may be displayed in hepatic cells. It is easier to impregnate it in the livers of amphibians than in mammals: probably the storage and hydrolysis of glycogen causes changes in the mammalian liver altering the rates of diffusion and the concentration of reducing substances at interfaces in the cells. Ludford[3] obtains impregnations of a Golgi apparatus in the hepatic cells of mice, rats and Guinea pigs after experimentally rendering the cells more or less glycogen-free. The apparatus forms a net on the side of a nucleus toward a bile canaliculus; during the secretion of bile it extends from the nucleus to the canaliculus and if the cell is in relation to more than one canaliculus, the apparatus is divided correspondingly. Vital dyes and particulate matter

[1] FORSGREN, 1929. [2] RUSJANZEV, 1927. [3] LUDFORD, 1928.

are stored near the apparatus, but usually only after several injections. Deposits of reduced silver and osmium on the surfaces of pigment granules and on droplets of secretion have often been interpreted as a Golgi apparatus. The pigments derived from this cellular destruction have been rather appropriately termed 'wear and tear' pigments and they can be stained by fat soluble dyes as Sudan III.

The liver possesses a high regenerative capacity. Large areas of the organ may become injured or destroyed and be quickly repaired through division of the unimpaired cells. Experimentally as much as three-

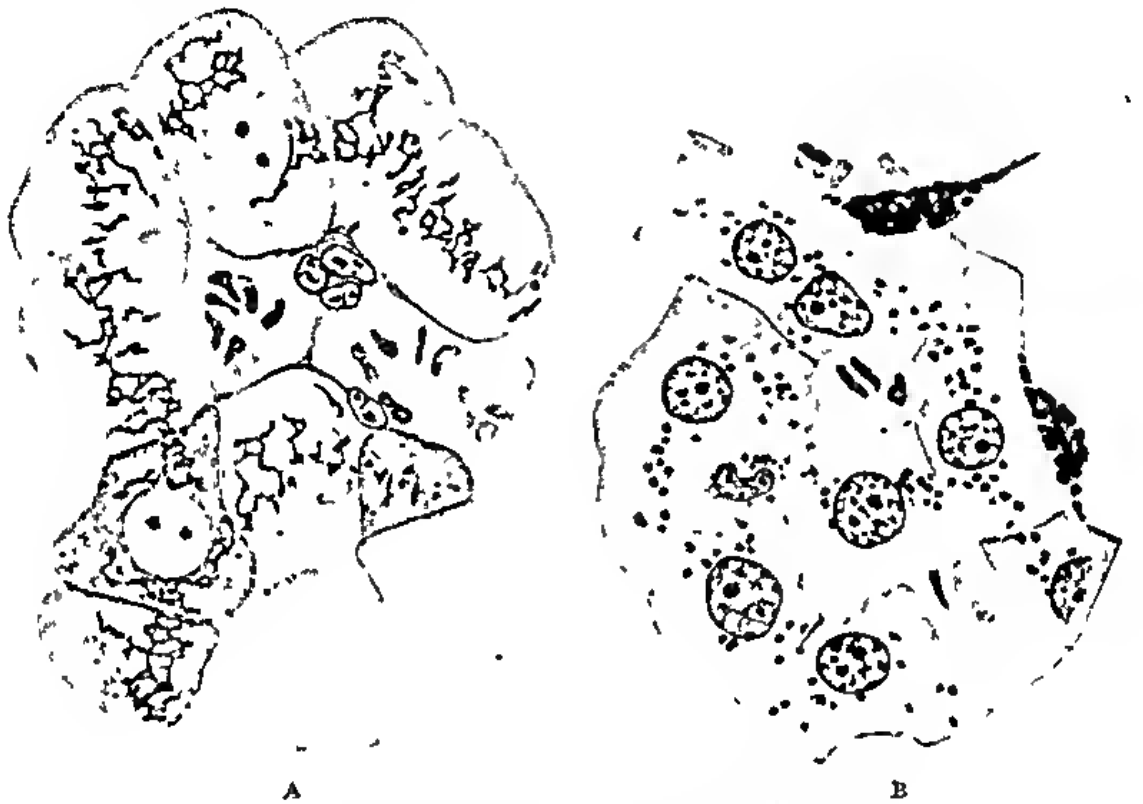


FIG 342.—LIVER CELLS OF THE WHITE MOUSE.
A, Golgi apparatus, B, storage of granules of trypan blue. (Nassonov)

fourths to seven-eighths of the liver may be removed and within the course of a few weeks the organ has been restored to its original size. Mitosis, rarely seen under normal circumstances become numerous in two or three days after operation and lead to the restoration. It has been much debated whether amitosis occurs in hepatic cells or not. Nuclear constrictions and the presence of binucleate cells are not a valid enough criteron for amitoses.

Biliary Passages. In man and the higher vertebrates (birds and mammals) the bile canaliculi begin as cuticula-lined spaces between the cells of the hepatic parenchyma. They form a continous network of minute tubules which may be observed in specially stained, impregnated and injected preparations. At the periphery of each lobule the cana-

liculi connect with the bile ducts in the interlobular septa and in the portal canals. The union between a bile canaliculus and a bile duct is not abrupt but the two are separated by a short connecting piece (canal of Hering[1] or connecting piece of Clara[2]) comparable to an intercalated duct in a salivary gland. Only three or four cuboidal epithelial cells surround the first part of each connecting piece, the cells becoming more numerous and taller as the duct is reached. The cytoplasm of the cells of the connecting piece is clear and homogeneous and the nuclei smaller and darker staining than in the hepatic cells. In the bile ducts the cells are columnar the heights varying in different animals and each cell contains a round to oval nucleus toward its base. Near the surface secretion granules often appear, which may lead to droplet formation. The mode of discharge of the secretion is apocrine. Goblet cells have been observed in the epithelium of the bile ducts in some animals, being seen frequently in the Guinea-pig. Fatty droplets have been described also, they have been stated to occur regularly in the epithelial cells of carnivora and to be rich in the rabbit after the feeding of fat. The bile ducts are embedded in collagenous connective tissue, the fibers running partly circularly and partly longitudinally with fine elastic tissue fibers intermingled. Smooth muscle occurs in the walls of the larger ducts near the hepatic portal in some animals.

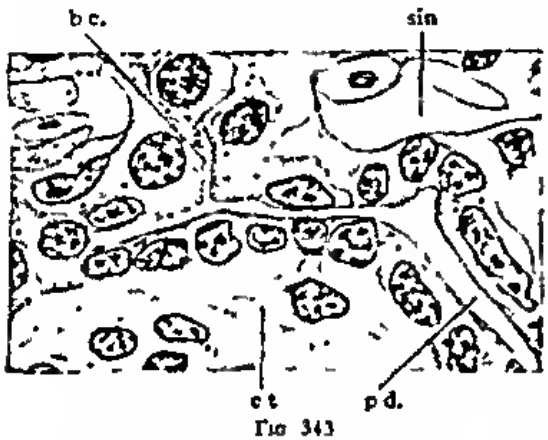


Fig. 343

Entrance of bile capillary, b. c., into periportal duct, p. d.; sin., sinusoid, c. t., connective tissue of portal canal.—Nechamus.

The *hepatic, cystic* and *common bile ducts* all have simple columnar epithelium, with occasional globlet cells and branching mucous glands. Around the hepatic duct there is a wide zone formed by the ramifying ducts of these mucous glands, as they extend into the surrounding connective tissue. The connective tissue layer is said to contain many elastic fibers. It is followed by a tunica muscularis consisting chiefly of circular fibers. These form a sphincter around the common bile duct, at the duodenal papilla. In the cystic duct there are folds of mucous membrane, containing muscle fibers, and forming the 'spiral valve.'

Lymphatics. The liver is provided with abundant superficial and deep lymphatic vessels. The superficial vessels form loose plexuses in the subserous covering of the organ which make connections with the deep lymphatic vessels leaving at the hilum and in the walls of the hepatic veins. They drain superiorly to the diaphragmatic and inferior vena

[1] Hering, 1871. [2] Clara, 1930b.

caval groups of nodes and inferiorly to the hepatic nodes. In the human liver, these superficial plexuses are better developed than in mammals having thinner external capsules.

The deep lymphatic vessels are present in two groups. Fine capillary plexuses surround the lobules and in the portal canals communicate with extensive networks in Glisson's capsule and with plexuses in the walls of the tributaries (collecting veins) of the hepatic veins. In the capsule, the networks encircle the branches of the portal vein, hepatic artery and the bile duct and connect with plexuses in the walls of the larger portal branches. These periportal lymphatic vessels on leaving the

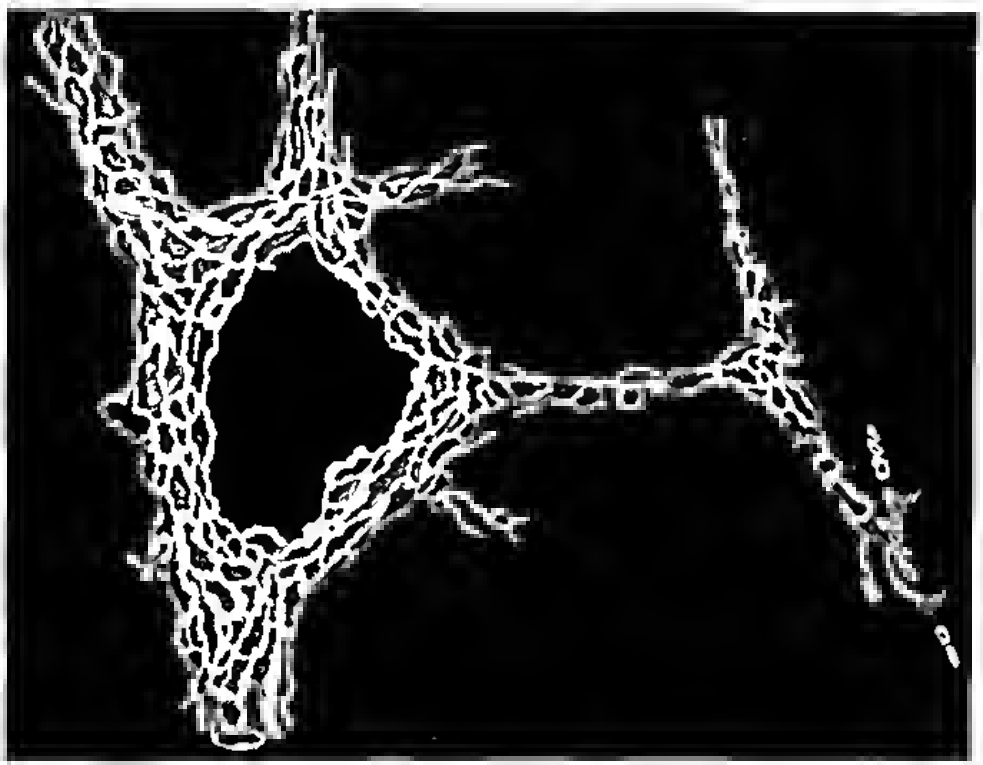


FIG. 344.—A DEEP NETWORK OF LYMPHATIC VESSELS SURROUNDING BRANCHES OF THE PORTAL VEIN AND CONNECTING WITH THE SUPERFICIAL LYMPHATICS. × 50 (Teichmann.)

hilum enter the hepatic nodes. Smaller networks of vessels in man surround the large collecting veins; but in the dog they are more extensive, being present in the thickened walls of the larger central veins and in the collecting veins. They drain to the group of lymph nodes around the inferior vena cava, either above or below the diaphragm.

There has been a prolonged discussion whether or not lymphatic spaces exist within the hepatic lobules. Attempts to fill any such potential spaces from injections into the capsule of the liver, the portal vein or the bile duct have led to conflicting results. Pressures sufficient to fill the vessels usually lead to extravasations of the injected fluid between the sinusoidal lining and the hepatic cells. Lee (1923) obtained a retrograde injection of the lymphatic vessels in the liver of a live cat, by ticing off

the thoracic duct and injecting pilocarpine intra-abdominally. After three days, the lymphatic vessels were filled to their finest terminals in the border sheath between the lobules; but *no* lymphatic vessels or spaces were ascertained within the lobules.

Nerves. The liver possesses a rich innervation derived from the sympathetic and vagus nerves which enter the organ at the hilum. In ordinary histological preparations a few bundles of non-medullated nerve fibers may be seen, particularly in the larger portal canals; but in special silver and methylene blue preparations, small groups of ganglion cells and interlacing plexuses are observed around and supplying the

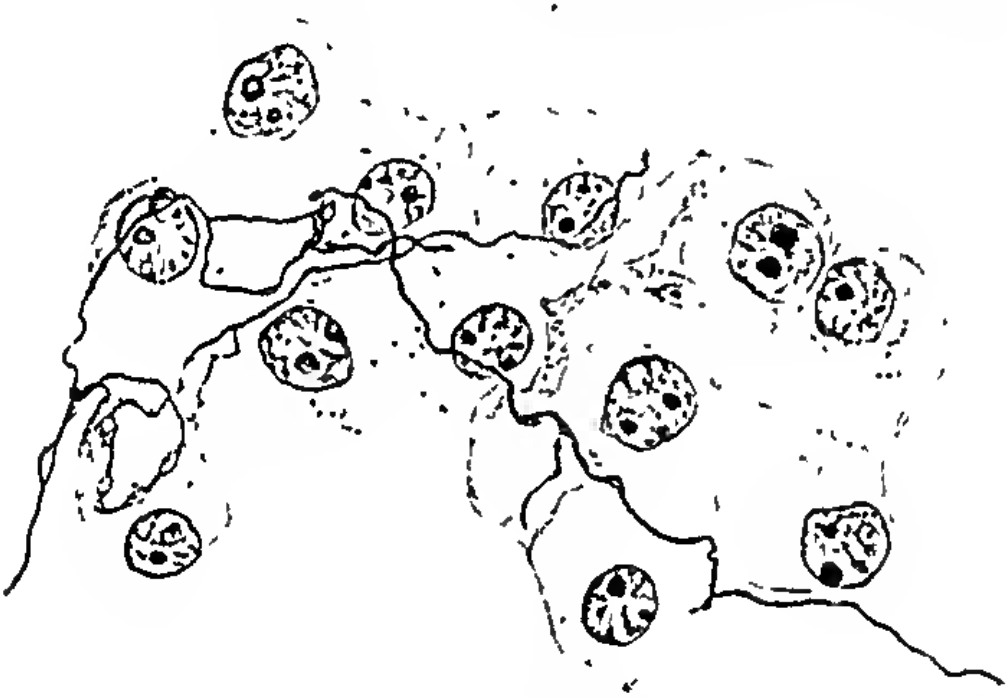


Fig. 345.—Non-medullated Nerve Fibers between the Hepatic Cells in the Human Liver. Bielschowsky's Method (Riegele).

branches of the vein, artery and bile duct in Glissons's capsule and its extensions. Thin varicose nerve fibers penetrate the lobules and are found everywhere between the parenchymal cells.[1] Several histologists have described also, intracellular endings even terminating within the nuclei, but the presence of such intra-cellular nerves has been emphatically denied by others as an erroneous interpretation of structures lying at different levels in the field.[2]

Gall Bladder. The *gall bladder* is a pear-shaped muscular sac lined by a simple columnar epithelium which rests on a connective tissue layer rich in blood vessels. This mucosa is ordinarily thrown into deep folds. Outside the mucosa is a muscularis of scattered smooth muscle and a highly developed serosa. The epithelial cells are tall and narrow with

[1] Riegele, 1928. [2] Wolff, M., 1905.

basal nuclei either oval or occasionally spherical. They show secretory granules in the upper part of the cells, and small masses of secretion, probably mucoid, may be seen breaking through the top plates. The secretion is apparently continuous, as different phases are evident in neighboring cells (Fig. 346). Terminal bars have been observed; true goblet cells are absent, though small mucous glands may occur infrequently. A plexus of blood vessels lies immediately beneath the epithelium. The muscularis consists of obliquely circular fibers arranged in a plexiform layer. Among them are groups of sympathetic nerve cells

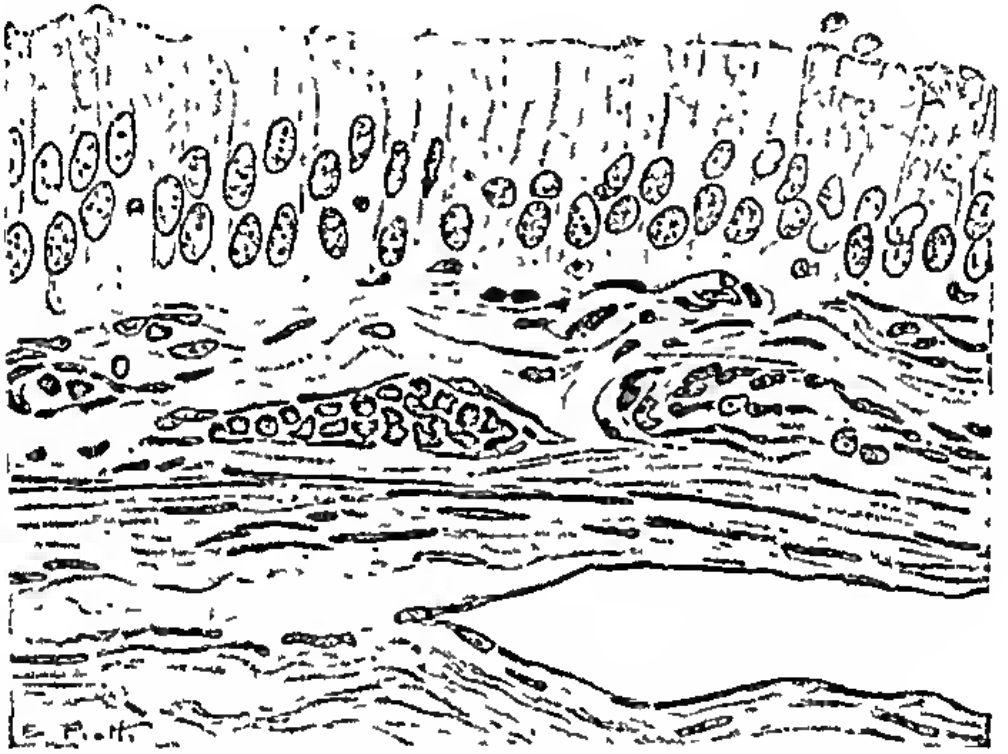


Fig. 346.—Epithelium of Gall Bladder, Cat. Secretion Products Are Evident.

which supply the muscle, and medullated fibers which end in the epithelium. The subserous tissue is highly developed and contains large lymphatic vessels.

The function of the gall bladder, beside the storage of bile, is also to remove certain of the bile fluids and probably to add other constituents, as shown by the secretory granules. Hepatic bile and bladder bile differ in color and consistency. It has been suggested that, in animals without gall bladder, similar alterations are carried on in the intrahepatic ducts, which are said to be enlarged.[1] The gall bladder becomes distended between meals, to such an extent that the muscular layer is reduced in thickness and the folds or rugæ of the mucosa nearly obliterated. The ingestion of fats provides a stimulus for its contraction,[2] and in the contracted state the muscularis may be ten times as thick as before, the mucosa nearly twenty times.

[1] Higgins, 1926. [2] Boyden, 1928.

PANCREAS

Development and General Features. Although the pancreas in the adult is a single gland, it arises in the embryo as two entirely distinct entodermal outgrowths, known as the dorsal and ventral pancreas respectively. The dorsal pancreas grows out from the dorsal wall of the intestinal tube, a little below the level of the common bile duct in most mammals, but a little above it in man. The ventral pancreas grows down from the common bile duct at its junction with the intestinal tube. The ventral pancreas may be more or less bi-lobed. Usually it grows to the right of the intestine and there meets the dorsal pancreas, which approaches it in close relation with the portal vein.

The left lobe of the ventral pancreas sometimes grows around the left side of the intestine and joins the dorsal pancreas, so that the intestine is encircled by pancreatic tissue (annular pancreas); sometimes it grows out beneath the gall bladder where it ends in a cystic enlargement. Usually the left lobe is scarcely indicated. As a rather frequent abnormality, accessory pancreases of small size, but sometimes of very typical structure, are found along the intestine, or even in the wall of the stomach, especially at the constriction between its cardiac and pyloric portions. Such glands may or may not extend through the tunica muscularis The anomalies of the pancreas are reviewed by Boyden.[1]

After the dorsal and ventral pancreases have come in contact, they are related to one another as shown in Fig. 347, B. The dorsal pancreas is much larger than the ventral pancreas, and grows across the body toward the left until it reaches the spleen. Thus it gives rise to the *body* and *tail* of the pancreas of the adult, and forms also the ventral part of the *head* of the gland, which fills the concavity in the duodenal loop. The ducts of the two portions anastomose at one point, and thereafter the duct of the ventral pancreas becomes the main outlet for most of the gland, the *pancreatic duct*, which was first figured by Wirsung (1642). It opens at the duodenal papilla either into the duodenum close beside the common bile duct (Fig. 347, C), or, retaining its embryonic relation, into the common bile duct itself. An intricate arrangement of smooth muscle surrounds the common bile duct near its opening into the duodenum. The development and disposition of the muscle fibers which form a sphincter, often called the 'sphincter of Oddi,' has been ably presented by Boyden.[2] The originally more important duct of the dorsal pancreas drains only a portion of the head of the organ, and becomes reduced to the accessory pancreatic duct, discovered by Santorini (1775). In the human adult it opens into the duodenum 1–3 cm. above the orifice of the common bile duct. In some cases the accessory duct becomes

[1] Boyden, 1925. [2] Boyden, 1937.

impervious, but it is generally functional, and if the outlet of the main duct were blocked by gall-stones or otherwise, the presence of this accessory duct would be of considerable importance. In some mammals, as in the pig, it is normally the chief duct. It will be noted that a large part of the dorsal pancreatic duct, extending through the body and tail, becomes incorporated in this main duct of Wirsung; the ventral pancreas supplies only its outlet.

In the adult no histological distinction has ever been found between the two pancreases, but although alike in structure and close together, there is no general anastomosis between them. Rarely they remain entirely separate. Usually, on injecting the ducts, only one connection

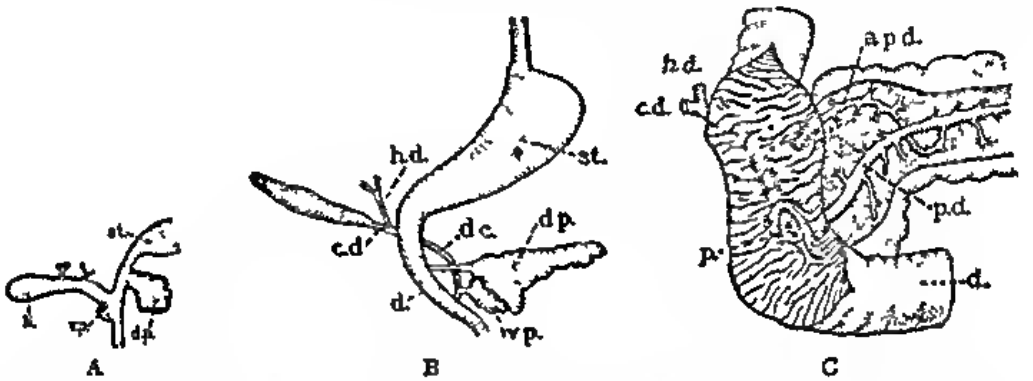


FIG. 347.—A AND B DIAGRAMS OF THE PANCREAS OF HUMAN EMBRYOS OF 10 MM AND 15 MM. C DISSECTION OF DUODENUM AND PANCREAS OF ADULT

a. p. d., Accessory pancreatic duct, *c. d.*, cystic duct, *d.*, duodenum; *d. c.*, ductus choledochus, *d. p.*, dorsal pancreas, *h. d.*, *hepatic duct*, *li.*, *liver diverticulum*, *p.*, *duodenal papilla*, *p. d.*, *pancreatic duct*, *st.*, *stomach*, *v. p.*, ventral pancreas

is found between the dorsal and ventral pancreases. In the pig Corner[1] found that the ducts in fetal stages formed an anastomosing network, but that this gradually developed into a tree-like system of trunks and branches, leading in the adult to terminal ducts, each supplying a definite structural unit consisting of a group of acini.

Microscopic Structure. As a whole the pancreas somewhat resembles the parotid gland. It is divided into angular lobes and lobules by connective tissue septa containing blood and lymphatic vessels, nerves, and interlobular ducts. The lobules are composed chiefly of short tubules or acini, which in models appear pear-shaped. In sections they are cut at all possible angles. Irregularly distributed among the acini are rounded or irregular areas of paler cells, called *pancreatic islets*, or islands of Langerhans, peculiar to the pancreas. They may be at the center or periphery of the lobule, or occasionally in the interlobular connective tissue. These two parts of the pancreas will be described separately.

The main part of the pancreas consists of a branching tree of ducts leading to acini of serous cells. The larger ducts, interlobular, are lined

[1] CORNER, 1914.

by a simple columnar epithelium, with occasional goblet cells or pockets of mucous cells. The epithelium becomes lower as the ducts branch. A thick connective tissue coat with some muscle fibers extends along the ducts and penetrates with them into the center of the lobules, where it is lost as the ducts branch. The smallest ducts, called *intercalated ducts* or 'neck pieces,' consist of small, low cuboidal or flattened epithelial cells, whose nuclei are oval and directed along the duct wall; the lumen is not much wider than the nuclei. These ducts branch repeatedly, and lead to the secreting acini, but the acini are not always at the end of the ducts, as

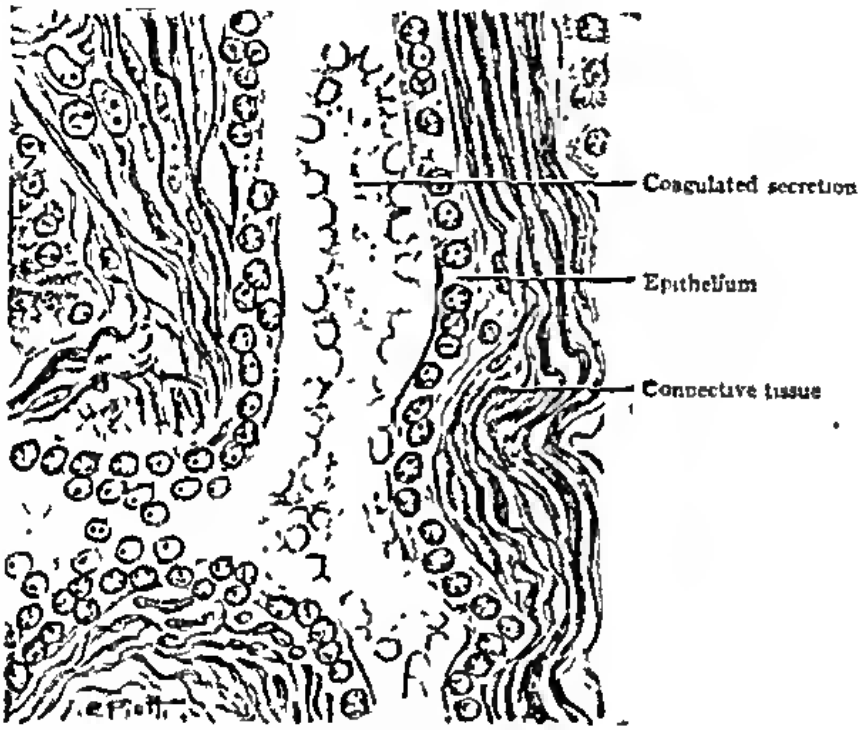


FIG. 348.—A SECTION OF A HUMAN PANCREATIC DUCT. Formaldehyde fixation, Azan.

is usual in the salivary glands; they may bulge from the side of the duct, partially surrounding it with their larger cells, or a few acinar cells may occupy one side of the duct along a considerable distance.[1] The tendency of the large acinar cells to expand over the smaller duct cells makes it appear in certain sections as though the duct cells were in the lumen of the acinus (Fig. 349), and gives such duct cells the name of centro-acinar cells. The true lumen of such an acinus is reduced to intercellular secretory canaliculi, reaching to every acinar cell. The acinar cells rest upon a basement membrane containing 'basket cells.'

The duct cells have vesicular nuclei and clear non-granular cytoplasm. The acinar cells are typical serous cells, containing zymogen granules, mitochondria and Golgi apparatus. The nucleus is large,

[1] ZIMMERMANN, 1927.

round, and vesicular. In fact the pancreatic cell may be considered the type of enzyme-secreting cells, since it has probably been more studied than those of other glands. Claude Bernard in 1856 was the first to figure the shiny, refractive granules in the fresh pancreatic cells. The size and number of the granules vary with the activity of the gland. In the resting condition they are large and closely packed in the inner end of the cell, down to the level of the nucleus. During digestion or after artificial stimulation of the gland the granules are discharged until only a few are

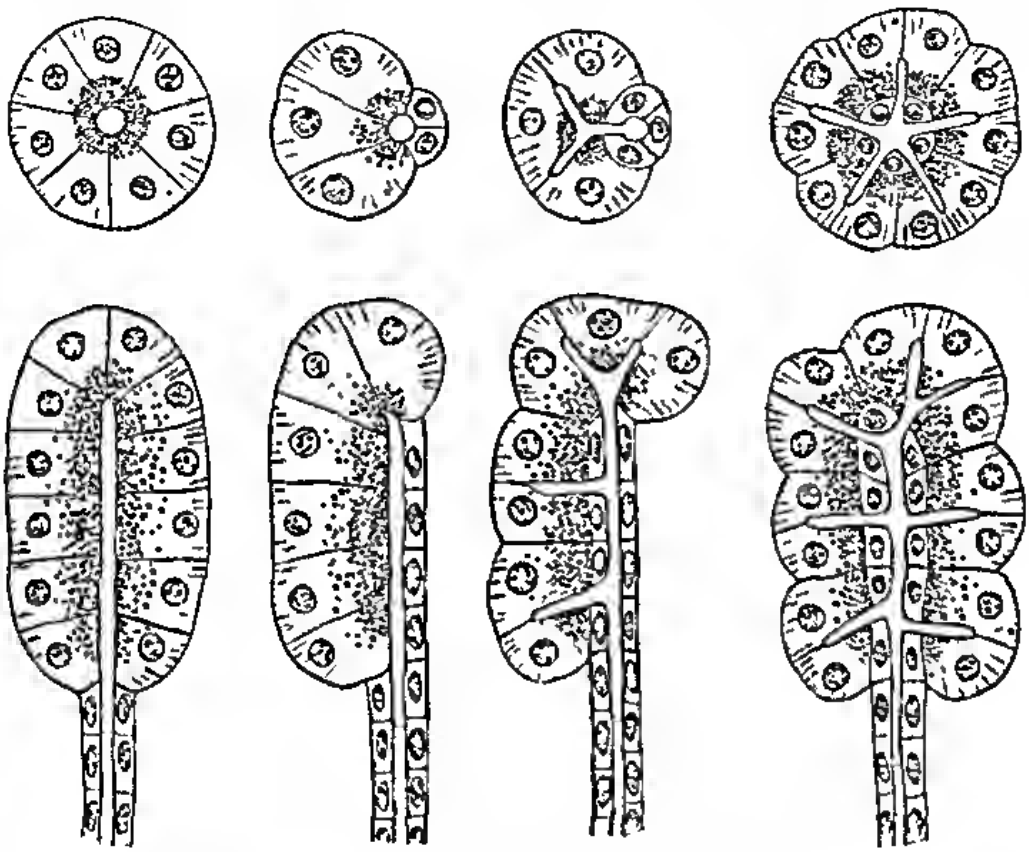


FIG. 349.—DIAGRAMS OF DIFFERENT RELATIONS OF DUCT AND GLAND CELLS IN THE PANCREATIC ACINI; TRANSVERSE SECTIONS ABOVE, LONGITUDINAL BELOW (Zimmermann)

present in the tip of the cell[1] (cf. Fig. 61). The base of the cells is occupied by mitochondria and by *chromidial substance*, a diffuse material staining with basic dyes and at one time considered as chromatin particles extruded from the nucleus. This chromidial substance may cause the basal end of the cells to have a striated appearance due to avoidance of spaces occupied by poorly fixed mitochondria.

The cells of the pancreatic acinus are peculiarly suitable for studies of secretion, as the gland secretes only when food reaches the duodenum, or under the action of certain drugs. At other times the cells are resting. By removing the organ at definite periods, various stages in secretory activity can be obtained. The long mitochondria, which are the cause of the basal striations, the granules, the Golgi apparatus, and other

[1] COVELL, 1928.

constituents of these cells have been frequently investigated. The secretion contains a proteolytic enzyme, trypsin, and a lipolytic enzyme, steapsin.

The secretory granules, also called zymogen granules because they seem to be the fore-runners of the specific pancreatic enzyme, trypsin, may be either preserved in the specimen or lost by dissolution in certain fixatives. In the first case they are stainable, but if dissolved the section shows a negative picture, the cytoplasm of the inner pole of the cell forming a delicate spongy reticulum, in the meshes of which the granules formerly rested.

The smaller, but equally important part of the pancreas is the islet tissue. Langerhans[1] in 1869 distinguished 'small cell heaps,' scattered throughout the organ, differing from the acini in the arrangement and character of their cells. These islets of Langerhans occur in great numbers, and vary in size from a single cell to groups of many hundreds. They are usually most numerous in the tail of the pancreas, and least frequent in its head. Their number was first adequately recognized by Bensley,[2] who found that after perfusion of the pancreas with neutral red or Janus green B the islets were differentially stained and could be counted in whole mounts of separated lobules. For the Guinea-pig pancreas the number of islets ranged from 15,000 to 40,000 or more.

The relation of the islets to the ducts of the pancreas was also brought out in a striking manner by Bensley's perfusion method. By the addition of pyronine or methylene blue to the neutral red the ducts also are differentially stained. Besides the ducts leading to the acini there are present other smaller ducts often with obliterated lumens, which form anastomoses frequently with the islets. In ordinary sections this secondary duct system remains unsuspected, the small duct cells lying unnoticed in the interacinar connective tissue. From these inert cells new acini and new islet cells probably develop.

Though attached to this duct system, the larger islets are composed of irregular cords of cells, without lumen, separated from each other by large capillaries, which come in close contact with the bases of the cells. The cells of the islets show little differentiation after the usual histological fixatives and stains, but by special methods at least three cell types can be differentiated [3] Two of these contain fine granules, the other is non-granular, with smooth clear cytoplasm. The granules differ chemically; if the tissue is fixed in alcohol, or in formol, the granules of the A (or Alpha) cells are preserved; if fixed in other watery fluids, another type of granule in the B (or Beta) cells is present. The A cell is large and polygonal, its nucleus usually large and elliptical, with little chromatin. The

[1] LANGERHANS, 1869. [2] BENSLEY, 1911. [3] LANE, 1907.

B cell is smaller, much more numerous, and with a smaller spherical nucleus, centrally placed and with considerable chromatin, therefore appearing much darker in stained specimens. Some of the smaller islets are composed entirely of this type.

In the pancreas of the Guinea-pig fixed in an aqueous chrome-sublimate mixture and stained with neutral gentian and acid fuchsin, Bensley was able to differentiate the two types of cells, the granules of the A cells

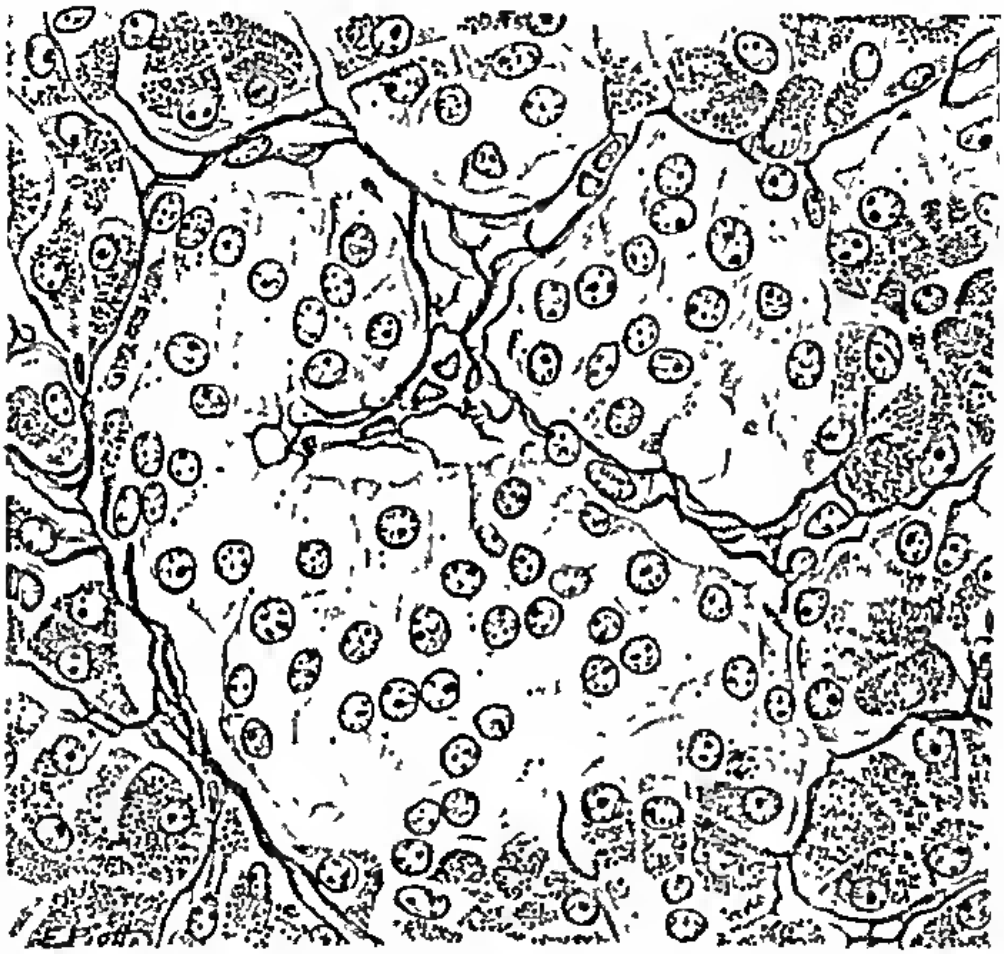


FIG 350.—AN ISLET OF LANGERHANS IN THE HUMAN PANCREAS.
Tissue removed at operation Formaldehyde, Azan The surrounding acinous cells contain red staining zymogen granules

staining red, those of B cells violet, and to distinguish a third cell type without granules, called the C cell. Bloom,[1] by other techniques, distinguishes in man a fourth type of cell, a *D* cell, with very small granules; but this may prove to be in fact the clear cell, C, under another guise, since four types are never recognizable in the same preparation. These D cells have been identified also, in the pancreas of many different mammals and other vertebrates.[2]

The relations of these cells to each other are not definitely known. The granules of the A cells vary greatly in number in different cells, sometimes

[1] BLOOM, 1931. [2] THOMAS, 1937 AND 1940.

being few and at the end of the cell nearest the blood vessels, at other times filling the whole cell cytoplasm. The C cell may represent a younger form of the A cell, its clear cytoplasm showing as yet no granules. The nuclei of these two types closely resemble each other in oval shape and vesicular character. The B cells are much smaller, usually are completely filled with granules, and no relations between these granules and those of the A cells have been suggested.

The islets are morphologically distinct from the rest of the pancreas. Though the islet cords may in some instances be continuous with the acini, there is no proof, according to Bensley, that one type of cell may be converted into the other or is a developmental derivative of the other. Some authors, however, note definite transition stages between acinus cell, granular islet cell, and clear islet cell, and consider all these related.[1] Van Campenhaut,[2] on the other hand, finds that the islets develop from individual cells of the ducts, which are in special relation with sympathetic nerve cells, and which migrate from the epithelial layer along the course of the nerves. One is reminded of the similar relations of the argentaffin cells of the intestine (p. 348). In the lobules of the pancreas the relation between islet cells and ganglion cells is soon lost, but the few islets found in the septa remain, according to him, connected with sympathetic ganglia. Physiologically also the two structures are quite distinct. The acini send their secretion through ducts to the intestine to aid in digestion; the islets are discrete ductless glands delivering their secretion, *insulin*, to the blood to regulate sugar metabolism. From the fact that the B granules are dissolved in alcohol, and that insulin is derived by alcoholic extraction, it may be inferred that the B cells of the islets are responsible for this active principle. The function of the A cells is not known.

Glycosuria, or the presence of sugar in the urine, denotes a failure of the normal carbohydrate metabolism. Attempts to isolate a hormone which would restore this metabolism were unsuccessful until Banting and Best,[3] reasoning that the digestive pancreatic secretion might destroy the islet secretion in extracts of the whole gland, sought pure islet secretion of the fetal pancreas, in which the activity of the alveolar cells had not commenced. This was successful. Similar hormones have been secured from certain fish, in which the islet tissue is entirely separate from the acinar tissue.

The blood supply of the pancreas comes from several sources and the larger vessels run in the interlobular septa. There are differences of opinion about the finer distribution. According to one view[4] two types of arterioles are given off from the intralobular arteries, one to the acini,

[1] Tschassownikow, 1934. [2] van Campenhaut, 1925.
[3] Banting and Best, 1922 [4] Beck and Berg, 1931.

the other to the islets. The acinar arterioles are long and the capillary network of small caliber and of wide mesh; the arterioles to the islets are short and divide into wide, tortuous, frequently anastomosing capillaries between the islet cords. The larger islets may have several efferent arterioles, while the smallest may make use of the acinar net. Anastomoses between acinar and islet capillaries are frequent. The efferent veins follow the course of the arteries. According to the other view[1] all the blood to the acini must first pass through the islets, the presence of special arterioles to the acini being denied. Thus the islets stand in this respect

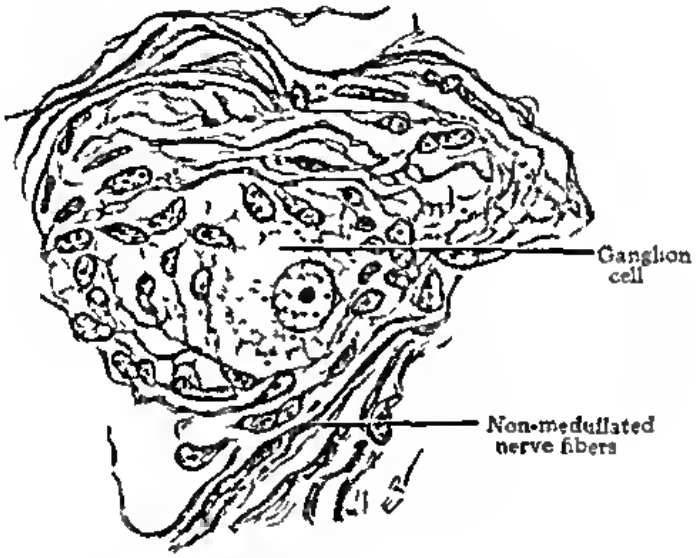


FIG 351.—A NERVE GANGLION CELL IN THE HUMAN PANCREAS. *Formaldehyde fixation, Azan*

somewhat in the same relation as the glomeruli of the kidney. In both cases the islet tissue would seem to have the fresher blood supply.

The lymphatics follow the larger vessels in the septa, not being demonstrable within the lobules. The nerves end around the blood vessels, ducts and pancreatic cells. They are chiefly non-medullated sympathetic fibers from the cœliac plexus, associated with scattered nerve cells found within the pancreas. Lamellar corpuscles occur in the connective tissue, being often numerous in the cat. The islets are said to be richly supplied by special nerves, not connected with those to the exocrine portion of the gland.

RESPIRATORY APPARATUS

Development and General Features. The respiratory apparatus consists of the larynx, trachea, bronchi and lungs; the upper respiratory passages, often included with these, will be considered as the site of an organ of special sense (p. 670). The lungs develop as a median ventral outgrowth of the fore-gut, immediately behind the last pair of pharyngeal

[1] WHARTON, 1932.

pouches. This lung bud, originally pear-shaped and directed caudally, soon subdivides (4 mm. human embryo) into two lateral pouches, representing the right and left primary bronchi, from which develop the paired lungs. The single trachea is not an elongation of the lung bud. At the stage when the bud first develops the fore-gut is flattened laterally so that its lumen is a dorso-ventral cleft. This portion grows in length until the lung bud has been carried far below the fourth pouches. The gut is then divided, from below upward, into two tubes, the dorsal œsophagus and the ventral trachea, the latter retaining connection with the bronchi. The division is accomplished by the growth of two lateral ridges of mesoderm, which extend orally beyond the region of the fourth pouches. The irregular upper ends of these ridges mould the larynx, through which the trachea connects with the pharynx, and the failure of the ridges to meet in any region may lead to anomalous connections between the trachea and the œsophagus.

The tracheal and bronchial tubes are lodged in a mass of connective tissue, situated above and behind the pericardial cavity, and since this tissue stands in the middle of the thorax it is known as the *mediastinum*. It is comparable with a broad mesentery. As the bronchi push out laterally, they occupy right and left folds bulging from the mediastinum, called by Ravn the *pulmonary wings* (*alæ pulmonales*). Into these the bronchi extend and produce branches after the manner of a gland. The pulmonary wings consist of mesenchyma, covered by the epithelium which lines the body cavity. At first they project into the part of the body cavity which connects the peritoneal with the pereardial cavity; later, by the development of the pleuro-pericardial and pleuro-peritoneal membranes respectively (the latter being a part of the diaphragm), the chamber into which the pulmonary wings project is entirely cut off from the rest of the body cavity. On each side, it forms a pleural cavity. The epithelium and underlying connective tissue covering the pulmonary wings constitute the *visceral pleura;* and the similar layers toward the thoracic wall form the *parietal pleura*. The lung is connected with the mediastinum by a short and broad stem of connective tissue, through which the bronchi, vessels and nerves extend. This is the *root* of the lung, and the vessels enter at the *hilum*.

Incomplete development of the pleuro-peritoneal membrane may lead to a diaphragmatic herma, with some of the abdominal viscera invading the pleural cavity.

The branches which are given off by the stem-bronchus within the pulmonary wings are formed with great regularity, and they have been carefully studied in many mammals. Very early in development, the human lungs become asymmetrical, and at the stage shown in Fig 352, C, the three lobes of the right lung and the two lobes of the left lung are already indicated In the pig the asymmetry is greater, since on the

right an unpaired lobe proceeds directly from the trachea; in certain animals, as in the seal, the right and left lungs have symmetrical bronchi. Whether the symmetrical

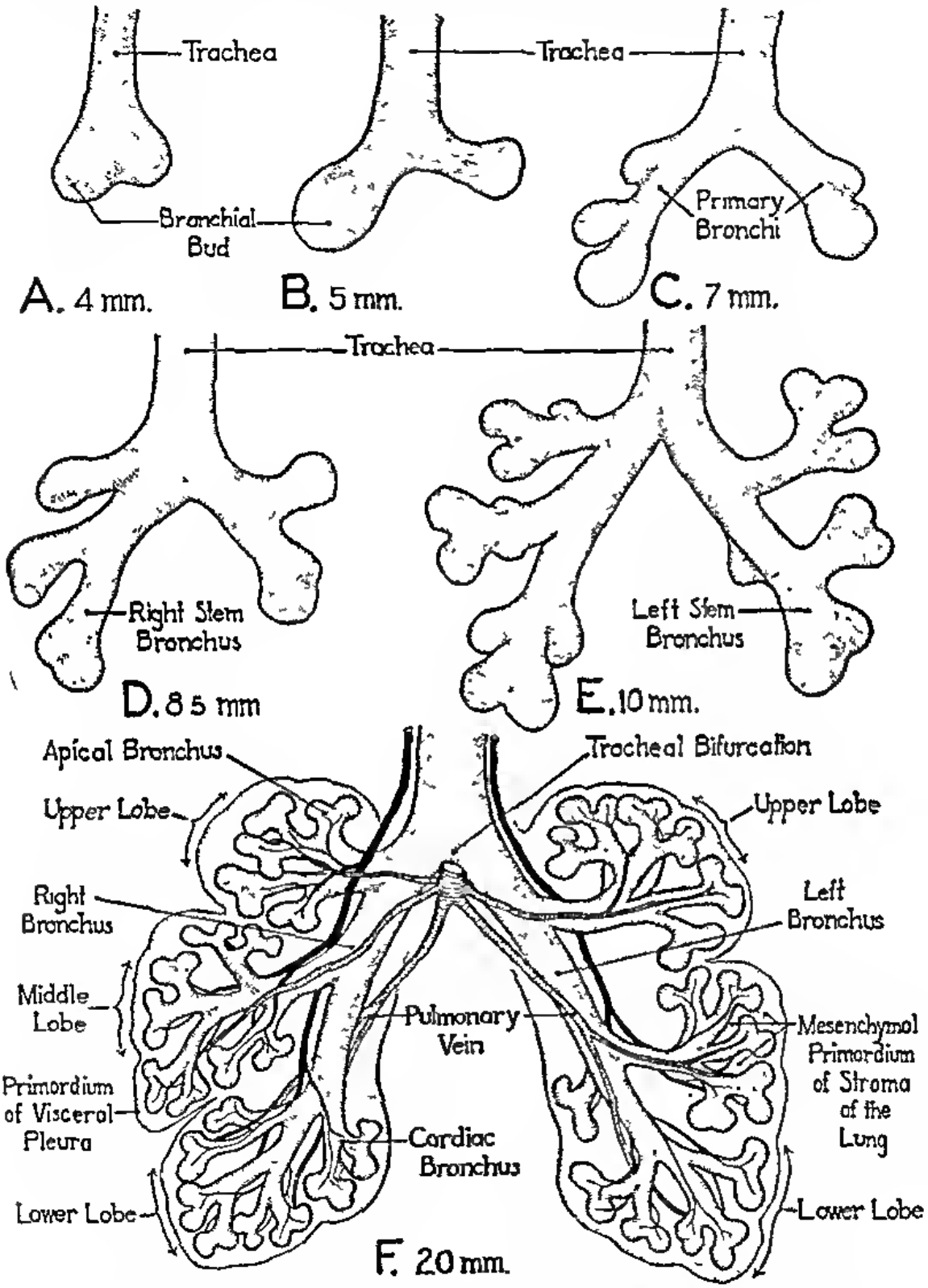


FIG 352.—DIAGRAMS SHOWING, IN VENTRAL ASPECT, THE DEVELOPMENT OF THE MAJOR BRONCHI OF THE HUMAN LUNGS (*From a forthcoming "Human Embryology," by Bradley M. Patten.*)

condition is the primary one, and how the bronchi of one lung should be homologized with those of the other are questions which have been much discussed. For the com-

parative anatomy of the bronchi, see **Huntington**;[1] for their development, especially in the pig, see Flint.[2]

The blood vessels of the lungs are derived from several sources. They include the large pulmonary arteries and veins, which are the principal vessels of the lung, and the small but important bronchial arteries and veins. The pulmonary vessels are shown in Fig. 353, which represents the trachea and right lung of a human embryo, seen from the left side; the left lung has been cut away at *l. br.*

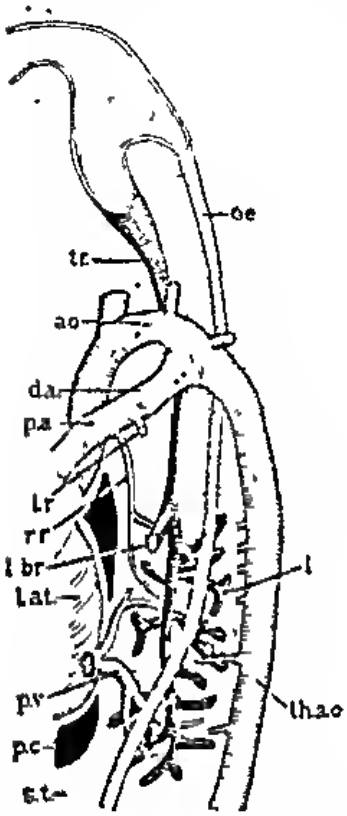


FIG. 353.—RECONSTRUCTION OF A PART OF A HUMAN EMBRYO OF 13·8 MM. (F. W. Thyng.)

ao, Aorta; d. a, ductus arteriosus; l, entodermal part of the lung; L at., left atrium; l br, left bronchus; L r, left ramus of pulmonary artery; p. a, r. r, its right ramus; oe, œsophagus; p c, pericardial cavity; p v, pulmonary vein; s. t., septum transversum; th. ao, thoracic aorta; tr, trachea.

The *pulmonary arteries* develop in connection with the *pulmonary arches*, which are two vessels, one on each side, passing from the ventral aorta to the dorsal aorta. Approximately midway in its course, each of these arches sends a branch to the lung of the corresponding side. Subsequently the trunk of the ventral aorta becomes spirally subdivided by a septum, so that the portion leading to the pulmonary arches is split off from the rest; the way in which its root becomes connected with the right ventricle only has been described with the development of the heart. As a result of this subdivision, the pulmonary artery leaves the heart and divides into right and left arches, each of which sends a branch to the lung on the same side and then passes on to the dorsal aorta. The connection between the right arch and the right dorsal aorta is soon lost, however, so that the vessel to the right lung (Fig. 353, *r.r.*) appears to be given off from the main pulmonary artery. In the pig embryo the same effect is reached by the fusion of the two branches and then the loss of the root of the right one.[3] The left pulmonary arch enlarges, and until birth it forms a great vessel, known as the *ductus arteriosus*, which conveys most of the blood from the pulmonary artery into the aorta. The amount of blood which goes to the inactive lungs may be inferred from the relative size of the vessels shown in the figure. Soon after birth, when respiration has begun, the ductus arteriosus closes, becoming a fibrous cord, and then the volume of blood going through the pulmonary artery equals that in the aorta.

The *pulmonary veins* are at first represented by a capillary plexus around the lung bud, which receives its blood in part from the pulmonary

[1] HUNTINGTON, 1898. [2] FLINT, 1906. [3] BREMER, 1902.

arteries already described, and in part from branches of the dorsal aorta, some of which persist as the bronchial arteries. The capillary plexus is drained partly by branches of the posterior cardinal or azygos veins, representing the future bronchial veins, and partly by a minute vein which has grown out from the left atrium and is destined to become the great pulmonary vein. At a certain stage these veins, two from each lung, have a common orifice in the left atrium; but in later stages, as the heart enlarges, their short common stem is taken up into the wall of the atrium, so that the four pulmonary veins acquire separate openings. The early stages in the development of the pulmonary veins in the cat have been studied by Brown.[1]

The small *bronchial arteries*, one or two on each side, are branches of the upper part of the thoracic aorta (Fig. 353); sometimes one of them proceeds from an intercostal artery. They enter the hilum of the lung and pass into the fibrous tissue in the walls of the bronchi. The main stems branch with the bronchi. They produce capillary networks in the bronchial mucous membrane, and send branches to the peribronchial connective tissue, supplying it with capillaries and becoming the vasa vasorum of the main branches of the pulmonary artery.[2] In some animals Miller finds that the bronchial arteries pass on into the pleura, as in the horse; in others, like the dog, terminal branches of the pulmonary arteries supply the pleura; and in the human lung the pleura receives both pulmonary and bronchial vessels.[3]

The *bronchial veins* are small branches of the azygos vein. They do not receive all the blood from the bronchial arteries, since some capillaries from the latter are drained by the pulmonary veins.

Larynx. The mucous membrane of the larynx is a continuation of that of the pharynx, and accordingly consists of epithelium and lamina propria. A submucosa connects it with the underlying parts. In most places the epithelium appears to be stratified, the top layer being either cuboidal or columnar. It may be pseudostratified, with nuclei at several levels, but it is difficult to determine whether or not all the cells are in contact with the basement membrane. The columnar type, which occurs also in the trachea, is ciliated. The stroke of the cilia is toward the pharynx. A stratified epithelium with squamous non-ciliated outer cells is found on the vocal folds (true vocal cords), on the anterior surface of the arytænoid cartilages, and on the laryngeal surface of the epiglottis. The distribution of the two sorts of epithelium above the vocal folds is subject to individual variation. The stratified squamous epithelium often occurs in islands, and is said to replace the ciliated after local infectious desquamation. The lamina propria is composed of

[1] Brown, 1913. [2] Miller, 1906. [3] Miller, 1907.

fibrous connective tissue with many elastic fibers, and beneath the epithelium it forms a basement membrane (*membrana propria*). It includes reticular tissue containing a variable number of lymphocytes, which are gathered in solitary nodules in the wall of the laryngeal ventricle (sinus of Morgagni). Connective tissue papillæ are found chiefly beneath the stratified squamous epithelium. At the free border of the vocal folds and on their under surface, the papillæ unite to form longitudinal ridges.

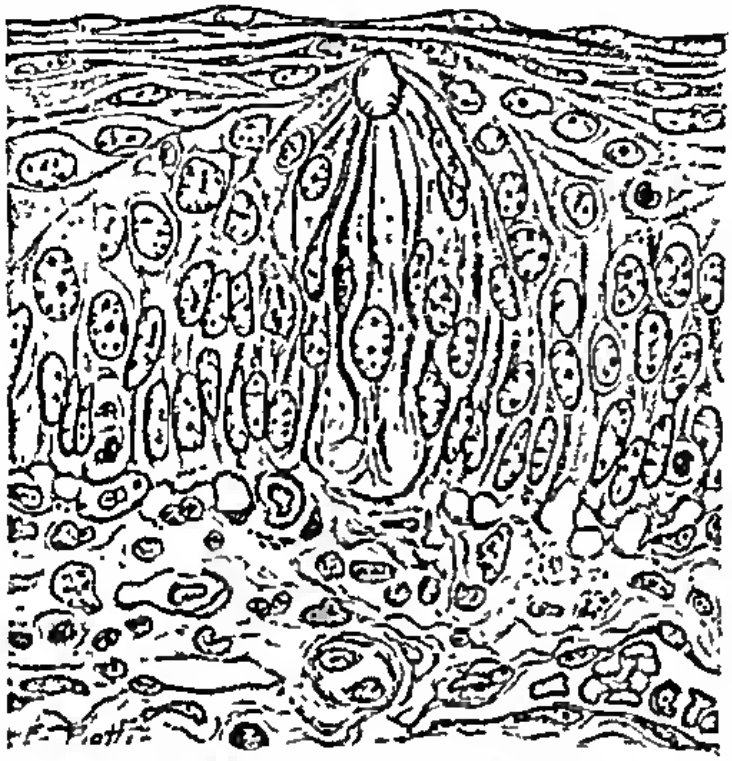


FIG. 354.—A TASTE BUD IN THE EPITHELIUM OF THE HUMAN EPIGLOTTIS. Zenker fixation, hæmatoxylin and eosin.

On the laryngeal surface of the epiglottis there are only isolated papillæ, against which rest the short taste buds.

The submucosa contains mixed, branched, tubulo-alveolar glands, measuring from 0.2 to 1.0 mm.; they are abundant in the ventricular folds but are absent from the middle parts of the vocal folds. The ventricular folds (false vocal cords) consist of a loose vascular fatty tissue, often containing small bits of elastic cartilage about 1 mm. long, and similar cartilages measuring 2–3.5 mm. are sometimes found in the anterior ends of the vocal folds.

The cartilages of the larynx are mostly of the hyaline variety, resembling those of the ribs. To this class belong the thyroid, cricoid, the greater part of the arytænoid, and often the small triticeous cartilages. Elastic cartilage is found in the epiglottis, the cuneiform and corniculate cartilages, the apex and vocal process of the arytænoids, and generally in the median part of the thyroid. In women this portion is not involved in the ossification (chiefly endochondral) which begins in the thyroid and

cricoid cartilages between the twentieth and thirtieth years. The triticeous cartilages (nodules in the lateral hyothyroid ligaments, named from their resemblance to grains of wheat) are sometimes composed of fibrocartilage.

The blood vessels form two or three networks parallel with the surface, followed by a capillary plexus just beneath the epithelium. The

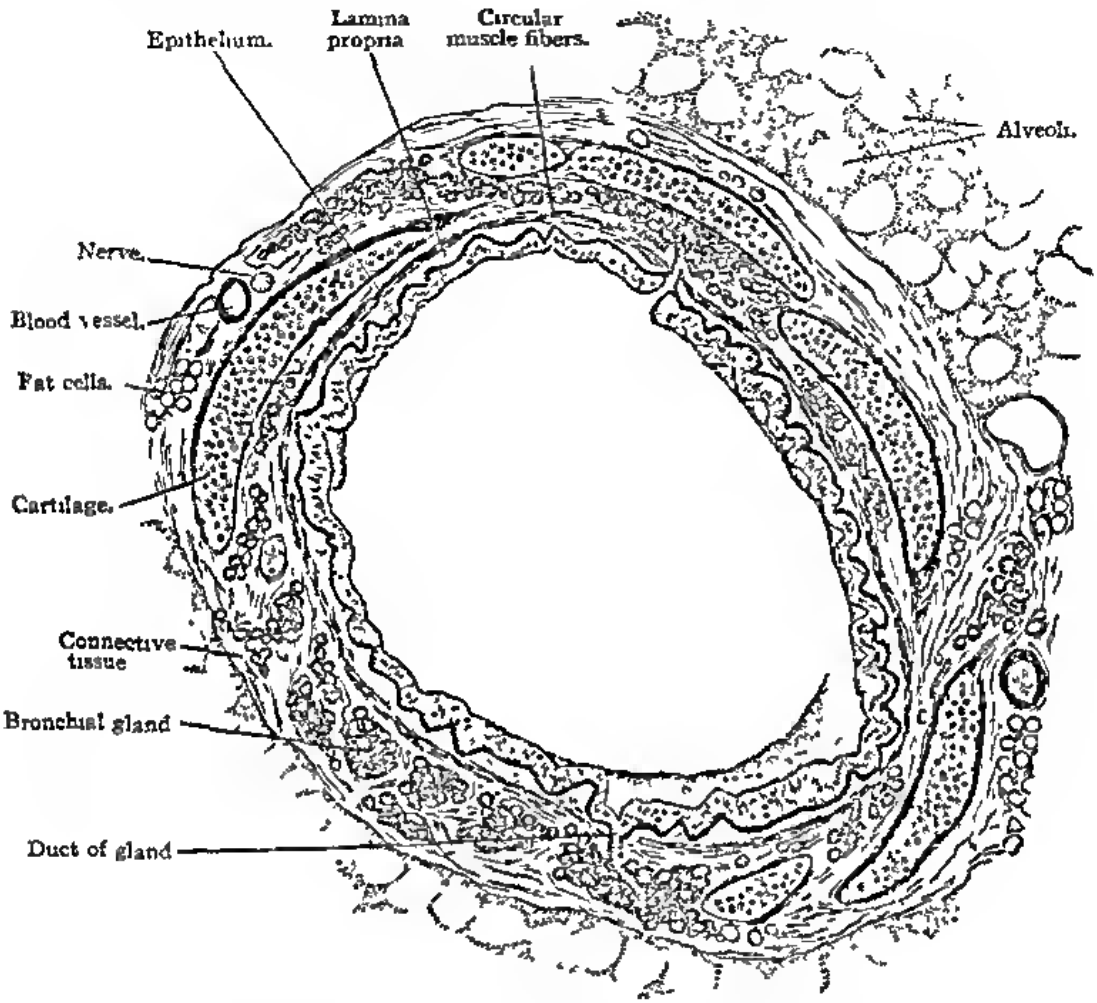


Fig. 355.—Cross Section of a Bronchus 2 mm. in Diameter, from a Child.

lymphatic vessels similarly form two communicating networks, of which the more superficial consists of smaller vessels and is situated beneath the capillary plexus. The nerves form a deep and a superficial plexus which are associated with microscopic ganglia. Non-medullated fibers end either beneath the epithelium in bulbs and free endings with terminal knobs, or within the epithelium in free ramifications and in taste buds. Below the vocal folds, subepithelial nerve endings and buds are absent, but many intraepithelial fibers occur, which surround individual taste cells. The nerves and vessels of the larynx are numerous, except in the dense elastic tissue of the vocal folds.

Trachea and Bronchi. The trachea consists of a mucosa, submucosa, and a fibrous and muscular outer layer containing the tracheal

cartilages. The general arrangement of the layers is the same as that found in the large bronchi (Fig. 355).

Mucosa. The mucosa consists of a pseudostratified columnar epithelium with cilia proceeding from distinct basal bodies (Fig. 356) and numerous non-ciliated mucous cells resting upon a broad basement membrane. Beneath this membrane is a layer of reticular tissue containing many lymphocytes and forming a lamina propria. Outside the reticular layer and occupying a position comparable to the muscularis mucosæ in the intestine there is found an elastic membrane formed of interlacing, coarse longitudinal elastic fibers which may readily be seen in preparations stained with hæmatoxylin and eosin. This membrane is rather indefinitely represented in amphibians and reptiles, is present in birds, but reaches its highest development in mammals. When dissected out the complete outline of the bronchial tree remains. It is strong above in the trachea and larger bronchi but gradually thins out in the terminal twigs where it joins the elastic tissue around the air chambers, stretching in inspiration and contracting in expiration.[1]

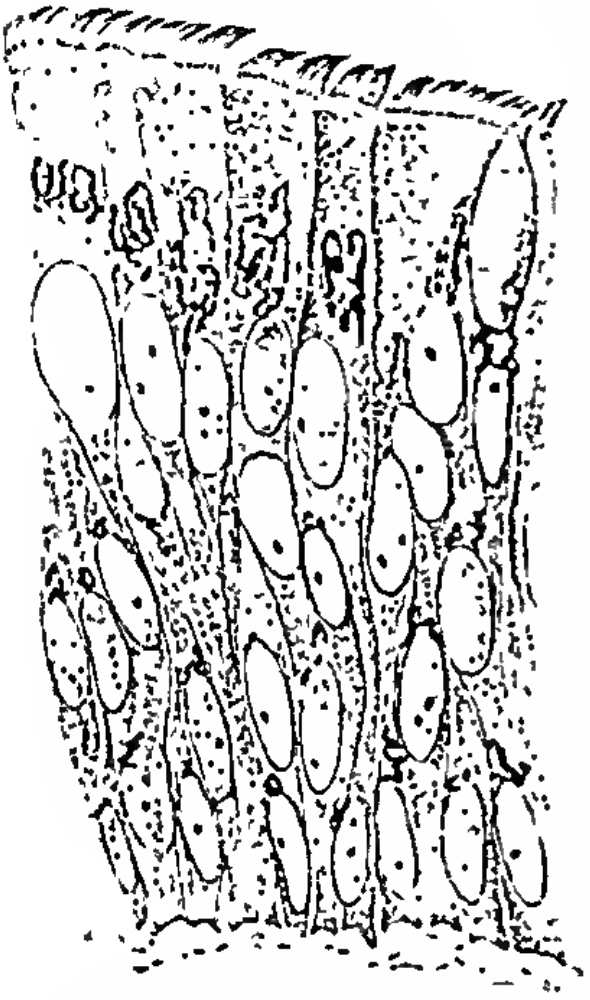


FIG. 356.—A SECTION OF PSEUDOSTRATIFIED CILIATED EPITHELIUM FROM THE HUMAN TRACHEA. A GOLGI APPARATUS LIES CLOSE TO THE NUCLEUS ON THE SIDE TOWARD THE CILIATED BORDER OF THE CELLS. CHROME-OSMIUM, × 1500 (Kopsch)

Submucosa. The submucosa is a layer of loose fatty connective tissue extending to the perichondrium of the tracheal cartilages. It contains the bodies of the tracheal glands, which include mucous cells and serous crescents.

The mucus from the surface goblet cells and from the glands is poured out on the inner faces of the tubes and tends to catch the dust particles in the inspired air, while the cilia sweep the mass toward the pharynx. It has been suggested that the serous cells supply an underlying film of watery fluid in which the cilia may move more freely. Exceptionally, the upper part of the trachea is lined by stratified squamous epithelium.

[1] MACKLIN, 1922.

resting on the connective tissue papillæ, but this is probably the result of some chronic irritation.

The outer layer of the trachea is continuous with the tissue of the mediastinum. It contains abundant blood and lymphatic vessels, and nerves, both medullated and non-medullated. Internally it forms the perichondrium around the succession of C-shaped hyaline cartilages, the

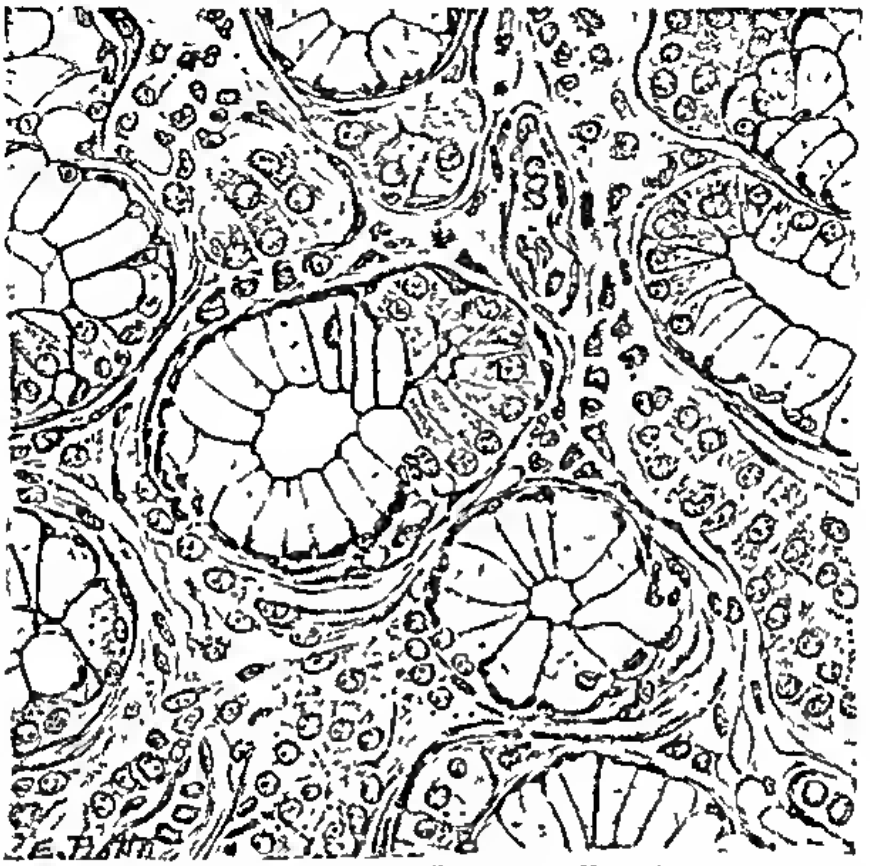


Fig. 357.—A Section of Mixed Glands in the Human Trachea. *Zenker fixation, hæmatoxylin and eosin*

free ends of which are toward the œsophagus. In the intervals between these ends there is a layer of circular smooth muscle fibers, usually accompanied by outer longitudinal fibers. Elastic fibers are abundant among the muscle cells. In old age, the hyaline cartilages show fibrous degenerative changes, and may become partly calcified.

The primary bronchi have the same structure as the trachea, but in their subdivisions changes occur. The epithelium is gradually reduced to a single layer of cells, with fewer goblet cells. The C-shaped rings of cartilage are replaced by irregular plates found on all sides of the tube. These diminish in size as the bronchi become smaller, and disappear in those about 1 mm. in diameter. Usually the cartilages are hyaline, but elastic cartilage may occur in the smaller branches. The circular muscle fibers form a layer completely surrounding the tube internal to the cartilages, thus replacing the dense layer of elastic fibers found in this

position in the trachea. Many elastic fibers are found, however, mingled with this muscle. Branched tubulo-alveolar *bronchial glands* extend further down the tubes than the cartilages. In the larger bronchi they are present in great numbers, and their bodies lie outside of the muscular layer and project into the spaces between the cartilages. The mucosa is covered with ciliated epithelium containing goblet cells and resembling that of the trachea. Lymphocytes are numerous in the lamina propria, sometimes collecting in solitary nodules and wandering into the epithelium.

The small bronchi, 0.5–1.0 mm. in diameter, are known as *bronchioles*. They are lined with ciliated or non-ciliated columnar or cuboidal epithelium, without glands, and are free from cartilage.

The musculature of the bronchi and bronchioles is a continuous net of bundles of smooth muscle fibers arranged partly circularly but mostly diagonally, so that contraction will both constrict and shorten the tubes, thus allowing them to conform to the changing volume of the air sacs and alveoli among which they lie.[1] By their contraction the mucosa is often thrown into longitudinal folds. The muscle net is accompanied by elastic tissue throughout its extent; in the larger air passages this consists of an inner membrane (subepithelial) and a finer network among the muscle fibers. In the bronchioles the latter alone persists.

Lungs

The lungs comprise all the branches of the bronchi that have just been described, representing in the adult about thirty generations or orders of branches, and also the ultimate portions of the bronchial tree, which are the active parts of the lung and form its parenchyma. The bronchi and bronchioles may be likened to the duct system of a gland. Following the terminal bronchiole the next generation of branches exhibits small thin-walled, rounded pouches, called *alveoli*, projecting at intervals from the walls. A bronchiole of this character is called a *bronchiolus respiratorius* (respiratory bronchiole) because by means of the alveoli it takes a certain part in the act of respiration. According to Miller[2] there are usually two or three orders of respiratory bronchioles in the human lung. The more distal orders divide into two branches, which have a larger number of alveoli opening into them; these are called the *ductuli alveolares* (alveolar ducts). Each alveolar duct branches at its distal end into from two to five spheroidal cavities, the *atria;* each atrium has opening into it a variable number of air spaces, the *sacculi alveolares* (air sacs); and each sacculus has projecting from its surface numerous smaller air spaces, the *alveoli pulmonis*. Alveoli are thus connected with all the air passages distal

[1] Macklin, 1929. [2] Miller, 1925

to the terminal bronchiole. 'A ductulus alveolaris, the air spaces connected with it, and all their associated vessels and nerves, constitute the primary lobule (anatomical unit).'

Though the foregoing description and nomenclature are probably correct for the majority of lung units and can be proved by reconstructions, in sections the distinction between the alveolar passages, atria, and

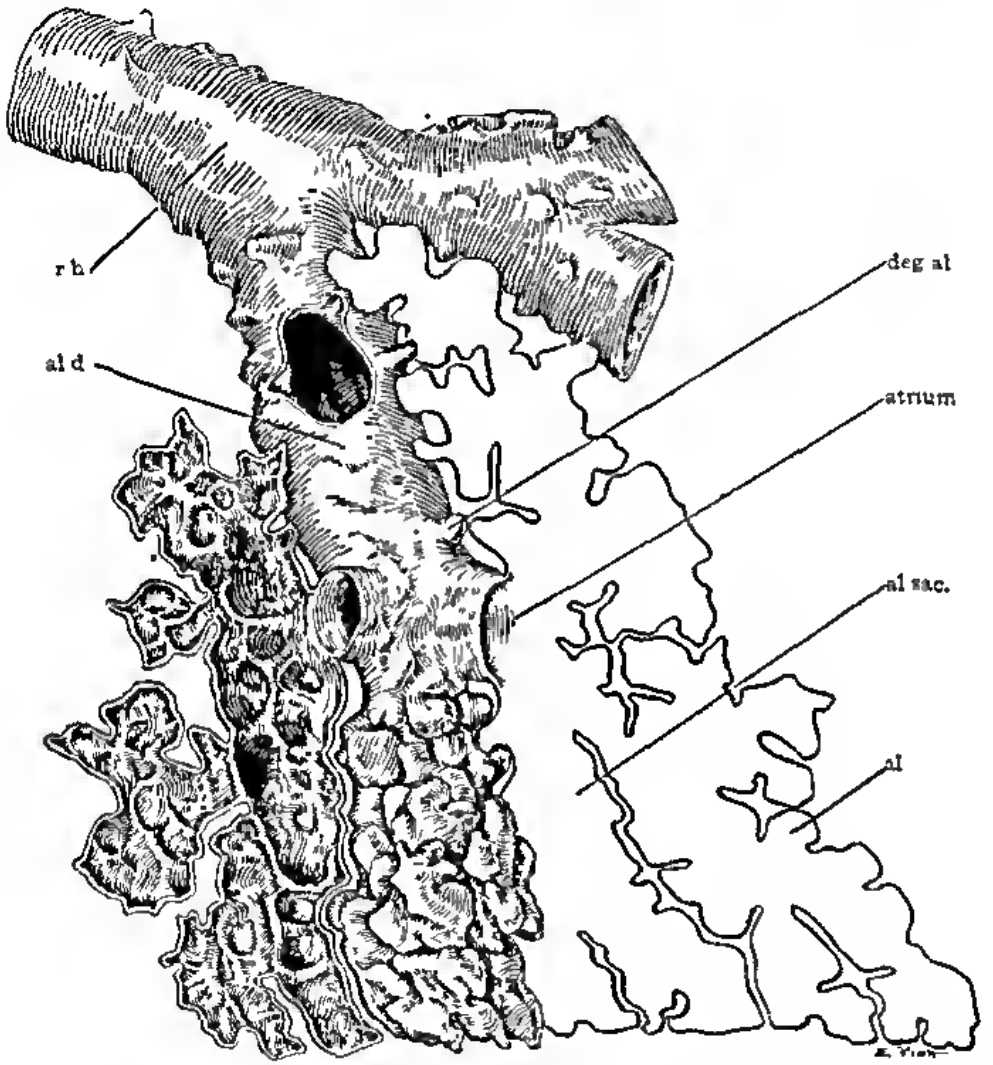


FIG 358.—LOBULE OF LUNG, DIAGRAM BASED ON RECONSTRUCTIONS OF LUNG OF KITTEN.
al, alveolus, al d, alveolar duct, deg al, degenerating alveolus, r b, respiratory bronchiole

air sacs is difficult to make, as all of them are thin-walled cavities studded with alveoli, differing only in their shape and relation to other parts. In practice it is wiser to group them all under the term alveolar duct or the older term infundibulum. Although these are actually at the ends of the bronchial tree they are not to be considered as necessarily peripheral for the lung as a whole; the spreading bronchial branches frequently course backward toward the center of the lung or toward the hilum, and the terminal units may be found everywhere throughout the organ. There is no division into a cortex and medulla in the usual mammalian lung.

In sections very little can be found out concerning the relations of the alveoli to the bronchial ramifications, except in favorable instances when a portion of a unit happens to be cut lengthwise. The following structures are all that can easily be identified: (1) alveoli; (2) spaces bounded by alveoli (alveolar sacs, atria, and alveolar ducts); (3) small bronchioles

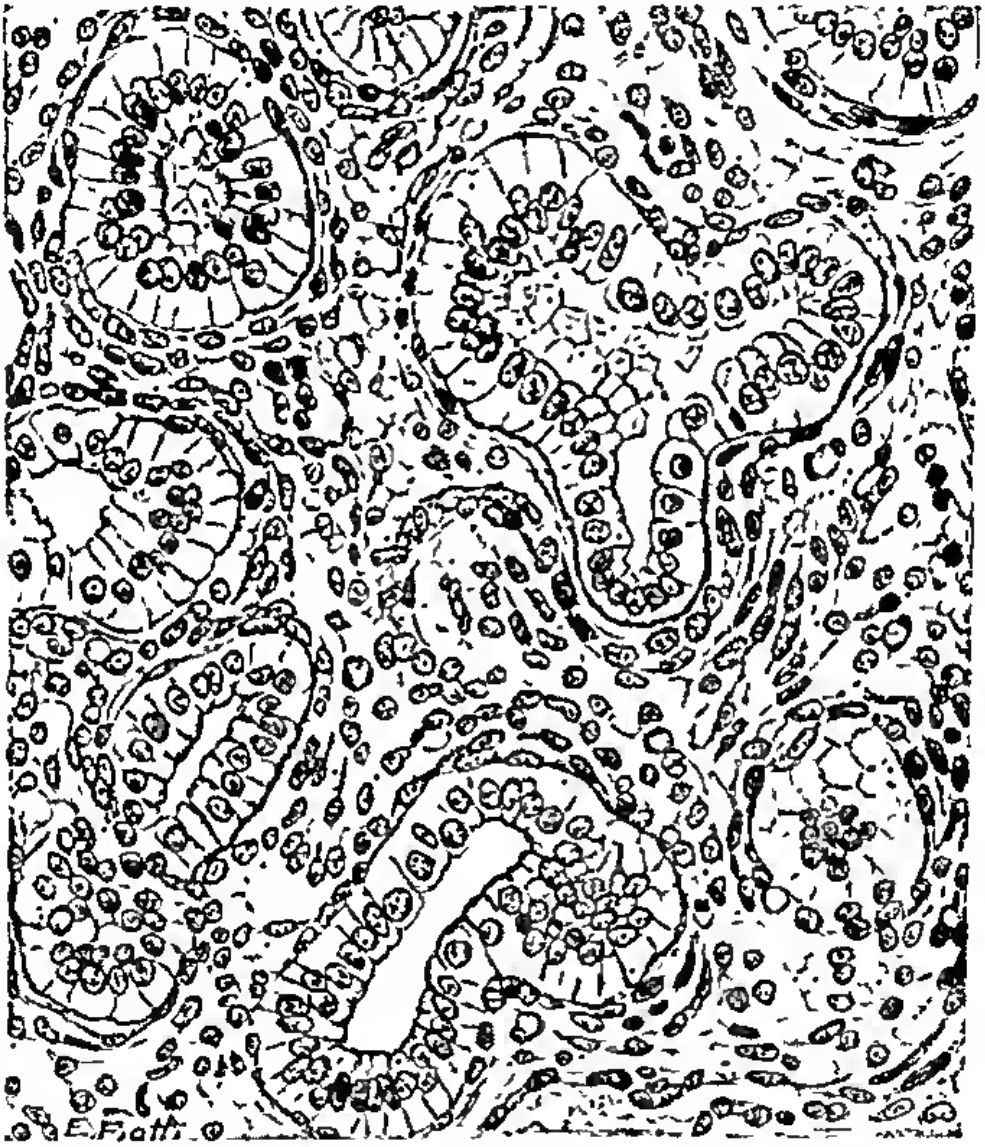


FIG. 359.—A SECTION OF THE LUNG OF A HUMAN FETUS—4 MONTHS OLD
Zenker fixation, hæmatoxylin and eosin

lined by cuboidal epithelium interrupted here and there by thin-walled alveoli (respiratory bronchioles); and (4) bronchioles with no alveoli. Frequently the alveoli are so closely ranged along the wall of an air sac that their openings are side by side and their lateral walls in contact; and the air sacs are so closely crowded that the alveoli of two adjacent walls interdigitate, the outer wall of the alveolus from one air sac abutting against the actual wall of another air sac. Alveoli sectioned transversely show as small enclosed spaces.

The musculature of the terminal bronchioles continues peripherally as slender strands along the walls of the air sacs, surrounding the mouths of the alveoli but not their walls, and thus making a wide-meshed network around all of the passages. As in the bronchioles these muscle strands are accompanied by elastic and reticular fibers, both of which are also present in the alveolar walls.

The disposition of the muscle net is such that contraction of the fibers would shorten and constrict the finer air passages, and narrow the mouths of the alveoli. The shortening of the finer bronchioles tends to condense the clusters of alveoli, thus preserving their relative position in the contracted chest. In expiration a contraction wave is said to start at the periphery of this system, and proceed toward the trachea, thus helping the chest muscles and diaphragm to expel the air.[1] Nerve cells may be found among the muscles of the larger tubes, recalling the myenteric ganglia of the intestine. Spasmodic contraction of the musculature of the terminal bronchioles, in the absence of cartilaginous plates, may lead to complete exclusion of the air from the functional parts of the lung, especially *if the mucosa is also thickened by disease. Such asphyxia, found in one form of asthma, is* automatically relieved when the blood returning by way of the bronchial arteries reflects the lack of oxygen in the lungs by an insufficient supply of the muscles, causing them to relax.

The study of sections of the adult lung is facilitated by comparison with those from an embryonic lung. Comparable sections, including the pleura and drawn at the same scale of magnification, are shown in Figs. 361 and 362. In the lung of the embryo of four months, the terminal branches of the bronchioles are found in the centers of lobules, one of which is shown in Fig. 361 (bounded by *b. v.* and *lym.*). The axial bronchioles break up into ramifying tubules lined with cuboidal cells (Fig. 359). The main arteries run with the axial bronchioles in the centers of lobules; and the large veins and lymphatic vessels are at their periphery. This arrangement is retained in the adult. Deep in the lung, the small bronchi are surrounded by considerable connective tissue, containing arteries, veins and large lymphatic vessels.

In sections of the adult lung the bronchioles have become inconspicuous, though still accompanied by connective tissue and vessels. The ramifying tubules have given place to voluminous, branching, thin-walled air spaces, with very little connective tissue between them. The difference can be explained by the changes incident to birth.

During late fetal life the cuboidal or columnar epithelium lining the rounded end pieces undergoes a fatty degeneration, the cytoplasm becoming hyaline and losing its mitochondrial content.[2] The connective tissue, also through fatty degeneration, partly disappears, making space for the expansion of the air passages, which takes place either by gasping movements in utero, with the inhalation of amniotic fluid, or more rarely

[1] MACKLIN, 1929. [2] STEWART, 1923.

by the first respirations of air. The expanded passages meet their neighbors and by mutual pressure become angular. The connective tissue is reduced to a thin layer in which the vascular net often occupies the whole thickness.

FIG. 360.—A LONGITUDINAL SECTION OF THE UPPER LOBE OF THE RIGHT LUNG OF AN EMBRYO PIG—6 CM. LONG, IN WHICH THE LYMPHATICS WERE INJECTED WITH PRUSSIAN BLUE THROUGH THE RETROPERITONEAL SAC, AND THE VEINS WERE INJECTED WITH INDIA INK THROUGH THE PULMONARY VEIN. SECTION IS 400 μ THICK AND UNSTAINED. CLEARED BY THE SPALTEHOLZ METHOD. × 39. (Cunningham.)

Histologists differ in their conceptions as to the fate of the epithelium and the ultimate character of the alveolar walls. According to one group the softened epithelial cells are flattened by the expansion and those overlying the capillaries become squamous, or more usually produce a thin flange-like process covering the vessel, the part of the cell between

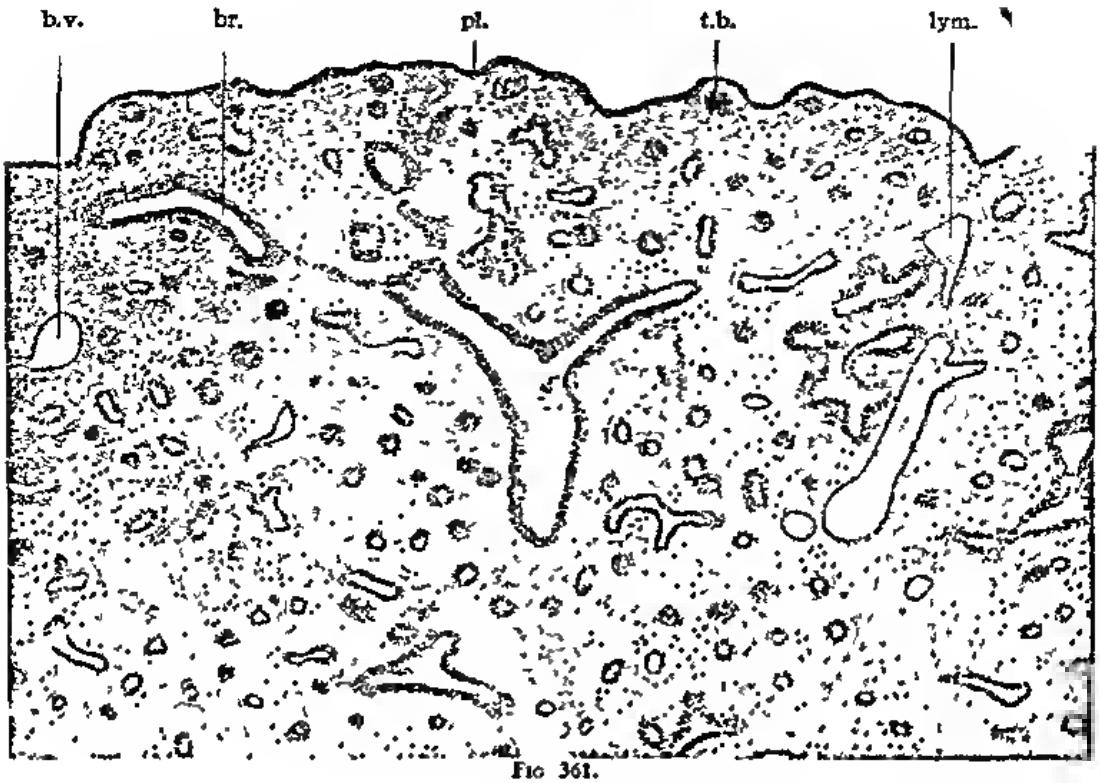


Fig 361.

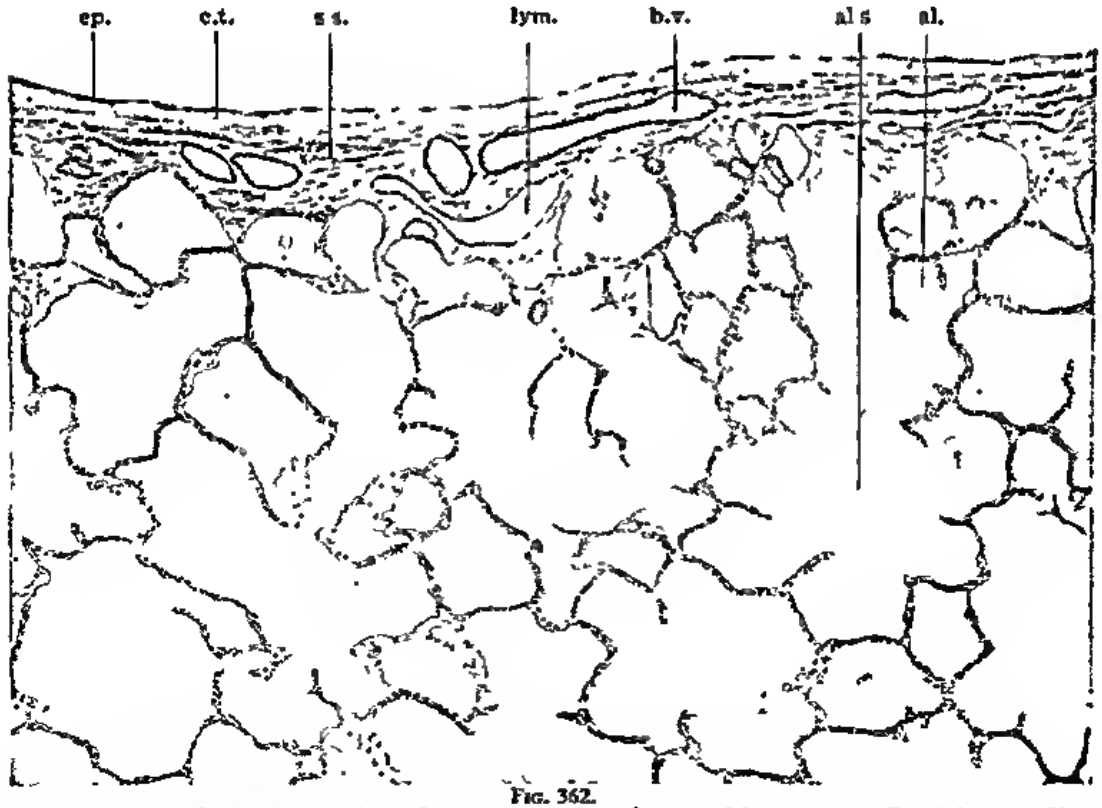


Fig. 362.

Figs. 361 and 362.—Sections of the Lung Drawn on the Same Scale of Magnification; Fig. 361 from a Human Embryo of Four Months, Fig 362, from an Adult.

al., Alveolus, al. s, alveolar sac, br., bronchiole, b v, blood vessel, c. t., outer layer of pleural connective tissue, ep, pleural epithelium, lym, lymphatic vessel; pl, pleura, s. s, subserous connective tissue, t. b, terminal branch of the bronchiole

vessels remaining cuboidal or rounded in shape and containing the spherical nucleus. The nucleated portions of several cells are thus grouped in the meshes of the capillary net, their flanges or films of protoplasm radiating over the surrounding vessels. In silver nitrate preparations the flanges often seem separated from the body of the cells. This peculiar type of simple epithelium consisting of groups of rounded cells alternating with 'non-nucleated plates' was called by Kölliker[1] the

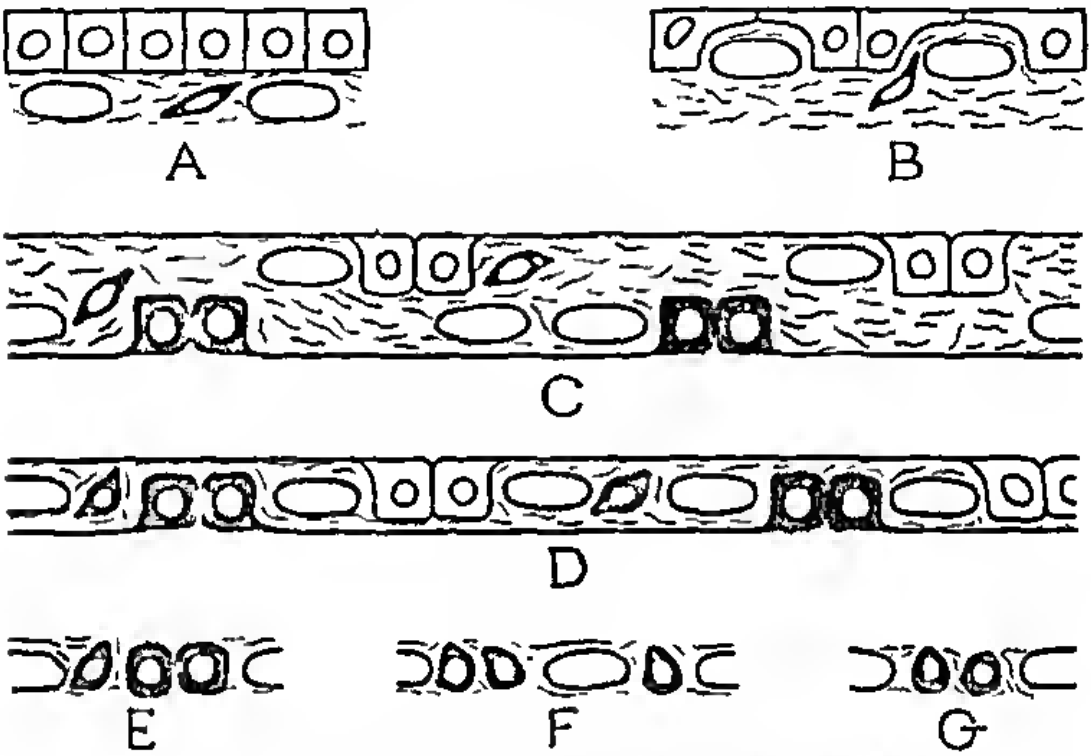


FIG. 363.—DIAGRAM TO SHOW RELATION OF EPITHELIUM, CONNECTIVE TISSUE AND BLOOD VESSELS IN ALVEOLAR WALL ACCORDING TO DIFFERING CONCEPTIONS. CONNECTIVE TISSUE IN BLUE.

A, B, and C, stages leading to D, representing 'respiratory epithelium' on both sides of an alveolar septum. E, F, and G, different views of the loss of entodermal elements and basement membrane.

respiratory epithelium. As the walls of two alveoli come together the cuboidal cells of one alveolus meet the plates of the other and form props for a network of flattened spaces in which runs the capillary net. The exchange of gases can take place on both sides of the capillaries; the endothelial nuclei are usually found near the epithelial cells. This economically excellent arrangement is not, however, always attained, as shown in Fig. 364. Sometimes the epithelial plates are merely flattened cells, and often the capillaries can function only on one side. Besides the epithelium and endothelium the alveolar walls contain connective tissue cells, wandering cells, many elastic fibers, and the contents of the blood stream. The richness of the capillary network in the alveolar wall is seen in injected specimens, and may be compared with similar injections of the intestinal villi. Respiration takes place by the transfer of gases

[1] KOLLIKER, 1881.

between the blood in these vessels and the air in the alveoli, therefore through the endothelial cells and alveolar plates, together with the trivial amount of connective tissue which may intervene. There is also a thin layer of fluid always present in the alveoli, so that vertebrates resemble fish in that they still receive oxygen through a watery medium.

The 'plates' are not, however, visible in adult lungs, so that the capillaries often seem naked. If they are obviously covered, a second

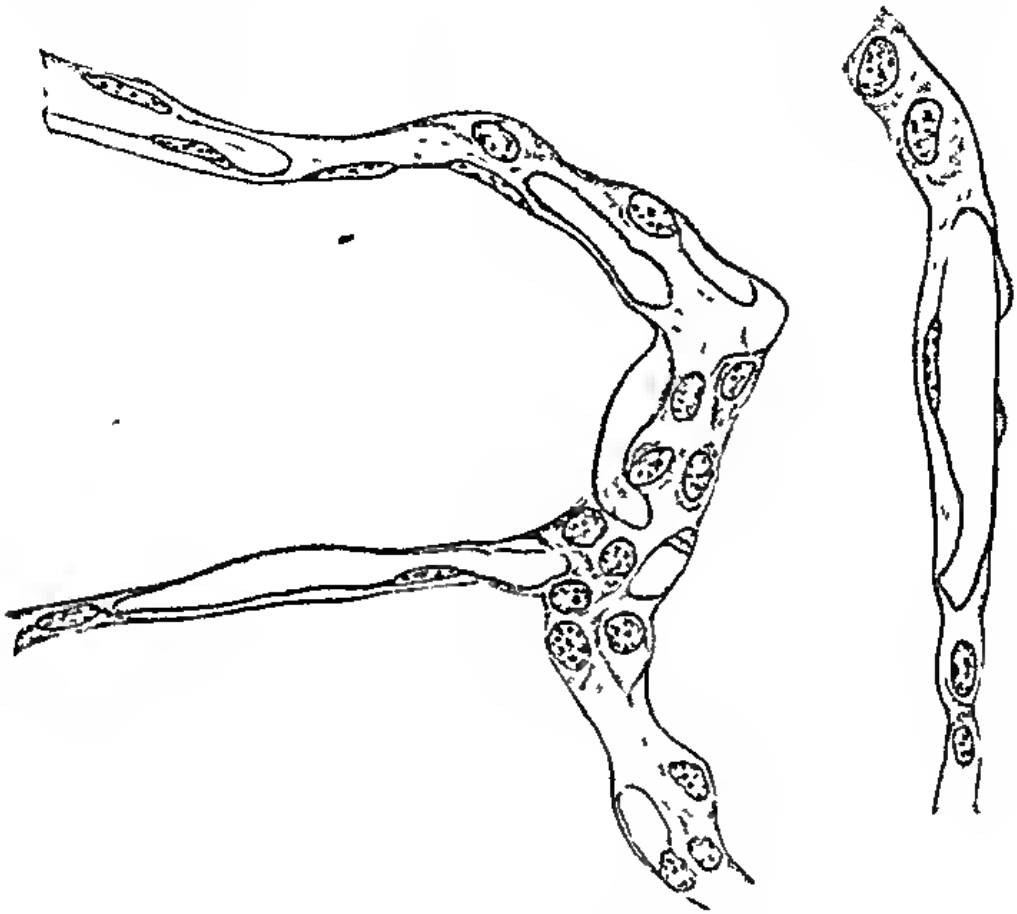


FIG 364.—PORTIONS OF INTERALVEOLAR SEPTA, HUMAN LUNG

group of authors considers that the surface material is of connective tissue origin, a sort of basement membrane.[1] The epithelium in this case would be discontinuous, and consist only of separate groups of cuboidal cells (Fig. 363, E).

A third school regards the alveoli and alveolar ducts as modified tissue spaces, which have either entirely lost their entodermal lining, or are the result of the distending action of the air coming from the open ends of the bronchial tree. This conception, originating formerly because of the abrupt change from the cuboidal epithelium of the bronchiole to the thin walls of the air spaces, has been revived because of the character of certain cells in the alveoli.

[1] SEEMANN, 1931.

Within the air spaces of the new-born are certain rounded, granular cells which have been identified as from the amniotic fluid. These disappear within a few days, and are replaced by others apparently identical in form and phagocytic activity with the tissue macrophages. In older lungs they are usually loaded with carbon particles, and therefore are designated *alveolar phagocytes*, or '*dust cells.*' They are probably derived from the cells lining the alveoli, and all stages of their enlargement, engorgement of foreign particles and final desquamation can be followed (Fig. 365).

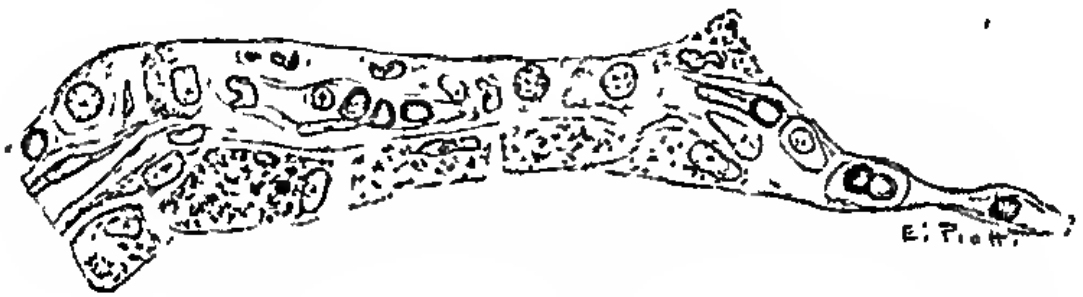


FIG. 365.—ALVEOLAR PHAGOCYTES WITH CARBON, IN HUMAN LUNG. APPARENTLY THEY ARISE FROM THE LINING EPITHELIUM AND THEN BECOME FREE.

The alveolar phagocytes ingest only material brought to them by the air, and are thus a protection for the lungs from external invasion. Their response is so rapid that it seems improbable that they could come from any but surface cells. Particulate matter injected intravitam into the pulmonary or bronchial vessels passes through the lung untouched, or is ingested by a very few tissue macrophages lodged in the interalveolar septa and in the peribronchial connective tissue. In appearance and behaviour the alveolar phagocytes are so similar, however, to the tissue macrophages of other parts of the body that many histologists consider them identical, and, since the tissue macrophages are known to be derived from the mesoderm, have postulated that the lining of the alveoli, from which the alveolar phagocytes are derived, must also be of *mesodermal origin.*[1] *These cells, called septal cells, are considered resting* histiocytes. Lewis[2] thinks they may be renewed from the store of Kupffer cells, brought from the liver by the blood; the capillaries are considered naked (Fig. 363, G).

There is as yet no incontrovertable proof of the correctness of any of these views. If the septal cells are entodermal, representing part of the original pulmonary outgrowth, they can turn into true phagocytes, showing that cells of different origins may differentiate along similar lines for similar purposes, though entodermal cells are not usually very phagocytic. In certain diseases[3] a surface layer is lifted away from the capillaries by an effusion, but this layer may be the basement membrane.

[1] FRIED, 1934 [2] LEWIS, M. R., 1925*b* [3] MILLER, 1932.

In other abnormal cases the alveoli become partially lined by a continuous cuboidal epithelium, but this may be an aggregation of histiocytes, or entodermal cells which have grown down from the bronchioles.[1] The failure to detect the epithelial plates in the adult lung may be due to their extreme thinness or to a great irritability causing them to retract on contact with a fixative. The Bensleys[2] have made them visible 'in many

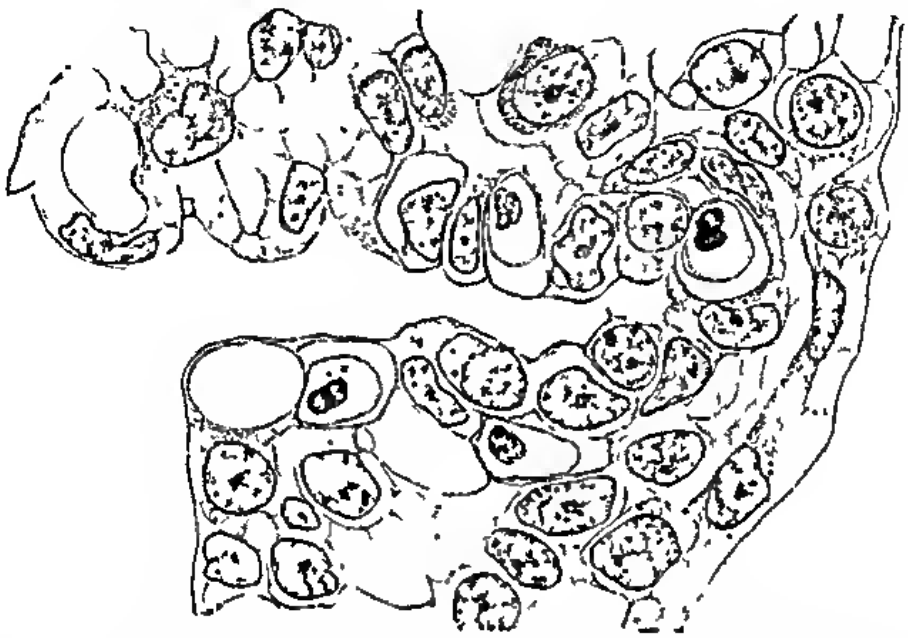


FIG. 366.—ANGLE OF AN ALVEOLUS OF LUNG OF OPOSSUM OF 12 MM. THE EPITHELIAL CELLS ARE PARTLY CUBOIDAL, PARTLY STRETCHED OVER THE CAPILLARIES, WHICH EITHER ARE EMPTY OR CONTAIN NUCLEATED CORPUSCLES

places' by injecting gold sodium thiosulphate intravenously into a rat, which so swells the ground substance of the alveolar septum that by proper staining the epithelial membrane can be differentiated from the endothelium of the capillaries by the intervening thickened basement membrane.

The formation of the alveolar plates or flange-like films can be followed occasionally in the late fetus, and especially in the opossum. This animal is only 10–13 mm. when born, and the lung is represented by only a few generations of bronchial branches. Not only are the end-buds enlarged and lined by a respiratory epithelium in which the flanges are often so thick as to be obvious (Fig. 366), but as new sprouts grow out from the angles of these end-buds forming new generations of branches, the parts which formerly breathed are converted into bronchioles and the epithelium reverts to the cuboidal type, as the closely investing capillary net disappears.[3] In the cat and rabbit also postnatal growth is accomplished by the development of new terminal alveoli and the reduction of older alveolar ducts to respiratory bronchioles by the downgrowth of muscle elements that choke and reduce the alveoli. The alveoli and lobules of the adult lung are more numerous, not larger than those of the infant, and the number of branches in the bronchial tree increases till adult life. In all this the epithelium acts like a continuous sheet, directly traceable from the embryonic entodermal tree.

[1] LOOSLI, 1935. [2] BENSLEY AND BENSLEY, 1936. [3] BREMER, 1935

The presence of pores or fenestra through the septa between adjacent alveoli is attested by Macklin,[1] who finds them present at all ages. They are rounded apertures with smooth edges, from 2 μ to 55 μ or more in diameter. They promote equalization in alveolar air pressure.

The pulmonary and bronchial blood vessels have already been described. The pulmonary arteries are axial vessels within the lobules, breaking up into terminal branches at the atria, and these branches become axial along the alveolar sacs. Each terminal branch has been described as the center of an ultimate lobule or structural unit. The veins are peripheral both in the units and larger lobules; between the latter they run through connective tissue septa.

The abundant lymphatic vessels are arranged in a superficial set draining into the pleura by way of the interlobular septa, and a deep set draining toward the hilum along the bronchi, accompanying the large vessels. Lymphatics of the deep set do not extend into the lobules; they terminate along the alveolar ducts. Around the larger bronchi and at the root of the lung, lymph glands are numerous. A conspicuous feature of the sections of the lung is the presence of black soot in the tissue around the lymphatic vessels. It penetrates the pulmonary epithelium in the smallest bronchioles, apparently passing between the epithelial cells. Some of it is taken up by the septal histiocytes, which receive it partly through the alveolar walls or from degenerated dust cells. Much of the inhaled material must be transported in the free phagocytes to the pharynx, the bronchial cilia helping. That entering the lymphatic vessels is distributed along their courses. On the surface of the lung it is seen in the interlobular septa, marking out the boundaries of the lobules. Because of the steady increase in this deposit, the color of the lungs changes from birth until old age.

The nerves of the lung are derived from the vagus and the sympathetic chain from both of which fibers enter the anterior and posterior pulmonary plexuses at the hilum. From these, three sets of nerves proceed further, to the bronchial tree, to the arterial tree, and to the visceral pleura. In the bronchi and bronchioles two plexuses can be recognized, the extrachondral and the subchondral,[2] the former of larger nerve bundles with frequent clusters of ganglion cells, often located between the cartilage plates, sending axons to muscles and glands; the latter made chiefly of non-medullated postganglionic fibers, with only a few clusters of cells supplying the epithelium. The deep glands only are under nervous control, the goblet cells being activated by local stimulation.[3] In the smaller bronchioles these two plexuses merge, and they extend no

[1] Macklin, 1935 [2] Larsell and Dow, 1933.

[3] Florey, Carleton and Wells, 1932.

further than the alveolar ducts. The periarterial plexus accompanies the vessels to the alveolar capillaries, and in certain places appears to mingle with the peribronchial plexus. The nerves to the pleura may reach it directly at the hilum or run through the septa between the primary lobules. Beside motor nerves to the muscles and glands, sensory endings

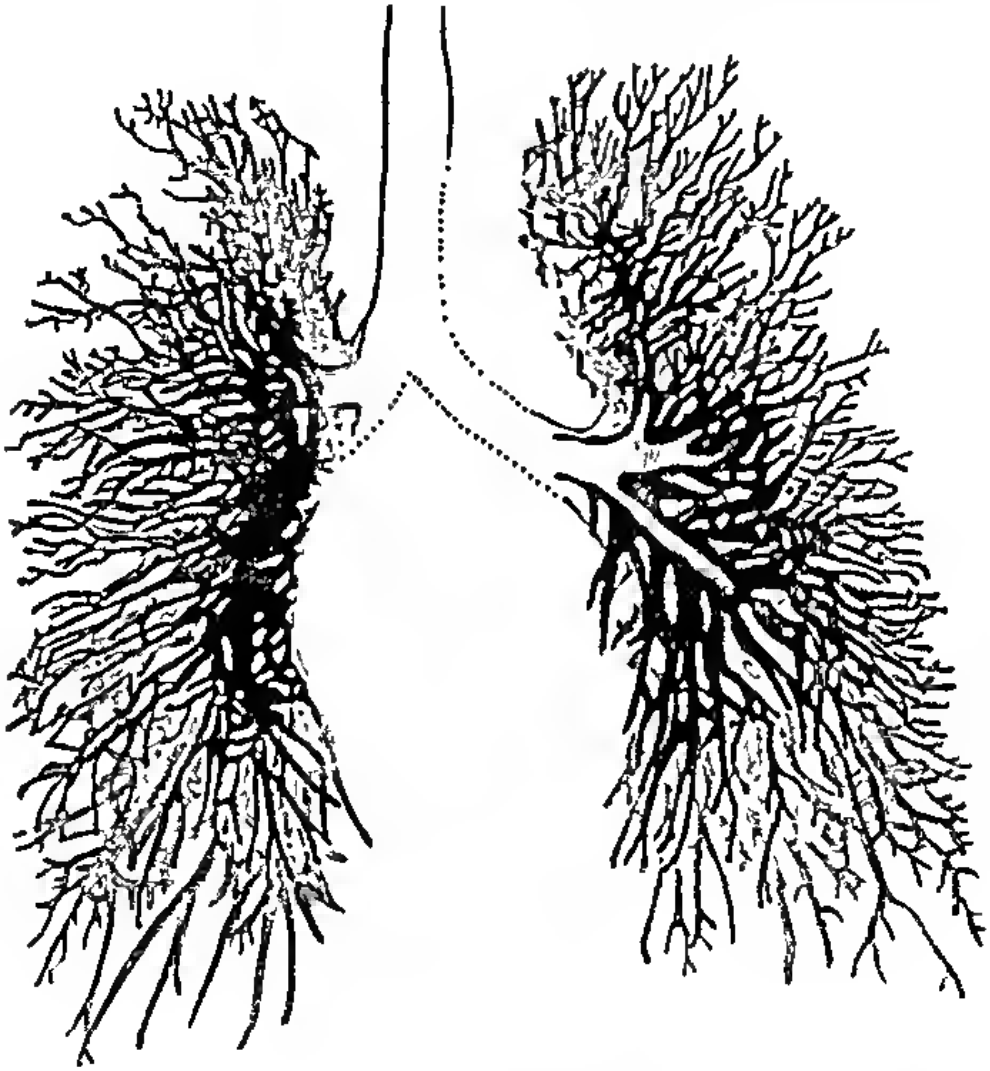


Fig. 367.—Bronchial Tree

Tracings made from x-ray shadows, gray, inspiration, black, expiration. (Redrawn from a colored illustration with permission of the author, C. C. Macklin.)

have been described in relation with the bronchial muscle and in the bronchial epithelium.

Pleura. The visceral pleura (*pleura pulmonalis*) is a thinner layer than the parietal pleura, and is closely attached to the lung. It is covered with a single layer of flat mesothelial cells, which in the collapsed lung become thicker and shorter. In specimens which have been handled, this layer is often lacking. It rests upon a thin layer of fine-meshed fibrous tissue, beneath which is the coarse subserous layer continuous with the interlobular septa of the lung (Fig. 362). This tissue is highly elastic. In the subserous layer, blood vessels, derived from both pulmonary and bron-

chial arteries, form an abundant capillary plexus. The superficial lymphatic vessels are very evident, and in relation with them lymphoid tissue is found, and occasionally lymph nodules.

The parietal pleura is a thicker and less elastic layer. Ventrally and below, toward the pleuro-pericardial membrane, it exhibits folds or villi,

FIG. 368.—ELASTIC NETWORK IN THE PLEURA. MIDDLE ZONE OF THE PLEURA PULMONALIS OF A 22 YR. OLD MAN. ORCEIN. × 230. (HAM.)

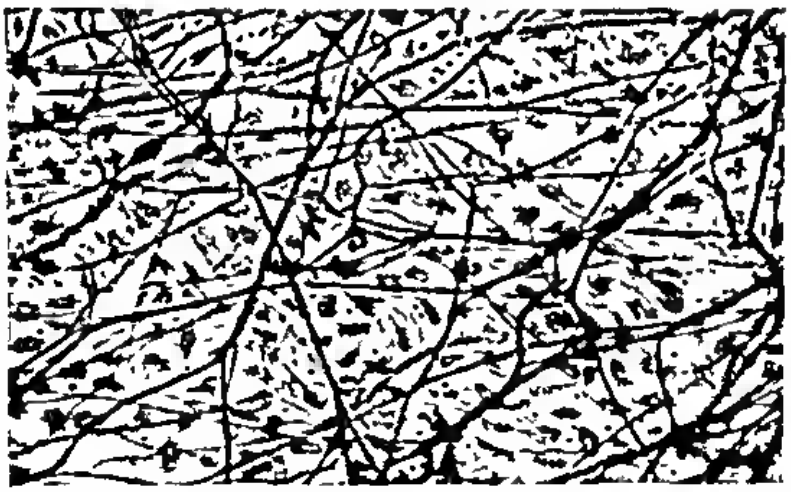


FIG. 369.—ELASTIC NETWORK IN THE PLEURA. SUPERFICIAL ZONE OF THE PLEURA COSTALIS OF A 22 YR. OLD MAN. ORCEIN. × 250. (HAM.)

containing fat (*plicæ adiposæ*). Fat may also be found in the pleura elsewhere. Milk spots (*tâches laiteuses*) similar to those found in the peritoneum and regarded as foci of macrophage, fibroblast, and fat cell production, have been noticed[1] in the human pleura at birth.

The nerves of the pleura are derived from the phrenic, sympathetic and vagus nerves. In the parietal pleura typical lamellar corpuscles may be found, together with the smaller variety, known as the Golgi-Mazzoni corpuscles.

[1] KAMPMEIER, 1928*b*.

THE ENDOCRINE GLANDS

In the classification of glands (p. 82) it was pointed out that certain epithelial outgrowths lose their ducts by obliteration during the course of development. In other cases masses of cells grow from an epithelial surface either as solid cords or by the migration of individual cells, which later become grouped; these also form endocrine or ductless glands. The lymph gland and spleen are also strictly ductless glands, but are better classed with the testis and ovary as cytogenic, in that their function is, in part at least, the production of cells.

The ductless glands may arise from any of the germ layers. From the ectoderm are derived the two parts of the pituitary body or hypophysis, the pineal body, the medulla of the suprarenal gland, and the chromaffin bodies. The mesoderm provides the cortex of the suprarenal gland, the interstitial cells of the testis and the corpus luteum of the ovary. From the entoderm come the thyroid (or thyreoid) gland, the parathyroid glands, the thymus and the islets of the pancreas. The glomus caroticum was formerly included in this group, but recent investigations show that its active cells are probably of ectodermal (sympathetic) origin, not from the entodermal pharyngeal pouches.

Endocrine glands vary greatly in appearance. In some the active cells are arranged in orderly rows or surround definite follicles; in others no order is observable, the cells lying in groups or even singly in the stroma. In some the 'gland' is merely an inconspicuous part of some organ, as in the testis. Apart from these scattered groups of cells, however, the recognized ductless glands have certain points in common. All secrete definite, but different, substances which are taken up by the blood or lymphatic vessels and carried in the general circulation to act on other cells of the body; and in most of them the cells are arranged, therefore, in close relation to the vessels. The active principle of the secretion is known as a *hormone*. The glands are called endocrine glands, or glands of internal secretion.

The recent studies of the glands of internal secretion have been the means of great advances in physiology and medicine. The recognized endocrine glands may each produce several hormones, some of which may act on other endocrine glands either as activators or inhibitors. The pituitary gland especially has been called the 'master gland' from its control of other endocrine organs. The literature on this subject is enormous; the reader is referred to the books by Sharpey-Schafer,[1] Allen,[2] and to many papers in the journal 'Endocrinology.'

Histologically the endocrine glands fall into four classes. (*a*) The thymus gives the appearance of lymphoid tissue. (*b*) The thyroid gland,

[1] Sharpey-Schafer, 1924. [2] Allen, 1939.

parathyroids, islets of the pancreas, cortex of the suprarenal gland, the anterior and middle lobes of the hypophysis, and the glomus caroticum show groups or cords of epithelioid cells in close relation with the vessels; in the hypophysis, and more fully in the thyroid gland, the cords become segmented and the cells of the resultant groups rearrange themselves to form hollow vesicles. (*c*) The posterior lobe of the hypophysis, the pineal body, the medulla of the suprarenal gland and the chromaffin organs are of nervous origin, and are alike in having no definite arrangement of cells. (*d*) The interstitial cells and those of the corpus luteum lie irregularly in groups in the stroma. The decidual cells of the pregnant uterus should probably be added to this group.

Hormones or somewhat similar substances are said to be given off by the epithelial cells of the duodenum (secretin) and of the pyloric end of the stomach (gastrin), acting especially on the pancreas and gastric glands respectively. It is probable that many other cells, as for instance the connective tissue cells, may contribute an 'internal' secretion as part of their function though it is not usual to class them as glands of internal secretion.

HYPOPHYSIS

The hypophysis (*i.e.*, growth beneath the brain), or pituitary body, is a rounded mass, about 1 cm. long in sagittal plane, 1.5 cm. wide and 1 cm. thick, and weighing about 0.5 gm., attached to the tip of the infun-

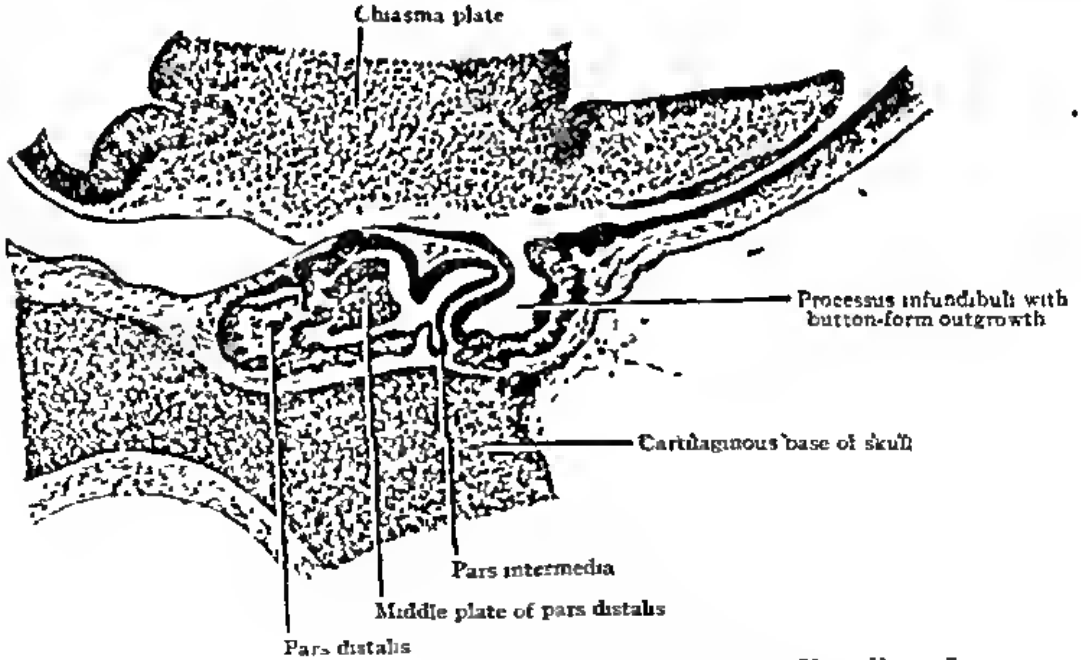


Fig. 370.—A Median Section through the Hypophysial Primordium of a 28 mm Human Embryo. Absolute alcohol fixation. Held's haematoxylin. × 45. (Schaffer.)

dibular process on the floor of the brain, and lodged in the sella turcica of the sphenoid bone. It is thus close to the optic chiasma, and its pathological enlargement is often accompanied by visual symptoms due to pressure on the optic tracts. Its stalk of attachment to the infun-

dibulum passes through the fibrous diaphragma sellæ, and in removing the brain the hypophysis is therefore usually torn from its stalk and left in the body excavation. It is a most important organ of internal secretion, consisting of two parts, distinct from each other in origin and function. The anterior lobe, or buccal or glandular part, is formed from Rathke's pouch[1] which grows upward from the oral ectoderm and encounters the knob-like posterior lobe or nervous portion which is an evagination of the floor of the third ventricle of the brain. The whole organ is thus ectodermal in origin. Both portions expand at their ends

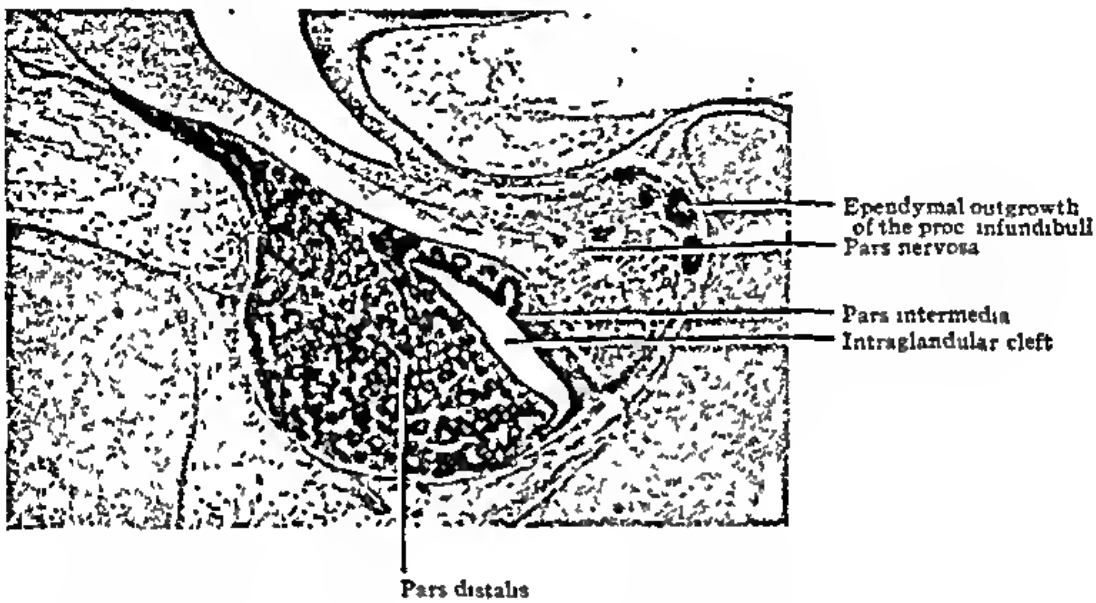


FIG 371.—A MEDIAN SECTION THROUGH THE HYPOPHYSIS OF A HUMAN EMBRYO OF 105 MM., CROWN-RUMP LENGTH, × 30 (Schaffer)

and become stalked. The anterior part then sends up a short process on each side which together enfold the infundibular stalk and spread over the base of the brain. The larger anterior part also partly enfolds the smaller posterior lobe laterally, so that Cushing ventured to describe *the posterior lobe as resting in the anterior lobe like a ball in a catcher's* glove.

The *anterior lobe* becomes separated from the roof of the mouth by the obliteration of its duct, which is reduced to a slender solid epithelial strand and ruptures in embryos of about 20 mm. A depression marking its former outlet has sometimes been found in the vault of the pharynx, and there may be a canal through the sphenoid bone, the craniopharyngeal canal, which follows the course of the former stalk. It is said that a small 'pharyngeal hypophysis,' having the structure of the anterior lobe, is constantly found near the pharyngeal end of this canal, and a similar accessory part is described in dogs, but not in man, beneath the dura of the sella turcica. The *posterior lobe* proper, or *pars nervosa*, at first a hollow

[1] RATHKE, 1838

outgrowth from the brain, loses its cavity in man and most mammals (the cat is an exception) by the thickening of its walls, so that the enlarged rounded end and narrow stalk leading to the infundibulum are solid.

The hypophysis can hardly be overlooked in examining the brain, and its existence is recorded by the earliest writers. The epiphysis, on the top of the brain, was called the pineal body from its resemblance to a pine cone, and according to Hyrtl the hypophysis below, being a round structure attached to a stem, was named the 'rose hip' by the Mohammedan physician Avicenna (ca. A. D. 1000). Vesalius introduced the name *pituitary gland* The pituita or phlegm was believed to be excrementitious material, eliminated by the brain and received by the naso-pharynx, and its possible origin by way of the olfactory nerves had been discussed. Vesalius and his followers believed that it was collected by the infundibulum and eliminated by the pituitary gland. If the sella turcica of a prepared skull is examined, four grooves may be traced from it, two passing forward to the optic foramina, and two passing backward to the lacerated foramina. Vesalius pictured these four channels as outlets for the pituitary gland, the two latter (which in life are closed by cartilage) being in relation with the naso-pharynx. Bartholin recorded another function of the pituitary gland, namely, 'to *close* the infundibulum lest vital spirits should escape,' and finally V. C. Schneider showed conclusively that the pituitary gland is not the source of phlegm. According to Hyrtl this was accomplished in five classic but lengthy books, *De catarrhis*, 1640–1642, and he adds—'No physician and no anatomist should leave this fundamental and learned work unread—if he has time for it.'

The anterior wall of the flattened buccal pouch becomes greatly thickened by the multiplication of its cells, and is known as the *pars distalis* of the anterior lobe. The forked process which encloses the infundibulum reaches in some animals to the tuber cinereum, a slight elevation of the brain floor, and hence is called the *pars tuberalis* of the anterior lobe. In man it may be only slightly developed in extent and thickness. The cavity of the pouch becomes reduced in childhood to a transverse slit, which in the adult is represented by disconnected flattened clefts, called the *residual lumen*. The posterior wall of the buccal pouch rests against the neural portion, and is known as the *pars intermedia*. While of considerable thickness in infants and in some animals, it is for the most part a thin, even discontinuous sheet of cells in the adult, and is moreover so closely applied to the pars nervosa and so inseparable from it, that the two together are usually spoken of as the posterior lobe, in spite of their different origins.

The pars distalis consists of solid, branched and uniting epithelial cords of irregular caliber and composed of irregularly arranged types of cells. Between the cords and in close relation with them are wide lacunar capillary vessels derived from several arterioles. The vessels are arteriovenous connections, not true sinusoids, though having a sinusoidal nature. Their lining cells are phagocytic, thus belonging to the reticulo-

endothelial system. The cords and vessels rest on a slight reticular framework, reinforced by larger septa of connective tissue.

The cells of the cords are of three main types,[1] the chief cells or chromophobes, the granular acidophils or eosinophils (Alpha cells), and the granular basophils (Beta cells). The chromophobes are small, rounded or polyhedral, and closely packed together. Characteristically

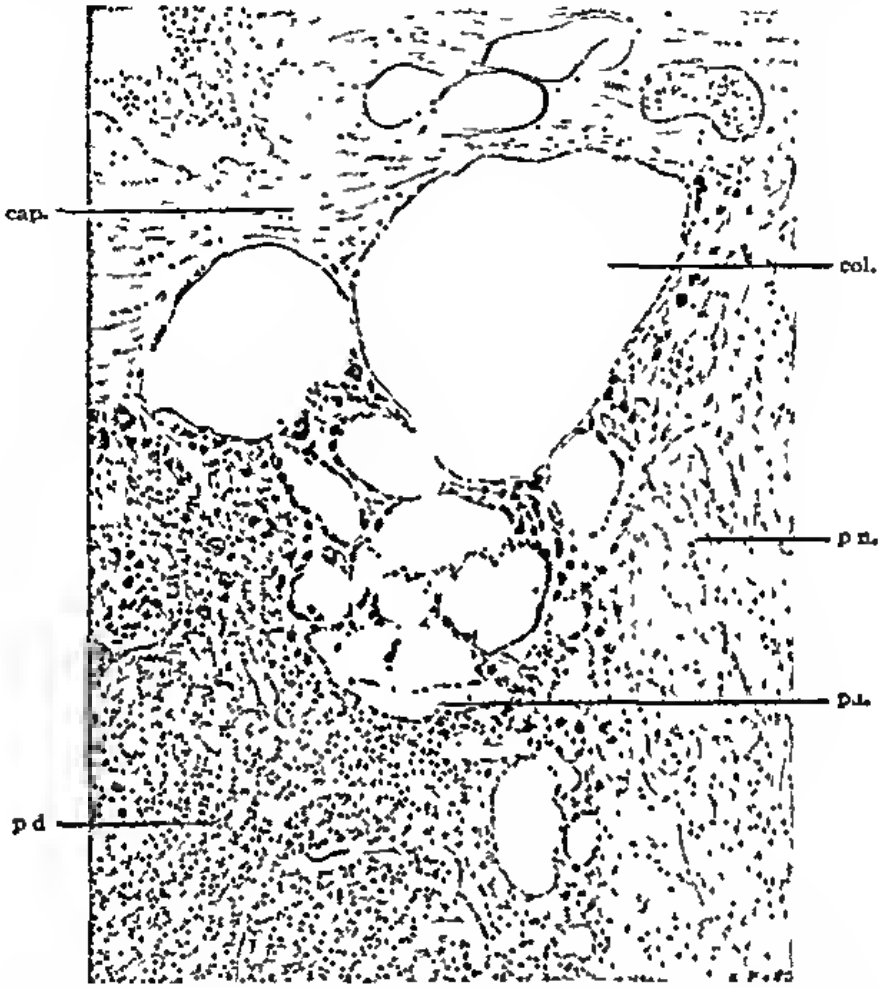


FIG 372.—JUNCTION OF GLANDULAR AND NERVOUS PORTIONS OF HYPOPHYSIS, HUMAN
cap., capsule; p. d., pars distalis, p. i., pars intermedia, p. n., pars nervosa, col., colloid in follicle of pars intermedia.

their cytoplasm is very clear, the cell walls inconspicuous. Occasionally they may take either an acid or a basic tinge, without definite granules. The granular cells are larger, rounded or columnar, and their cytoplasm contains specific granules exhibiting the affinities for basic or acid stains. The acidophilic granules are said to be larger than the basophilic (the reverse of the condition found in the blood cells), but this is not always apparent. The granules may fill the cells or be grouped at one pole. The nuclei of all the cells are usually round and vesicular, with little chromatin, but pycnotic nuclei are not infrequent. The distribution of the cells is irregular within the pars distalis and within the individual cords. The chromophobes are by far the most numerous; the acidophilic cells usually

[1] GEMELLI, 1906.

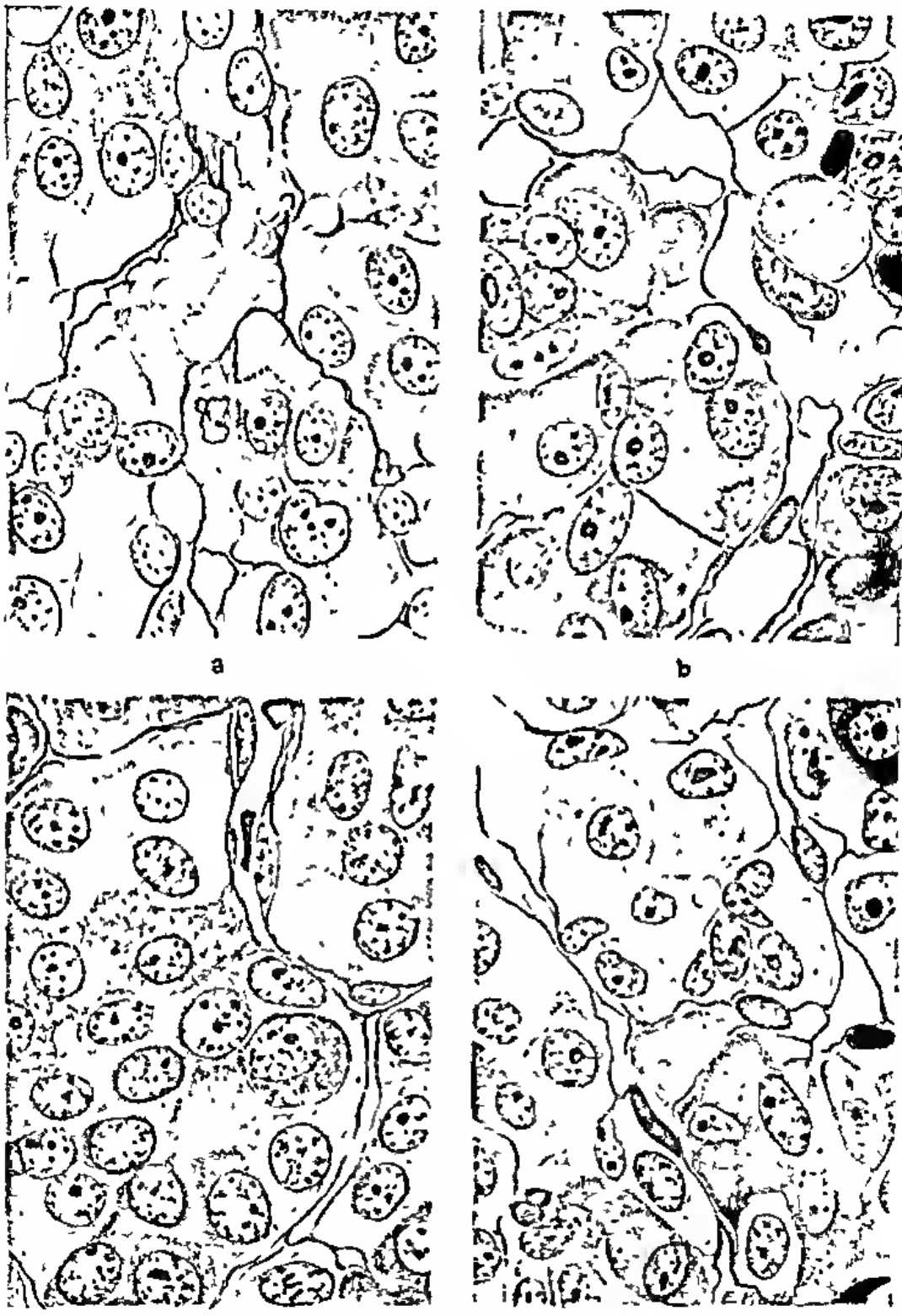


FIG 373.—PORTIONS OF SECTIONS FROM THE ANTERIOR LOBE OF THE HYPOPHYSIS.

a, cat-anestrous Ordinary acidophil cells are orange-yellow, chromophobe cells are pale blue, b, cat—last week of pregnancy, appearance of granular cells (acidophils) staining red with azocarmine, c, rabbit—preovulatory stage showing a localized carmine reaction, d, monkey—given daily injections of estrogen, castrated and killed 19 days later Numerous 'carmine cells' Sublimate-formaldehyde fixation, modified azan stain (Drawn from preparations of Dr. A B Dawson)

outnumber the basophilic. The chief cells usually occupy the center of the cords but also frequently extend to the surface, while both types of granular cells may occur singly or in small groups, frequently simulating a columnar epithelium at the surface of the cords, resting against the blood vessels. In the ox the basophils are said to be in greater concentration in the center of the anterior lobe, but in man they are perhaps more frequent at its periphery.

The genetic relations of the different cell types have been much discussed. The idea that they represent a single strain passing through a series of growth phases must be doubted, partly because of the different reputed activities of the two granular types. Tumors involving chiefly an increase in the number of acidophils produce increase in bone growth, leading to gigantism in the young subject and to acromegaly (increased thickness of the bones of the face and hands chiefly) after the epiphyses have united Basophilic tumors cause over-development of sexual characteristics,[1] and basophils are much increased in the hedgehog after hibernation,[2] when sexual activity is greatest. The anterior lobe becomes larger during pregnancy, owing chiefly to an increase of altered chromophobe cells, of a kind which might be considered transitional between chromophobes and eosinophils, and called 'pregnancy cells.'[3] In some animals cyclical changes, corresponding to œstrous periods, have been noted.[4] Friedgood and Dawson[5] differentiate two distinct types of acidophil cells; one the ordinary type and the other a special type, staining with azocarmine, which they designated as 'carmine cells.' Their numbers are correlated with the different stages of reproductive activity. The illustrations in Fig. 373 are drawn from some of their preparations. Atwell[6] discusses the interrelations from the point of view of the Golgi apparatus, and believes that the two granular types are developed separately from the chromophobes, which constitute a large 'reserve,' to which both types may return after discharging their secretion. Two distinct types of chromophobes, characterized by different forms of Golgi apparatus, have been described.[7] The hypophysial cells being highly differentiated, mitoses are seen but rarely in the 'normal' gland Under experimental conditions and under abnormal functioning numerous mitotic figures may be observed.[8,9,10]

At infrequent and irregular intervals the cells of the cords may be so arranged as to enclose a lumen, thus making small follicles, reminiscent of the thyroid gland. The walls may be one or two cells thick, and may include any of the cell types. The surface cells may even show slight top plates and terminal bars. The lumen contains a 'colloid,' either hyaline or granular in character. Small masses of this material may also be seen among the cells of a cord without evident follicle formation. It is also found in isolated portions of the residual lumen, which may closely simulate the true follicles. Not infrequently the cells bordering the original cleft show cilia, recalling their naso-pharyngeal origin. The

[1] Cushing, 1932. [2] Rasmussen, 1921. [3] Erdheim, 1926.
[4] Wolfe and Cleveland, 1933. [5] Friedgood and Dawson, 1938.
[6] Atwell, 1932. [7] Severinghaus, 1933. [8] Pomerat, 1941.
[9] Dawson, 1942b. [10] Hunt, 1943.

colloid has been regarded by some as one of the active secretory products of the gland.

The pars tuberalis and the intermediate lobe have a similar arrangement of cords and blood vessels, but the size and distinctness of the cords vary in the different lobes. The cells are apparently similar in character in all the buccal portion, though the size of the cells and of the granules in them is said to be greatest in the anterior lobe. Their proportionate numbers vary also. In the pars tuberalis most of the cells are chromo-

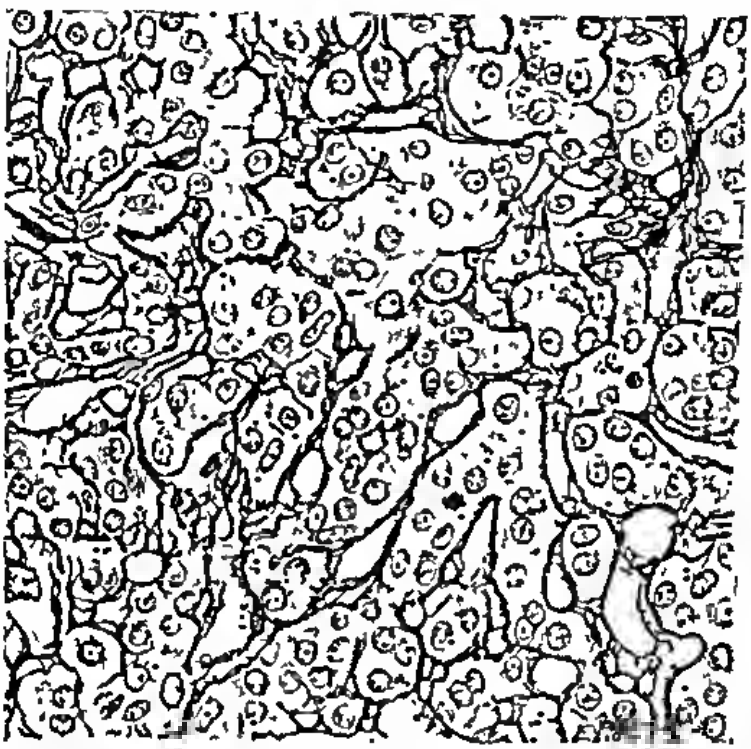


FIG. 374.—RETICULAR FIBERS IN THE ANTERIOR LOBE OF THE HYPOPHYSIS OF A RABBIT. Formaldehyde fixation, silver impregnation according to Pap and hæmatoxylin.

phobes; in the pars intermedia the basophils increase relatively, with only a few eosinophils; in the pars distalis about half of the cells are chromophobes, one-third eosinophils and one-sixth basophils, though the ratios vary greatly with age and sex.[1] A further distinction has been noted in the number and size of the follicles present, which are most common in the pars tuberalis, less frequent but larger in the pars intermedia, and infrequent and small in the pars distalis.

The basophils of the pars intermedia show a remarkable tendency to invade the pars nervosa, either singly or in groups, or in childhood in the form of the tubular outgrowths from the residual lumen.[2] This migration may account for the diminishing thickness of the intermedia with age. Only the basophils seem to migrate and their fate is unknown.

[1] RASMUSSEN, 1933. [2] LEWIS AND LEE, 1927.

The posterior lobe, or pars nervosa, contains ependymal and neuroglia cells, but no true nerve cells and only a few nerve fibers. The ependyma lines the cavity which extends downward into the lobe from the infundibulum. Otherwise one can recognize only two types of cells, mossy neuroglia cells and larger spindle-shaped or multipolar cells, which resemble somewhat nerve cells, but in which no Nissl substance is found, though they may contain pigment granules. Scattered throughout the lobe are hyaline or colloid masses,[1] the 'Herring bodies,' thought by some to be the secretion of the gland, arising in the follicles of the middle and anterior lobes, and 'activated' on its passage through the posterior lobe to the cerebrospinal fluid. This has been denied by others, who consider them the remains of degenerated cells. The nerve fibers descending into the hypophysis mostly accompany the blood vessels, though some apparently end in end-bulbs or complicated whorls around groups of cells. Others run to masses of the colloid material or hyaline bodies, and it is suggested that they are degenerating. The function of the nerves is not understood.

The functions of the different parts of this complex gland are apparently so numerous and so varied that no agreement has been reached as to the details of the mechanism. The extract of the pars tuberalis seems to promote diuresis; that of the distalis, antuitrin or pituitrin, promotes growth, increases adrenal and thyroid function, causes lactation, and also affects the sugar regulation of the blood. Perhaps its best authenticated action is that on the ovary. The extract of the pars intermedia causes or hastens the growth and maturity of the gonads The pars nervosa probably acts on the blood vessels and smooth muscles These are only some of the better authenticated claims. The distribution of the cell types in the different lobes has been correlated with these functions only in so far as they agree with the observations of the results of tumor formations, that the basophils, more numerous in the pars intermedia, probably govern gonad growth, and the eosinophils of the pars distalis probably secrete the hormone governing bone growth.

As to the path of elimination of these various hormones, some probably pass directly to the sinusoidal blood vessels (no lymphatic vessels have been recognized in the gland parenchyma). *Others may act on the nervous centers of the adjacent brain floor to* produce their results, and Cushing considers that the colloid seeps through the tissues of the posterior lobe and infundibular stalk to be delivered directly into the cavity of the third ventricle for this purpose. Popa and Fielding,[2] on the other hand, describe a 'hypophysio-portal' circulation which collects blood from both lobes of the pituitary gland and distributes it to the hypothalamus. On each side the inferior hypophysial artery, from the carotid artery, sends a branch to the posterior lobe, where it breaks up into capillaries. Vessels from the circle of Willis, the superior hypophysial arteries, supply the infundibulum, stalk and anterior lobe, where they open into sinusoids. According to these authors the blood from the anterior and posterior lobes is drained partly by ordinary veins opening into the cavernous sinus, but chiefly by a number of 'hypophysio-portal' veins, which pass without anastomoses up the lateral surfaces of the infundibular stalk to the floor of the brain (hypothalamus), where they form a secondary

[1] HERRING, 1908. [2] POPA AND FIELDING, 1933.

distribution-net of capillaries within the brain wall. 'Colloid' has been reported in these veins and in the parts of the brain supplied by them.

Wislocki and King,[1] by careful injections of the monkey hypophysis, offers another explanation. According to these authors the vascular infundibulum is sharply marked off from the brain floor (Fig. 375). The superior hypophysial arteries enter both infundibulum and anterior lobe. From the sinusoids of the former the blood is collected into the

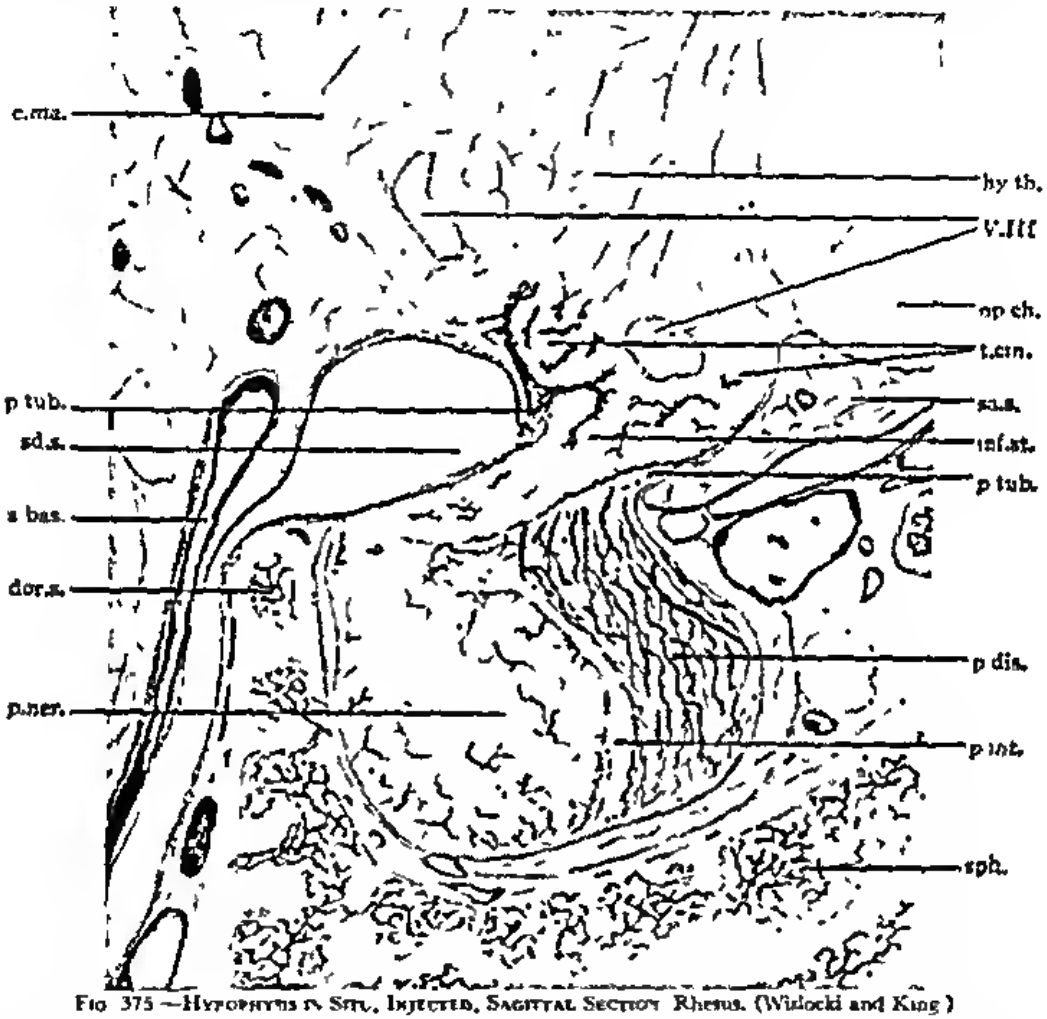


FIG. 375.—HYPOPHYSIS IN SITU, INJECTED, SAGITTAL SECTION. Rhesus. (Wislocki and King.)

A. bas., basilar artery, c. ma., mammillary body, dor. s., dorsum sellæ, hy-th., hypothalamus, inf. st., infundibular stalk, op ch., optic chiasma, p. dis., pars distalis, p. int., pars intermedia; p. ner., pars nervosa, p. tub., pars tuberalis, sa. s., subarachnoid space, sd. s., subdural space, sph., sphenoid bone, t. cin., tuber cinereum; v. III., third ventricle.

numerous 'portal' veins and flows down the stalk to the anterior lobe, where it again enters sinusoids. The anterior lobe thus receives both arterial and venous blood, and in this respect resembles the liver. It is interesting to note that the endothelial cells of these sinusoids ingest injected particulate matter as do the Kupffer cells. The anterior lobe drains laterally into branches of the cavernous sinus. The posterior lobe has its own, practically independent capillary circulation by way of the inferior hypophysial arteries and veins. Direct venous transportation from hypophysis to the hypothalamus would be denied in this conception.

PINEAL BODY

The pineal body or gland (epiphysis cerebri, conarium), is a median evagination from the caudal end of the roof of the forebrain, covered by

[1] WISLOCKI AND KING, 1936.

pia mater and overlapping the superior corpora quadrigemina. It is a slightly flattened cone-shaped structure 5–8 mm. in length and 3–5 mm. in breadth, attached to the brain by a hollow stalk lined by ependyma. The originally solid ectodermal outgrowth is invaded by connective tissue septa, so that it is later composed of irregular cords or masses of epithelioid cells. The septa contain numerous blood vessels and all the usual connective tissue elements.

The cells of the cords are of two types, neuroglial and parenchymal The neuroglial cells, when brought out by special methods, are stellate,

FIG 376.—A SECTION THROUGH A PORTION OF A HUMAN PINEAL BODY. Formaldehyde fixation, hæmatoxylin and eosin.

with long, branching processes making a close mesh on which the epithelioid cells rest, and extending to the septa.[1] Their nuclei are slightly smaller and darker than the others. The epithelioid cells, in sections stained by ordinary methods, seem crowded together, pale, with no clear cell boundaries. The distinctive characteristics of the parenchymal cells were first brought out by special staining methods. Instead of being simple polyhedral forms, most of the cells are provided with several long slender processes, mostly radially disposed in the cords, interlacing and often ending with bulb-like expansions among the connective tissue fibers of the septa or on the walls of blood vessels.[2] A few of the cells are without processes. Several types of parenchymal cells have been described —large clear cells with a homogeneous or fine granular cytoplasm, smaller cells some with acidophilic, others with basophilic granules, and cells

[1] BAILEY, 1932. [2] ACHÚCARRO Y SACRISTÁN, 1912.

containing lipoid droplets. Some authors regard the presence of cells with granules in the cytoplasm as an evidence of their glandular nature. Nerve cells have been reported, also both medullated and non-medullated nerve fibers.

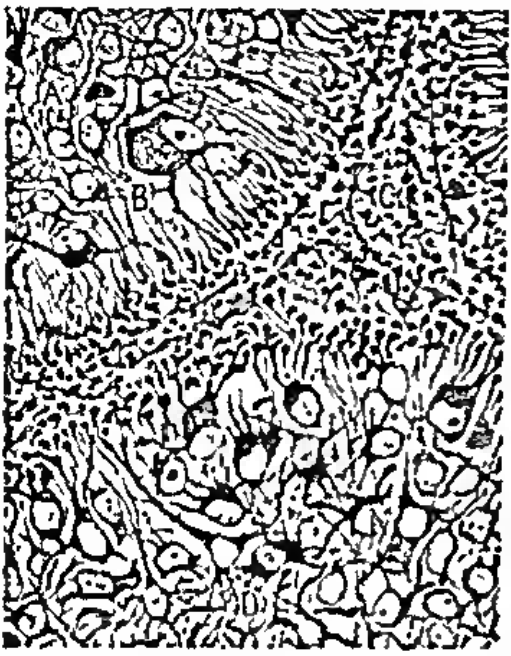


FIG. 377.—ADULT PINEAL BODY (SEMI-DIAGRAMMATIC)

A, D, lobules with polymorphic parenchymatous cells, B, neuroïd element, C, interlobular space with vessels, surrounded by club-shaped prolongations (Del Rio Hortega)

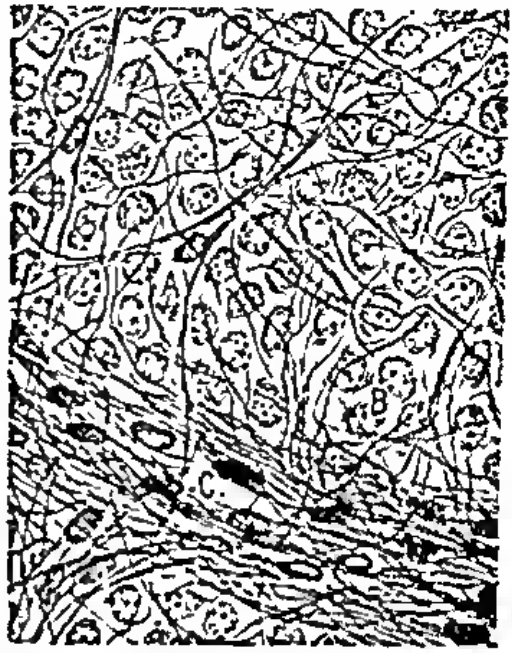


FIG. 378.—NEUROGLIAL SUBSTRATUM IN THE ADULT PINEAL BODY

A, astrocyte, B, parenchymatous cells, C, vessels surrounded by longitudinal neuroglia fibers. (Del Rio Hortega) Both figures are reduced slightly for reproduction

The pineal body was considered by the ancients as the seat of the soul. From the researches in comparative anatomy it became evident that in some of the lower forms a median dorsal eye develops in the same region, and that the pineal body represents not the eye, but a glandular structure developed in close relation to it. Anatomists generally thought of it as a functionless, vestigial organ in the higher animals and man. But Tilney and Warren[1] disagree with this idea; 'To consider the epiphysis in mammalia as a vestige in the light of the histological evidence here summarized seems to be an attitude which is wholly untenable.' In spite of the apparently glandular character of the pineal cells, however, attempts to isolate and test the action of a pineal hormone have only lead to indefinite and sometimes conflicting results. These have been summarized by Berblinger.[2]

Within the pineal body, *acervulus cerebri* or 'brain sand' is usually found consisting of round or mulberry-like concretions, 5 μ to 1 mm. in diameter. In specimens preserved in glycerin or balsam these concretions show distinct concentric layers. They have an organic matrix containing

[1] TILNEY AND WARREN, 1919 [2] BERBLINGER, 1926.

calcium and magnesium phosphates and carbonates, and they are sometimes surrounded by a thick connective tissue capsule.

Not infrequently, especially in old age, the brain substance contains rounded or elongated bodies, distinctly stratified, which are colored violet by tincture of iodine and sulphuric acid, and therefore are related to amyloid. These *corpuscula amylacea* are found almost always in the

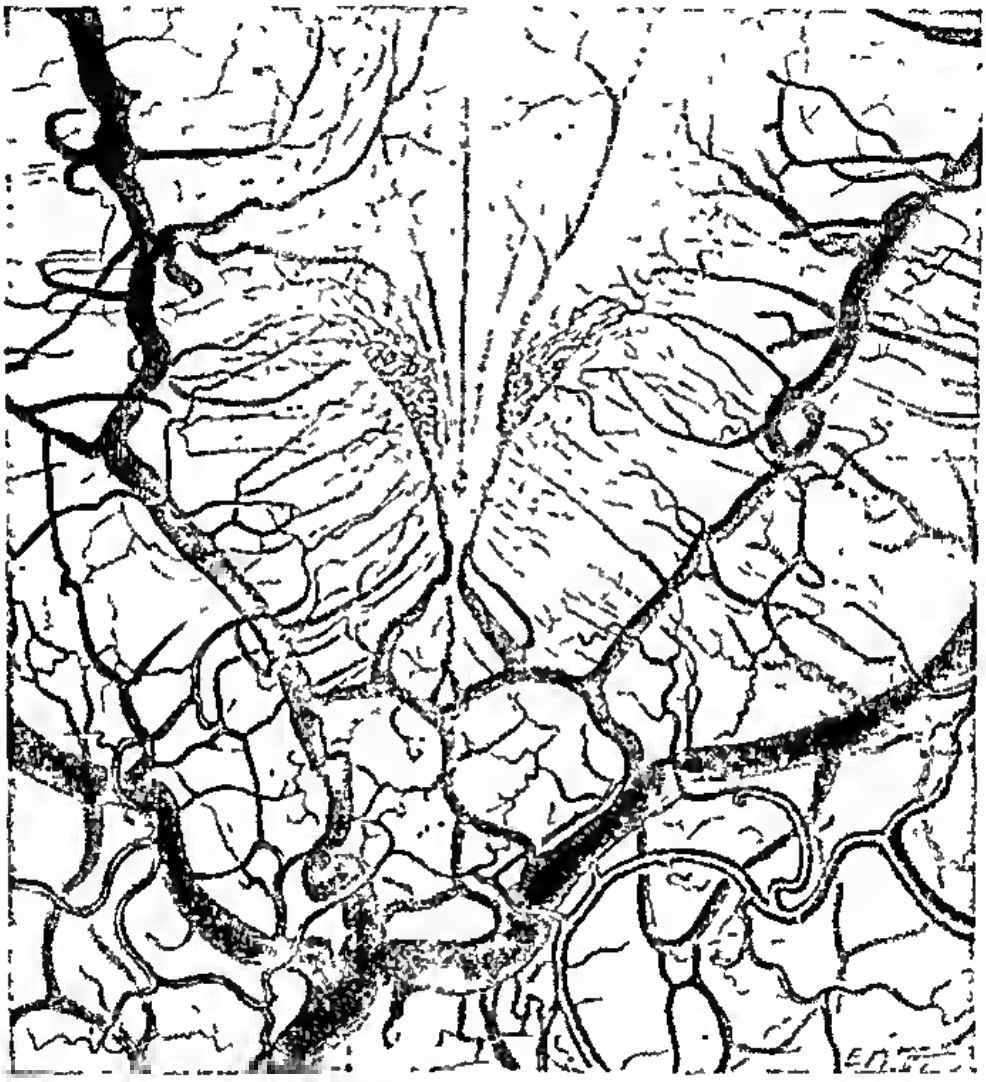


Fig. 379.—Injected Blood Vessels in the Area Postrema of a Monkey (Wislocki)

walls of the ventricles, and also in many other places in both gray and white substance, and in the optic nerve. They have a homogeneous capsule with occasional processes and are evidently neuroglia cells transformed by amyloid infiltration.

Elsewhere in the brain there are found several well defined nodular masses containing numerous neuroglial cells and fibers, and cells regarded as parenchymal. At each side of the mid-line in the most caudal portion of the fourth ventricle there is seen a small mass, the form and position of which varies in different species, and called the *area postrema*. These areas are richly supplied with blood (Fig. 379) and the adventitia

of the arteries, in distinction to the usual cerebral arteries shows an exceptional development. In this connective tissue sheath are found numerous histiocytes and in animals injected with trypan blue the whole area becomes deeply colored.[1] The neuroglia are peculiar, exhibiting a diversity of shapes, and they are said to resemble spongioblasts and astroblasts.[2] There is some disagreement on the presence of nerve cells: nerve fibers are extremely rare. Another small nodule occurs in the third ventricle between the anterior pillars of the fornix, about on the level with the superior border of the foramina of Monro. It was first described by Putnam who said 'it might therefore appropriately be called the *intercolumnar tubercle*.'[3] Pines named it the *subfornical organ of the third ventricle*.[4] It is described, in the fresh, as a pearly translucent nodule about 1 mm. broad and 0.5 mm. high, and varying very little in shape and size in man and different animals. The vascular arrangement and the histological structure are similar to that of the areæ postremæ. Both of these structures are covered on their ventricular surfaces with a modified ependyma—the cells are squamous to low cuboidal and blend gradually with the columnar cells characteristic of the ependyma elsewhere. The constancy, structure and vascular relationship of these bodies suggests some specialized function, but what it is remains unknown.

Neurosecretory cells are known to exist in fishes and some amphibians, but their presence has been doubted in mammals. Scharrer and Scharrer describe some unusual nerve cells in the nuclei supraopticus and paraventricularis of the human hypothalamus and discuss the possibilities of neurosecretion.[5]

SUPRARENAL GLANDS

Development and General Features. The suprarenal glands, or adrenal glands, are two flattened masses of cells, without lumen or ducts, *situated in the retroperitoneal tissue above the kidneys. They vary* considerably in size and shape, but are usually 0.5 cm. thick and 3–5 cm. tall, sometimes being wider and sometimes narrower than their height. The right suprarenal gland is generally described as triangular and the left as crescentic.

The gland resting upon the kidney (*glandula reni incumbens*) was first described by Eustachius.[6] It was apparent from the outset that the relation of the suprarenal glands to the kidneys was merely that of juxtaposition. Certain early writers supposed that they were renal structures and named them 'succenturiate kidneys.' Bartholin[7] perceived the medulla, which he described as a cavity containing a black humor; and he published an extraordinary figure in which the gland resembles a cocoanut cut across with the lid

[1] Wislocki and Putnam, 1920. [2] King, 1937. [3] Putnam, 1922.
[4] Pines, 1926. [5] Scharrer and Scharrer, 1940. [6] Eustachius, 1564.
[7] Bartholin, 1666.

lifted. In accordance with this conception he named the structures 'atrobiliary capsules,' and the name *capsule* is still often applied to them. Diemerbroeck,[1] following Wharton, states that 'the glands are found at a place where there is a plexus of nerves, to which they are firmly united.' In reviewing the various 'conjectures' as to their function he writes, 'Wharton thinks that in these capsules a certain juice is removed from the plexus of nerves on which they lie, useless indeed to the nervous system, but which, flowing thence into the veins, may serve some useful purpose.' The intimate relation of these glands to the nervous system, and the production of an internal secretion received by the veins have since been demonstrated, in certain recent works the glands have even been described as parts of the nervous system. Diemerbroeck concludes by hoping that physicians, through many autopsies, may find out to what diseases these glands give rise. In 1855, Addison described the disease, usually fatal, which is thought to depend upon the loss of function of these glands. Their physiological importance has been amply demonstrated, but they still present fundamental problems, both as to function and structure.

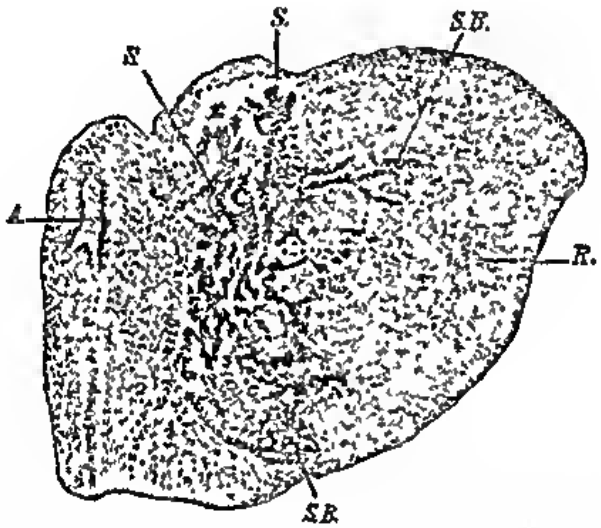


FIG 390.—SECTION OF THE SUPRARENAL GLAND OF AN EMBRYO OF 17 MM (Wiesel)
A, Aorta, R, cortical portion, S, chromaffin tissue, penetrating to form the medulla at S B. (From McMurrich's Development of the Human Body)

A section through a fresh suprarenal gland reveals at once the division into cortex and medulla. The cortex is yellowish, owing to the presence of lipoid substance, except next to the medulla where it is reddish-brown due to blood and pigment, while the medulla is dark gray. The color contrast is usually very striking, and it is shown also in unstained sections of tissue preserved in chromic acid solutions, although the medulla may then be lighter than the cortex. Not only do the cortex and medulla differ in gross appearance, but they are radically different in embryonic origin, and in the sharks they exist as separate organs. In sharks the medulla is represented by groups of ectodermal cells associated with the sympathetic ganglia, and the cortex takes the form of an 'interrenal gland,' composed of cords of mesodermal cells with a sinusoidal circulation. In human embryos corresponding parts arise separately, but they come together to form a single gland.

[1] DIEMERBROECK, 1672.

The cortex appears first, and is formed from cells which develop as buds of the cœlomic epithelium, growing into the mesenchyma on each side of the root of the mesentery, medial to the Wolffian bodies. In embryos of 8–12 mm., the buds or cords have become detached from the peritoneal epithelium (Zuckerkandl), and in cross sections they appear as round masses of cells penetrated by a network of slender veins. The cells of these masses rest directly against the vascular endothelium, so that the vessels are described as sinusoids.

Meanwhile cells from the sympathetic ganglia grow ventrally along the medial side of these masses, where they are conspicuous because of their dark stain (Fig. 380). These cells, which give rise to the medulla of the suprarenal gland, are considered gland cells, in spite of their origin. Similar cells are found in relation with other sympathetic ganglia, usually in small groups on the dorsal side, where they are known as paraganglia. They contain granules which stain intensely with chromium salts, and on account of this affinity the cells are called '*chromaffin cells*.' They produce the important internal secretion epinephrin (*adrenalin*). In embryos of 15–20 mm., strands of chromaffin cells are seen penetrating the cortical portion of the gland, but it is not until much later that they gather in a central mass which constitutes the medulla; even at 190 mm. the invasion is not complete (Zuckerkandl). As a whole, the gland acquires a relatively very great size in embryos.

Islands of medullary substance may occasionally occur in the cortex, and outlying portions of the gland may not contain any medulla. Moreover portions of the gland frequently become detached, forming *accessory suprarenal glands*. These may remain near the main gland or may be carried down, with the descent of the adjacent sexual glands, into the broad ligament, or epididymis.[1] Such glands usually consist entirely of cortex, but they may contain medullary substance. Isolated *paraganglia*, consisting entirely of medullary substance, are not regarded as suprarenal glands. There is no evidence that accessory suprarenal glands may arise from the cœlomic epithelium at a distance from the main glands.[2]

Adult Structure. The cortical substance consists of epithelioid cells arranged in continuous cords two or three cells thick without lumen, resting on a small amount of reticular tissue continuous with the connective tissue capsule of the gland; between the cords are capillary blood vessels. The cortex may be divided into three areas or zones; the *zona glomerulosa*, *zona fasciculata* and *zona reticularis*.[3] In the zona glomerulosa the arrangement of the cells, in the adult, is in irregular, rounded masses; in the fasciculata the cords are straight and radially disposed; and in the reticularis the cords join to form an irregular network and end blindly. The cells show evidence of the accumulation, storage, and secretion of

[1] Wiesel, 1899. [2] Zuckerkandl, 1912. [3] Arnold, 1866.

a fatty substance. In the glomerulosa small fat droplets appear in the cytoplasm; in the fasciculata the cells are loaded with larger droplets; in the reticularis the cytoplasm is darker, more compact, and may contain pigment. The nuclei remain in the center of the cells throughout, and the fat droplets do not coalesce, as in true fat cells. All the fat

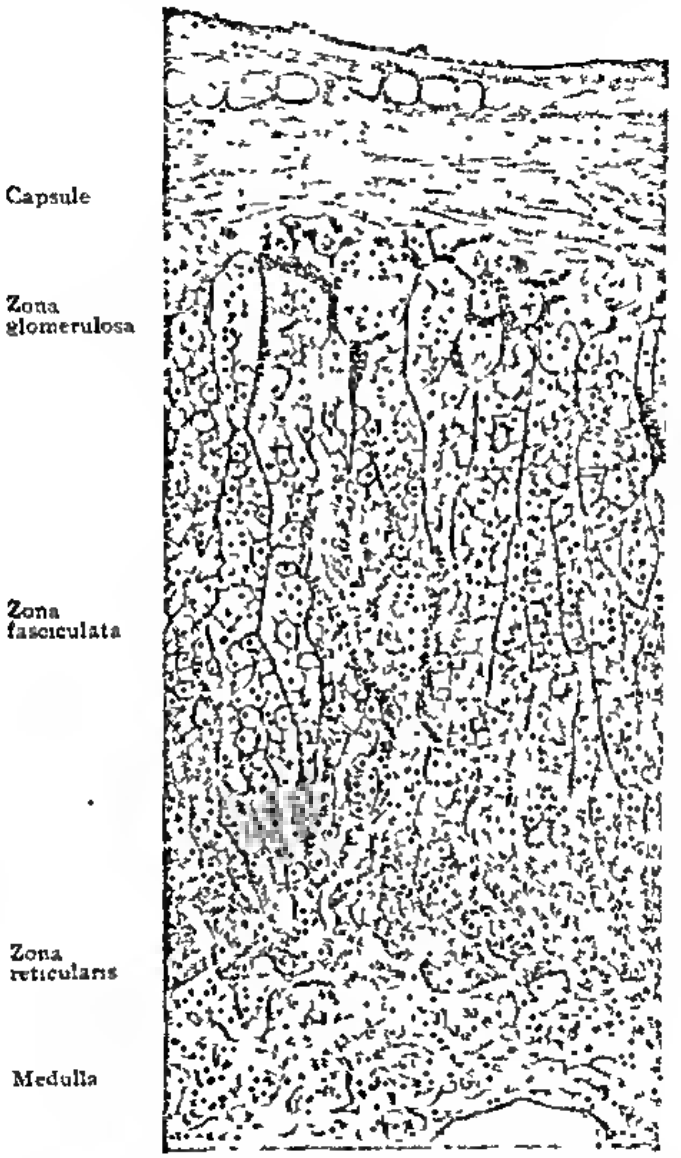


FIG 381.—SECTION OF HUMAN SUPRARENAL GLAND, THROUGH CORTEX TO MEDULLA.

droplets in any one cell tend to be uniform in size. With ordinary fixation the cells show a fine lace work of cytoplasm, the fat having been dissolved; this gave the name 'spongy layer' to the fasciculata. The cells are in intimate relation with the endothelium of the blood vessels. In portions of the suprarenal gland where the medulla is lacking, the zonæ reticulares of the opposite sides come together. The boundary between cortex and medulla may be irregular, because of the varying extent of the inner cords.

In fetal life the suprarenal glands are relatively enormous structures, chiefly due to the extent of the cortex. This fetal cortex degenerates during the last ten weeks of fetal life, and has entirely disappeared at twelve months. It is replaced by the downgrowth of the true cortex, situated just beneath the capsule, so that for a time two different, though ill-defined, cortical layers are present.[1] The cortical cells gradually become arranged in radial rows, perhaps through the influence of the blood vessels. The zona glomerulosa derives its name from the fetal arrangement of its cells, which

FIG 382.—SUPRARENAL GLAND, HUMAN
Above, section through the zona fasciculata, below, section of medulla

for a time take the form of hollow balls. In adult man they are inconspicuous solid masses, little larger than the cords of the zona fasciculata, with which they are continuous.

The medulla is composed chiefly of chromaffin cells arranged in strands and masses which unite to form a network, with lacunar veins filling the interstices. The cells are irregular in shape; usually they have an abundant cytoplasm, but they tend to shrink, even in well-preserved specimens, so that they may appear stellate. The cytoplasm is filled with minute granules which show the 'chromaffin reaction'; the nuclei

[1] KEENE AND HEWER, 1927.

are large and clear. Cells of a second type may be present in small groups; they are smaller and have dark cytoplasm, without specific granules, and small dark nuclei. These may be immature sympathetic cells not yet differentiated, or perhaps lymphocytes. Nerve cells of the sympathetic type have been reported. The slight framework of the medulla is of reticular tissue.

The chromaffin reaction[1] *consists of the reduction in the granules of dichromate to* an insoluble chromium trioxide(?). The granules are supposed to be, or at least to be largely made of, the actual epinephrin (adrenalin) which is the product of the medulla. They have been detected in this form in the lumen of the veins.

The function of the cells of the medulla is the secretion of epinephrin into the blood stream. The cortical cells have been supposed to supply the precursors of epinephrin which is only completed in the medulla, or to secrete some detoxicating substance, or to elaborate lipoids for general body use. Destruction of the cortex is not compatible with life, though animals may be kept alive by the extract called 'cortin,' or by the crystalline derivative 'corticosteron.' In both cortex and medulla mitoses are frequently seen, indicating that the cells are often destroyed in the performance of their function.[2] Correspondingly certain pycnotic nuclei and shrunken cells are present, and macrophages to remove the debris of dead cells. There seems to be no cycle of secretion, but the glomerular zone is said to increase markedly in pregnancy. Some relationship seems to exist between the suprarenal cortex and the gonads, since if the latter are normally large in a certain race or type of animal, the cortex is also large. The effect of epinephrin in general is the contraction of smooth muscle, but its action is selective, and different *on various blood vessels and organs* Thus it contracts the uterine muscles, but inhibits those of the intestinal tract. It also stimulates certain glands, and causes the contraction of pigment cells. Its rôle in the body under the influence of the emotions is described by Cannon.[3]

The capsule of the suprarenal glands is a connective tissue layer, said to contain smooth muscle fibers and small ganglia, in addition to vessels and nerves. Around the blood vessels especially, it contains elastic tissue. The capsule sends slender prolongations into the gland, and elastic tissue occurs in the medulla. The cortex contains very few if any elastic fibers, and its framework appears to consist of reticular tissue.

The arteries supplying the suprarenal glands are from several sources. They divide into many small branches in the capsule, and these penetrate the cortex, forming a long-meshed capillary network. In the medulla the meshes become round and the vessels collect to form veins, the larger of which are accompanied by longitudinal strands of smooth muscle fibers. Some arteries are said to pass directly from the capsule to the medulla, without branching in the cortex. Within the medulla the veins unite to form the *central veins* which are the main stems of the *suprarenal veins* in which the muscles are chiefly longitudinal (Fig. 206). They emerge at the *hilum;* the right empties into the inferior vena cava and the left joins the left renal vein.

[1] Kingsbury, 1911. [2] Hoerr, 1931. [3] Cannon, 1929.

Lymphatic vessels have been found in the capsule, where they may drain the cortex, and also in the medulla, emerging at the hilum.

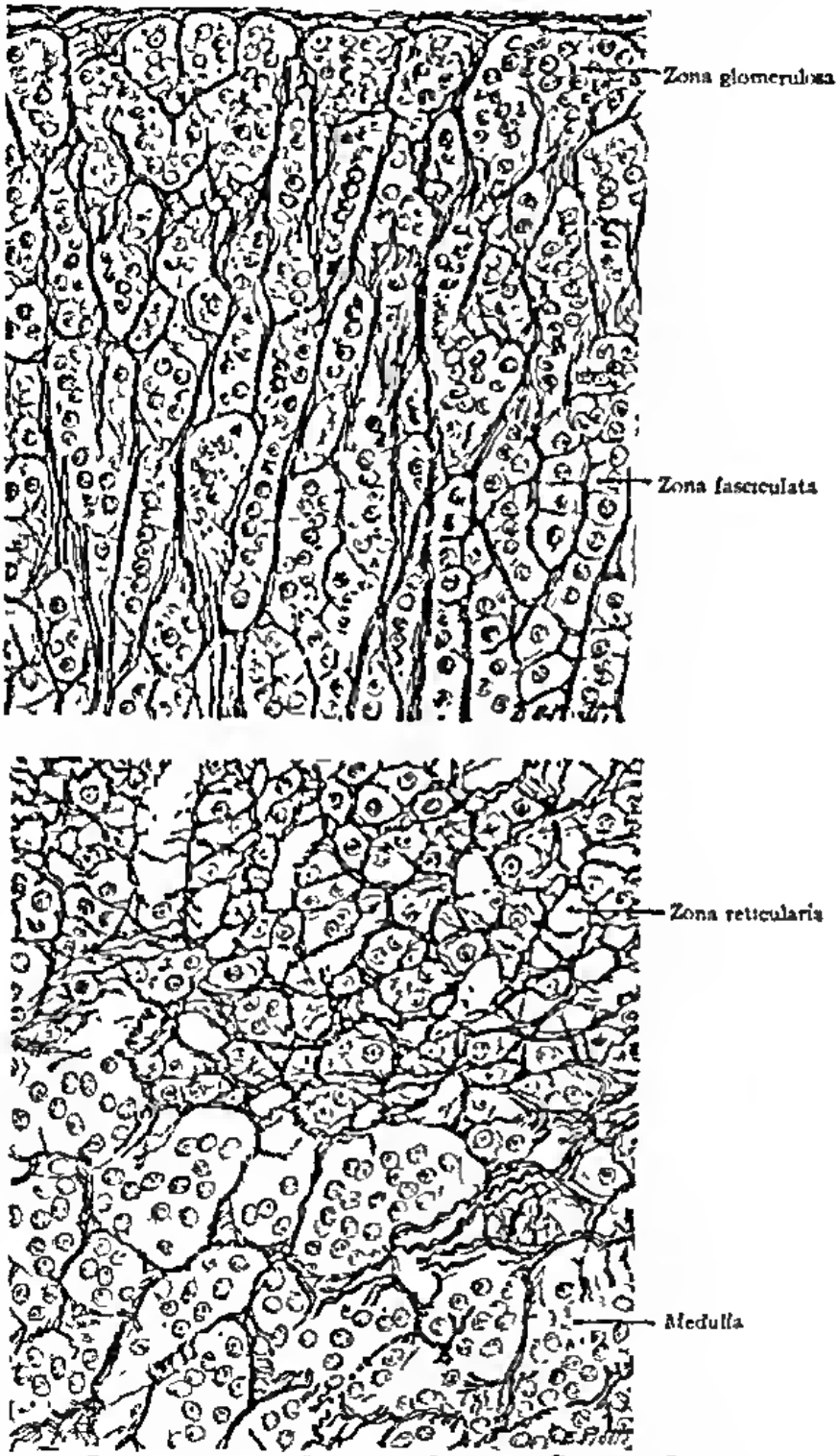


Fig. 383.—Reticular Fibers in the Suprarenal Gland of a Rabbit

Formaldehyde fixation, silver impregnation according to Pap and hæmatoxylin. Above, in the cortex, below, the cortico-medullary junction in the same preparation.

The numerous, mostly non-medullated *nerves*, of which a human suprarenal gland receives about thirty small bundles, proceed chiefly

from the cœliac plexus and pass with the arteries from the capsule into the medulla. Within the capsule they form a plexus, from which branches descend into the zona glomerulosa and zona fasciculata; there they end on the surface of groups of epithelioid cells, without penetrating between the individual cells. The plexus in the zona reticularis is more abundant, and is formed from fibers which descend directly through the outer zones; its fibers likewise terminate on the outer surface of groups of cells. In the medulla, the nerves are extraordinarily abundant and each cell is surrounded by nerve fibers. Groups of sympathetic ganglion cells are found here and there in the medulla but only rarely in the cortex. A part of the nerves terminate in the walls of the vessels.

The Paraganglia. The *paraganglia* (chromaffin bodies, organs of Zuckerkandl) are scattered minute masses found in the retroperitoneal region in relation with the sympathetic system and the great vessels, and composed of supporting cells and chromaffin cells resting against thin-walled blood vessels. They are therefore similar in origin and histological characteristics to the medulla of the suprarenal gland; but only when they are intimately connected with cords like those of the cortex should they be called accessory suprarenal glands. It is thought that they probably elaborate epinephrin. Their distribution in man is described by Zuckerkandl.[1] They grow smaller with age without actually disappearing.[2]

THYROID GLAND

The thyroid (*i.e.*, shield-shaped) gland is a median, entodermal downgrowth from the tongue; its thyroglossal duct becomes obliterated, leaving the foramen cæcum to mark its former attachment. The downgrowth is joined by cells from the postbranchial bodies (see p. 304). In some animals these lateral cells may fuse with and become a part of the thyroid gland, and Weller[3] considers that they do so also in man, but Kingsbury[4] states that in man, though coming into relation with the median gland, the lateral elements degenerate without adding to its substance, and that the term 'lateral thyroid' is not justified. The median structure comes to lie beside and in front of the upper part of the trachea. It consists of two lateral lobes, each about two inches long and an inch wide, connected by an *isthmus*, about half an inch wide, which crosses the median line ventral to the second and third tracheal rings. An unpaired *pyramidal lobe* extends from the isthmus or adjacent part of one lateral lobe toward the tongue. Irregular detached portions of the gland, such as occur especially along the course of the thyroglossal duct, are called *accessory thyroid glands.*

[1] Zuckerkandl, 1912. [2] Iwanow, 1932 [3] Weller, 1933.
[4] Kingsbury, 1935.

The proliferating mass of entodermal cells forms at first a network of solid cords or plate-like sheets.[1] This becomes separated into small masses, within each of which a lumen may appear. The lumen enlarges and becomes spheroidal; the entodermal cells which surround it form a simple epithelium, either columnar, cuboidal, or flat. Flat cells are said to occur especially in old age; usually the cells are low columnar or cuboidal. The mature thyroid gland consists, therefore, of rounded,

Fig. 384.—Normal Human Thyroid Gland. Anterior Surface of a Dissected Lobe. × 9. (Rienhoff.)

closed spaces, or *follicles*, bounded by a simple entodermal epithelium. The follicles vary greatly in diameter. Generally they are rounded, but sometimes they are elongated, and occasionally they branch or communicate with one another. Models show occasional follicles of most bizarre shapes, due to the incomplete separation of the original network or to later growth by budding. Among them are cords or small groups of cells which have no lumen, called the interfollicular cells.

Within the follicles, and forming the most conspicuous feature of the thyroid gland in ordinary sections, is a hyaline material which stains deeply with eosin and is named 'colloid.' The hyaline material in the thymic corpuscles, the hypophysis, and in the coagulum in the cervical blood and lymphatic vessels, has also been designated colloid. In sections

[1] Norris, 1916

of the thyroid gland it usually does not fill the follicle but has contracted, producing a spiny border. This condition, however, has been noticed by some in fresh tissue,[1] and may merely represent a difference in stainability of the more recent secretion but it may also be caused by fixation Granules, vacuoles and droplets, detached cells, leucocytes, and crystalloid bodies may be found in it. 'In some follicles of a particularly well-preserved human thyroid, each arch of the scalloped colloid corresponded exactly to one epithelial cell. Threads of the colloid were seen to be 'anchored' on the terminal bars. Evidently those parts of the

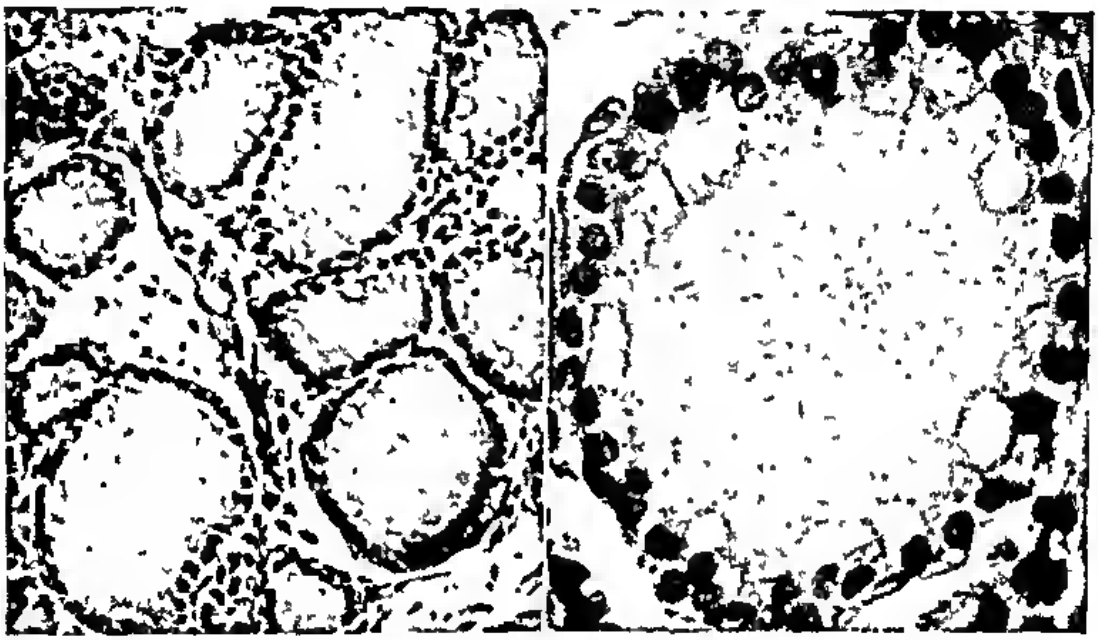


FIG 385.—PHOTOGRAPHS OF A SECTION OF THE THYROID GLAND OF WOOLLY MONKEY
On the left under low power, on the right under high power magnification Formaldehyde, hæmatoxylin and eosin.

colloid which had been adjacent to the terminal bars had been prevented from retracting.' (E. Mayer.)

The cells lining the follicles form a single row.[2] Most of them have large, vesicular nuclei and a pale cytoplasm often containing granules which stain like the colloid, located in the upper pole. Occasionally these granules may be seen leaving the cell as though to be added to the colloid. Fat droplets and colorless vacuoles may also be present. Mitochondria are rod-shaped and thin. The Golgi apparatus lies above the nucleus. Occasionally a cell shows a dark, shrunken nucleus, and cytoplasm staining homogeneously with acid dyes like the colloid. Bensley[3]

[1] UHLENHUTH, 1925.

[2] Langendorff (1889) differentiated between 'chief cells' and 'colloid cells' and named them. The first vary in height, contain a yellow to greenish-yellow pigment which blackens with osmic acid and dissolves in turpentine. The colloid cells found in inconstant numbers are often smaller than the chief cells, have a more homogeneous appearance and are regarded as indicating a *functional state of the secreting epithelium.*

[3] BENSLEY, 1914.

noted an oval type of cell, loaded with coarse granules, found in the opossum, to which he ascribed a special internal secretion; and Seecof[1]

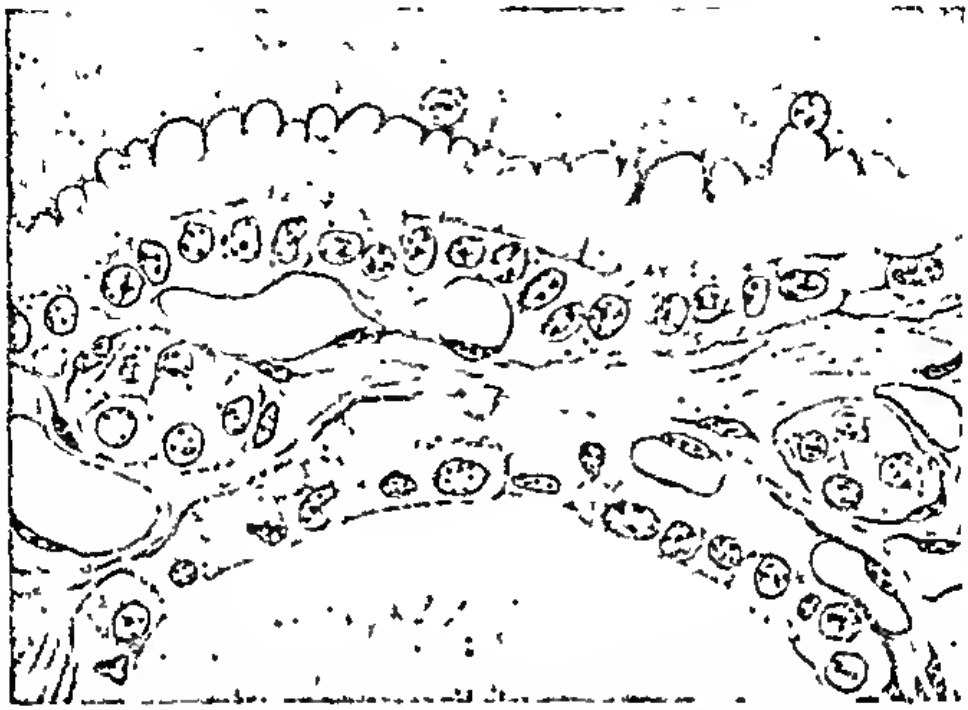


Fig 386.—Thyroid Gland. Parts of Two Follicles with Connective Tissue between.
The cells of the upper follicle show granules in the cytoplasm apparently forming the colloid. In the lower follicle the colloid seems to be 'leaking' past a few cells with pycnotic nuclei to the tissue spaces outside. Two small cell groups show no lumen.

described '*mitochondria-rich cells*,' most numerous in man. The cells seem to rest directly on a loose reticular tissue framework, with no noticeable basement membrane although a thin homogeneous line becomes stained by some methods (Azan).

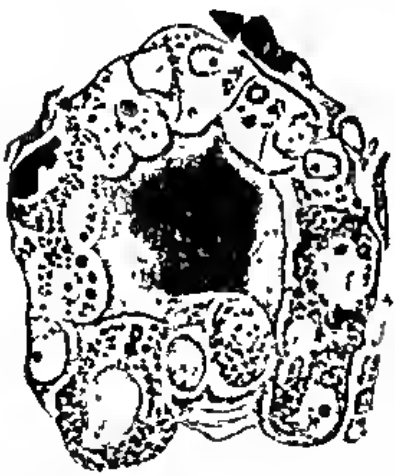


Fig 387.—A Section of a Human Thyroid Gland Follicle Showing Mitochondria Ludford-Schridde Technique (Thomas)

There has been much discussion as to the correlation of the cell type and arrangement with the function of this gland. The colloid contains the active agent of the secretion, yet while it is enclosed in the follicles it does not seem available for body use. It might be stored in the follicle and 'leak' out between the follicular cells or through certain of them that have passed their peak of activity. Williamson and Pearse[2] described a system of minute capillary channels between the cells, but Wilson[3] considers these merely especially prominent terminal bars and intercellular cement lines. The cells with pycnotic nuclei appear to be filled with colloid which also may be found in the neighboring tissue spaces.

[1] Seecof, 1927 [2] Williamson and Pearse, 1923. [3] Wilson, 1929.

The colloid might be passed outward through the cells by a process of reverse secretion. Grant[1] finds that, in Amblystoma, the stored secretion is absorbed through the apical cell membrane in a non-staining form, may become segregated into chromophilic bodies in the cytoplasm, but passes through the basal membrane in the non-staining state again. The colloid in the follicles may be merely stored, the usual secretion coming from the bases of the cells. In favor of this is the finding by Bensley[2] of

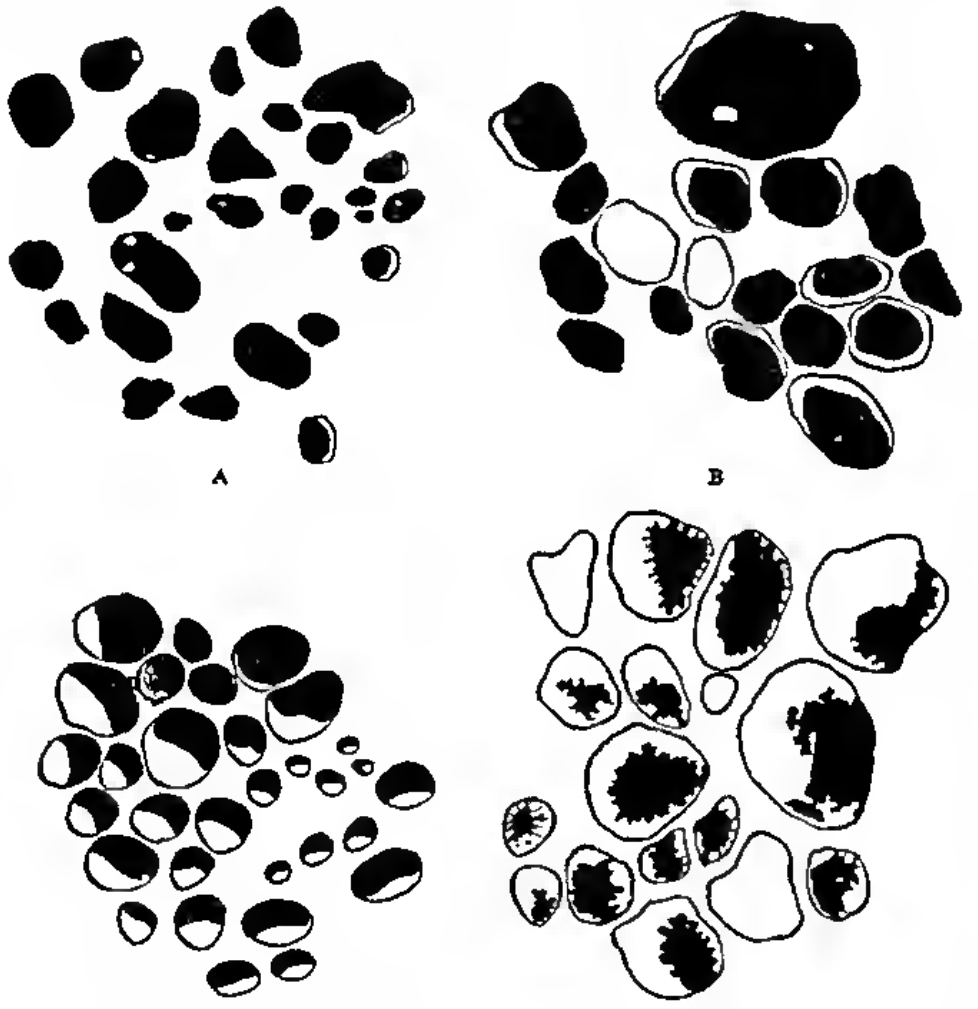


FIG 388.—OUTLINES OF COLLOID IN THE FOLLICLES OF THE THYROID GLAND OF A RABBIT, SHOWING THE EFFECTS OF SHRINKAGE AFTER FIXATION WITH

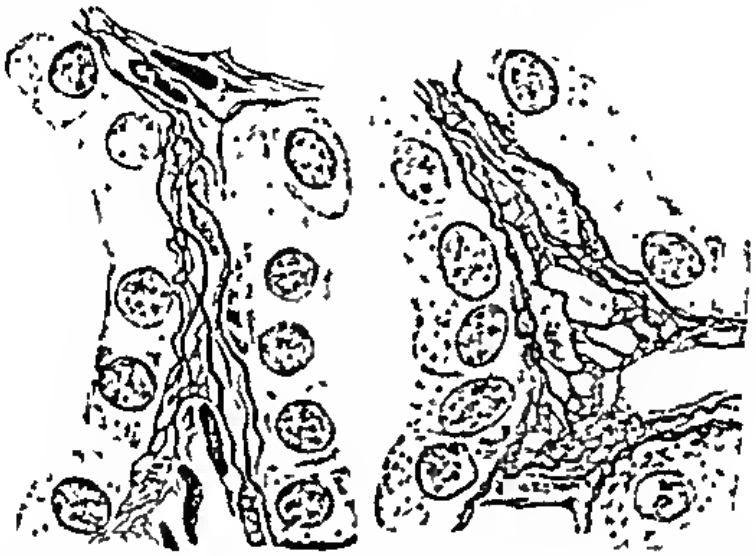
A, osmic acid, B, formaldehyde, C, alcohol, D, picric acid (Redrawn slightly modified from Bucher)

'dilute colloid' in the cell bases, and the occasional reversed position of the Golgi apparatus[3] near the base of the cell which may indicate reversal of polarity and secretion of the cell; but the colloid within the follicles does not increase with age, and in certain diseases this reserve is called into use. Secretion from the base, if it exists, seems to be only a secondary process.

The interfollicular cells have been variously interpreted as 'fetal cell rests' from which new follicles might be produced; as tangential sections

[1] GRANT, 1931. [2] BENSLEY, 1916. [3] COWDRY, 1922.

of bulging irregularities of the larger follicle walls; or as buds from the older follicles to form new follicles. Rienhoff[1] states that all the follicles, whether large or small, have smooth external epithelial walls; the irregular shapes found in many models being due to the irregular thickness of the walls, when the outlines of the lumens are drawn. Nonidez[2] considers that, in young puppies, the interfollicular cells are derived from the follicles. He recognized them at first singly or in small groups in the follicular epithelium, differentiated by the presence of argyrophil



A B

FIG. 389.—ARGYROPHIL RETICULUM IN THE INTERFOLLICULAR SPACES IN THE THYROID GLANDS OF TWO DIFFERENT DOGS.

A, formaldehyde alcohol fixation, Bielschowsky-Gros technique, B, formaldehyde fixation, silver carbonate and diluted acetic acid (Nonidez).

granules (perhaps Bensley's oval cells); they migrate from the epithelial row of cells to the underlying tissue spaces, where they gradually lose their granules. He calls them parafollicular cells, and suggests that they have some endocrine function connected with growth. Zechel,[3] on the other hand, finds all stages of transition between the normal follicles and the interfollicular cells. According to his findings the follicles may break up, the stored colloid may be released, and the same epithelial cells may be arranged in solid masses, from which new follicles may be formed. Perhaps the epithelial cells can produce colloid for only a limited period, and then need to rest as interfollicular cells. This would make the relation of the storing of the secretion to its release into the blood stream more intelligible. It is discussed by Nonidez.[4] Mitoses are seen but seldom in the follicular epithelium of the normal thyroid gland; they occur, however, frequently in hyperplastic epithelium, with evidences of pathology.

The thyroid follicles are surrounded by loose, elastic connective tissue, said to be reticular near the follicles, which contains very many

[1] RIENHOFF, 1931. [2] NONIDEZ, 1932*a* [3] ZECHEL, 1931. [4] NONIDEZ, 1932*b*.

blood and lymphatic vessels in close relation with the epithelium. The venous plexus lies close to each follicle,[1] the lymphatic plexus further away, serving several neighboring follicles. Denser connective tissue forms a capsule and lobular partitions. It contains small arteries, the media and intima of which are said normally to present local thickenings. The nerves from the cervical sympathetic ganglia form perivascular plexuses, and pass to the follicles, but there is no conclusive evidence that there are true secretory fibers reaching the cells.[2] Scattered ganglion cells are reported.

PARATHYROID GLANDS

The parathyroid glands[3] are normally four in number, two superior and two inferior. They are small, reddish, oval or pyriform bodies,

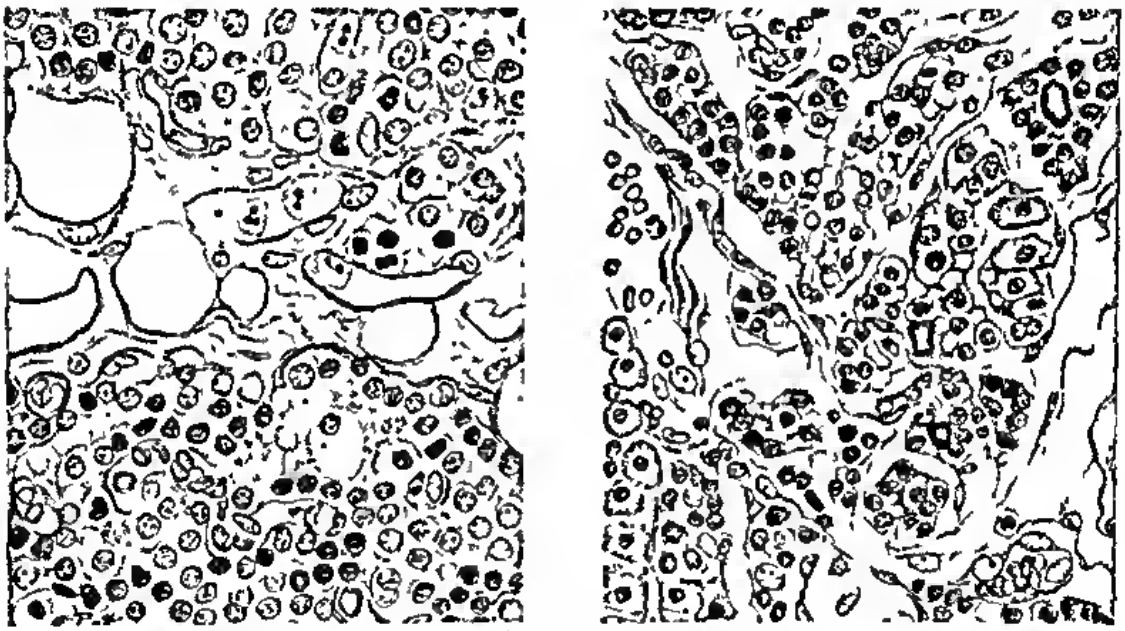


FIG 390.—SECTIONS OF PARATHYROID GLANDS FROM TWO DIFFERENT HUMAN INDIVIDUALS.
Left, a young adult, right, an old man, showing many colloid cells Zenker fixation, methylene blue and eosin.

6–7 mm. long and 2–3 mm. thick, attached to the postero-median borders of the lateral lobes of the thyroid gland. Each is enclosed in a fibrous capsule, which may be embedded in the capsule of the larger gland, making them very inconspicuous on superficial view. They are derived from local thickenings of the entoderm of the third and fourth pharyngeal pouches (called parathyroids *III* and *IV* respectively) and migrate downwards and forwards, those from the third pouch travelling

[1] RIENHOFF, 1931. [2] NOVIDEZ, 1935.

[3] The parathyroid glands were described and named by Ivar Sandström (Upsala Läkareförenings Förhandlingar, vol. 15, pp. 441–471, 1879–80). An English translation of this important paper was made by Carl M. Seipel and published with biographical notes by Professor J. August Hammar. Baltimore, 1938.

further and becoming the inferior pair.[1] Both pairs have a similar structure, resembling the corresponding *epithelial bodies* of lower vertebrates.

Variations in the position and number of these glands are common. One or more may be lacking, and as many as four have been reported on one side. They may be deeply embedded in the thyroid gland, or free in the surrounding tissue, or even attached to the thymus. In animals the variations seem to be more frequent than in man.[2]

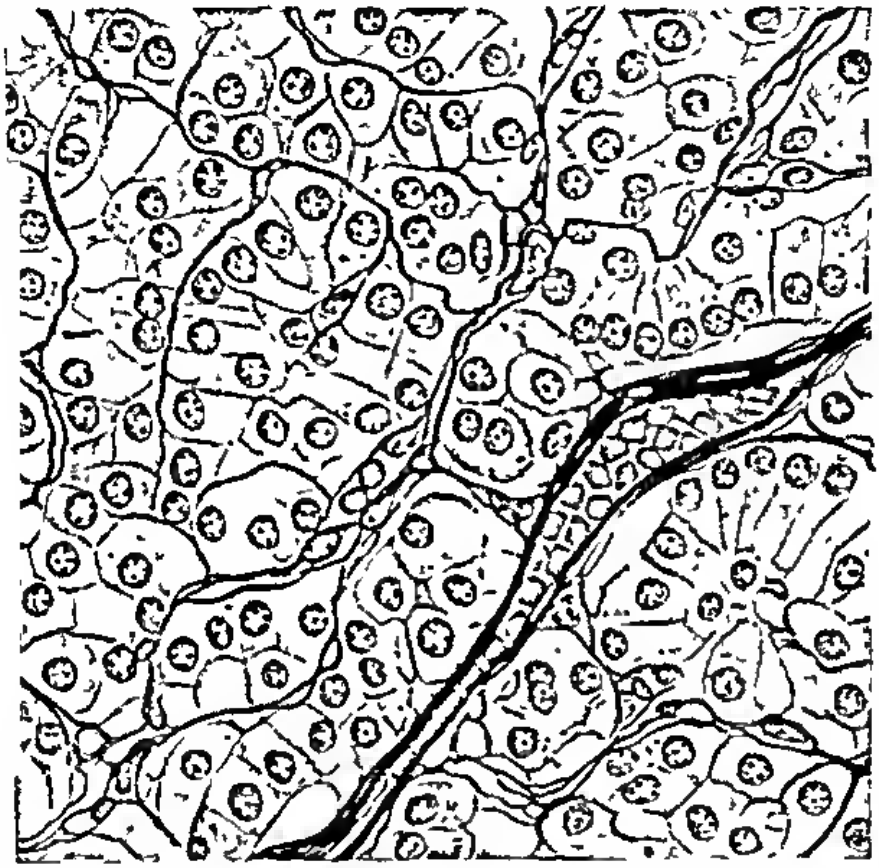


FIG. 391.—A SECTION OF A PARATHYROID GLAND FROM A WOOLLY MONKEY. THE ANIMAL HAD A SLIGHT PARATHYROID DISTURBANCE AND ONLY CHIEF CELLS ARE PRESENT.
Formaldehyde fixation, Heidenhain's azan stain.

The parenchyma of the glands is arranged in cords or solid masses of cells in close relation with sinusoidal blood vessels, surrounded by a minimal amount of reticular tissue. The principal cells are closely packed and polygonal, containing round or oval nuclei with networks of chromatin. The cytoplasm is pale, almost like that of plant cells, usually without granules; the cell membranes are prominent. In late childhood a second type of cell appears,[3] characterized by a granular, eosinophilic cytoplasm, larger size, and smaller and darker nuclei. A third type, intermediate between these two, has also been described. These three types may represent secretional phases,[4] and Rosof[5] finds

[1] WELLER, 1933. [2] MARINE, 1932. [3] WELSH, 1898. [4] MORGAN, 1936
[5] ROSOF, 1934.

the cycle evidenced by increase of the Golgi apparatus and the mitochondria. The eosinophilic cells are lacking in early childhood and in certain animals, and may represent wasted cells.

Variations of the character of the gland occur with age. The cells are small in the young, mitoses are frequent, the connective tissue sparse. With increasing age the cells become larger, and the septa increase, until the gland may represent a lobular appearance. Considerable fat may accumulate in the septa (Fig. 390). Follicles may develop, often containing a colloid material which, however, does not contain iodine These may become cystic, and have been found to increase in number after the experimental removal of the thyroid glands. The glands are very vascular, and each receives a special arteriole from the inferior thyroid artery. A capsule surrounds the entire structure, said to contain occasional smooth muscle fibers. Little is known of the lymphatics. The nerves, of sympathetic origin, have been traced to both vessels and cells.

The parathyroid glands produce *parathormone*, a hormone which regulates the calcium metabolism. Its lack, as after the complete removal of all of the glands, leads to tetany and death, which, however, can be prevented by the administration of calcium or of vitamin D.[1] It seems probable that the hormone helps in regulating the growth of the bones and the teeth.

THYMUS

The thymus (Gr. *θύμος*, thymus) arises from the two tubular prolongations of the third pharyngeal pouches, which meet in the median line and become bound together by their connective tissue coverings. The lumen is lost, and the cells proliferate. They form a broad, flat, bilobed mass with a tapering prolongation up each side of the neck. The bulk of the organ is in the thorax, beneath the upper part of the sternum. At birth it weighs generally between 5 and 15 grams (about half an ounce), and is relatively a large organ. Haller (1761) described it in older embryos as 'a huge gland, scarcely smaller than the kidney; but in the adult it is diminished, and having become constricted, dried up and much harder, it is almost buried in the surrounding fat.' Meckel found ordinarily no trace of it at twelve years. These older ideas, however, have been revised. The thymus grows very rapidly until the second year, and continues at a slower rate until puberty, then undergoes a certain involution and becomes progressively smaller, but persists even in the aged.[2] It actually degenerates apparently only as the result of chronic disease.

[1] Semsroth and McClugage, 1932. [2] Winiwarter, 1924.

Each tubular prolongation becomes thick-walled with a very small lumen, which is soon lost, and then sends out irregular branches into the surrounding mesenchymal tissue, forming the lobes of the organ, between which connective tissue septa develop (Fig. 392). Smaller secondary branches become the lobules.[1] The entodermal cells of the solid epi-

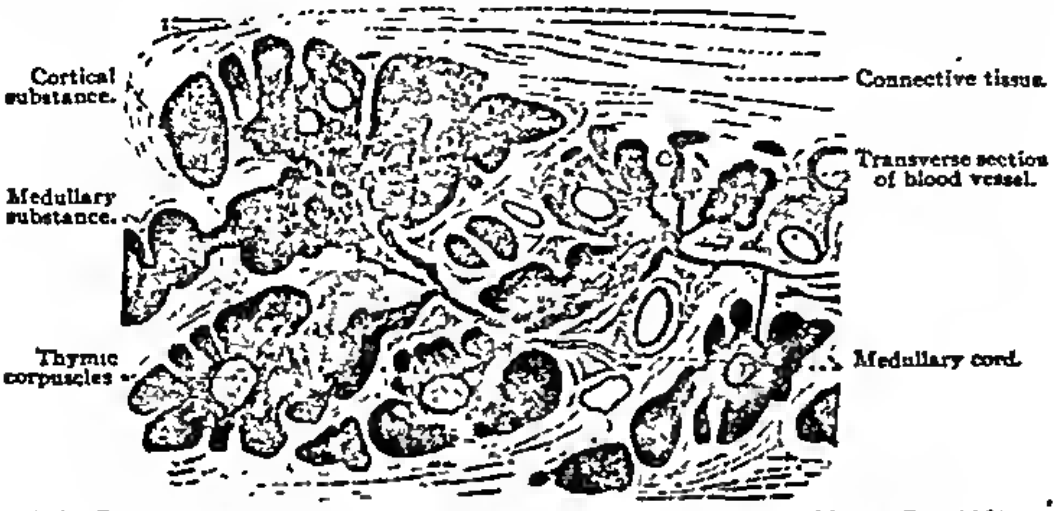


FIG. 392.—FROM A CROSS SECTION OF THE THYMUS OF A CHILD ONE YEAR AND NINE MONTHS OLD. × 21.

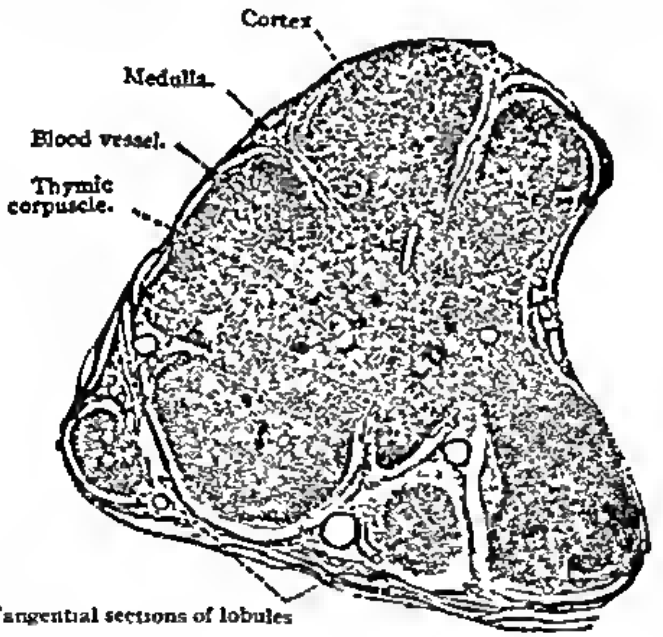


FIG. 393.—PART OF A SECTION OF THE THYMUS FROM A HUMAN EMBRYO OF FIVE MONTHS. × 50.

thelioid cords become changed to a stellate reticulum, looser at the periphery of the cords, and in the meshes of this net appear small cells like lymphocytes, called *thymocytes*. The bulk of them lie near the surface and form the cortex of each lobule, not sharply marked off from the medulla, which consists chiefly of the entodermal reticulum. In stained sections, then, each lobule consists of a pale medulla, extending from the cord, and a darker peripheral cortex, bordered by the capsule and septa. The

[1] HAMMAR, 1911.

entire structure somewhat resembles a lymph gland, but there are no germinal centers and no trace of lymph sinus.

At one time it was held that the stellate reticulum was of mesenchymal origin, and the thymocytes derived from the entodermal epithelium. Later it was recognized that the reticulum developed by vacuolization of the epithelium, but the thymocytes were believed to develop as a further differentiation of the same cells.[1] Others consider the thymocytes true lymphocytes, of mesenchymal origin, which invade the epithelium in the same way as in the tonsil.[2] This is perhaps the prevalent view. The thymocytes are first found at the end of the second fetal month, at about the same time embryologically that true lymphocytes develop in the lymph glands; they resemble lymphocytes very closely, and they could readily migrate into the reticulum. On the other hand, their arrangement in the thymus is not typical of lymphoid tissue. Recently the problem has been studied by tissue culture methods and by observing the regeneration of the gland after injury by X-rays and other agents. Popoff[3] thus confirms the lymphoepithelial theory, but Deanesly,[4] by similar methods finds that the thymocytes are derived from 'epithelioid' cells of entodermal origin, not connected with the reticulum, which she considers mesodermal. Thus the origin of the thymocytes is still in dispute.

FIG. 394.—A SECTION OF A THYMIC CORPUSCLE FROM A HUMAN FETUS OF 70 MM., CROWN-RUMP LENGTH. Picric acid-formaldehyde fixation; hæmatoxylin and eosin × 865. (Hammar)

Histologically the cortex resembles the denser portions of a lymph gland. The thymocytes show a small, spherical, dark nucleus, and only a rim of basophilic cytoplasm. The cells of the reticulum resemble true reticular cells with larger, paler, oval nuclei, and stellate cytoplasm mostly overlaid by the other cells. They are said not to ingest foreign particles, as do those of mesenchymal origin, and hence not to belong to the reticulo-endothelial system. A few macrophages are present, however, in both cortex and medulla, in the adventitia of the penetrating blood vessels. The medulla has few thymocytes and correspondingly more reticular cells, and also may contain leucocytes, especially eosinophils, and occasionally multinucleate giant cells. In birds and some mammals, but not in man, 'myoid cells' somewhat resembling muscle fibers have been reported. Sometimes in man, and normally in the cranial portions of the gland in cat and dog, the medulla contains cysts, which may be lined in part by ciliated cells. They are probably the remains of the ventral prolongations of the fourth pouches which degenerate in man but are said to form part of the thymus in the animals mentioned.

[1] STÖHR, 1906. [2] MAXIMOW, 1912. [3] POPOFF, 1927. [4] DEANESLY, 1929.

The most characteristic structures in the thymus are the *thymic corpuscles* (Hassall's corpuscles) which are found exclusively in the medulla. They are rounded bodies, at first few in number and small (12–20 μ in diameter), but they increase rapidly in size (to a diameter of 180 μ) and new ones are constantly forming. They are said to be present at about the fifth month, and at birth they are numerous. Small corpuscles may consist of a single cell. The nucleus enlarges and shows

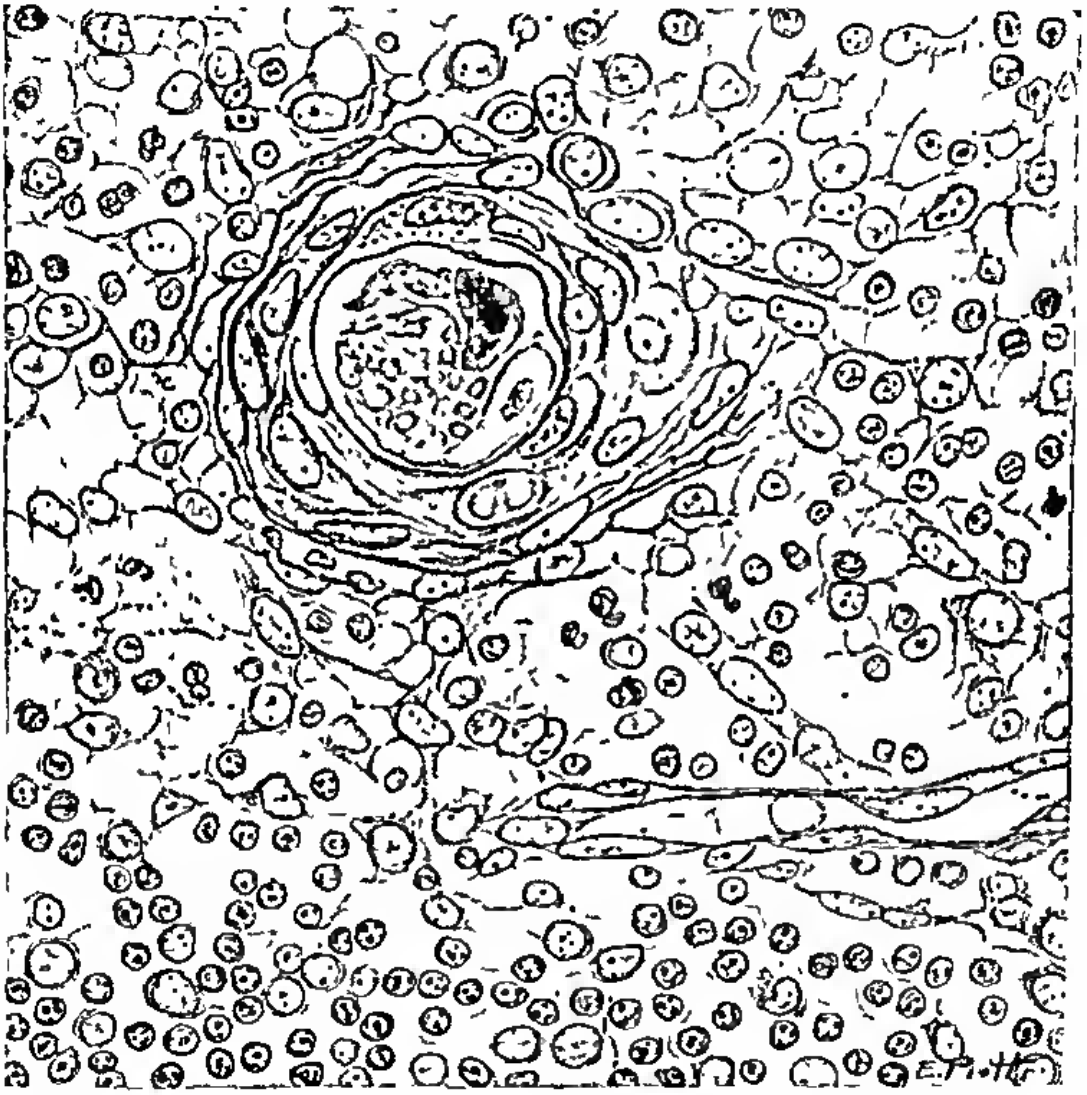


Fig. 395.—Thymic Corpuscle in the Medulla. Below the Blood Vessel a Portion of the Cortex Is Shown.

changes in its staining properties, and a layer of deeply staining hyaline substance appears in the cytoplasm. This increases until it fills the entire cell, often being arranged in concentric layers, and the nucleus becomes obliterated. Neighboring cells are concentrically compressed by the enlargement of this structure, and by hyaline transformation they may become a part of the corpuscle. The larger corpuscles are due to a fusion of smaller ones, or to hyaline changes occurring simultaneously in a group of cells. The central portion of a corpuscle may become calcified.

Sometimes it is vacuolated, containing fat. The hyaline substance may respond to mucous stains, but generally it does not; it has been considered similar to the 'colloid' of the thyroid gland. Leucocytes are said to become embedded in the corpuscles, or to enter them and assist in their disintegration. Thymic corpuscles have been regarded not only as degenerative products of the entodermal epithelium but also as concentric connective tissue masses, and as blood vessels with thickened walls and obliterated cavities. Injections show that they are not connected with the blood vessels. Although they have recently been described as active constituents of the thymus, they are generally regarded as degenerations. Jordan and Horsley[1] revive the old idea that the thymic corpuscles are degenerative phenomena of involuting blood vessels, while Kingsbury[1] maintains that they are like epithelial pearls, representing the keratinization of surface cells in the obliterated lumen of the original pouch.

The involution of the thymus consists of the reduction of the epithelial reticulum and thymocytes, and the increase of thymic corpuscles and the interlobular connective tissue. The septa often contain large amounts of fat, and the vascularity of the gland is diminished.

The arteries of the thymus enter it along the medullary strand, and extend between the cortex and medulla, sending branches into both but chiefly into the cortex. The cortical branches empty into veins between the lobules; the others into veins within the medulla. There are many interlobular lymphatic vessels, beginning close to the surface of the gland substance, and accompanying the blood vessels. There is nothing in the thymus to correspond with a lymph sinus. The nerves, chiefly sympathetic fibers, with some from the vagus, terminate along the vessels; a very few have free endings in the medulla.

The function of the thymus is not definitely known. Some think of it as an organ to supply lymphocytes. It has been suggested that the thymocytes serve as reservoirs of nuclein to be used in the mitoses of the growing body cells. Erythroblasts are said to occur in the cortex, and the thymus is therefore sometimes considered a blood-forming organ. Its relation to puberty is perhaps indicated by the fact that the same substances which cause early development of the gonads inhibit the growth of the thymus.[3]

[1] Jordan and Horsley, 1927. [2] Kingsbury, 1928. [3] Evans and Simpson, 1934.

URINARY ORGANS

WOLFFIAN BODIES AND WOLFFIAN DUCTS

Caspar Friedrich Wolff (1759) in a thesis entitled '*Theoria generationis*,' included an account of the development of the kidneys in chick embryos. From the diffuse *substantia cellulosa* along the ventral side of the spinal column, beginning on the third day of incubation, Wolff saw two elongated bodies gradually take form, and become the *kidneys*, each being connected with the cloaca by a *ureter*. These structures, however, are not the kidneys of the adult, and they are generally known as *Wolffian bodies;* their ureters are the *Wolffian ducts*. They are large and important organs in young human embryos, as shown in Fig. 396. The true or permanent kidneys of mammals arise later, and the Wolffian bodies degenerate, becoming vestigial in the female; in the male, however, they acquire new functions, and are in part retained as a portion of the genital ducts (namely the ducts of the epididymis). In the embryo they are renal organs built upon the same plan as the permanent kidneys, and moreover in the fishes and amphibia they are the kidneys of the adult.

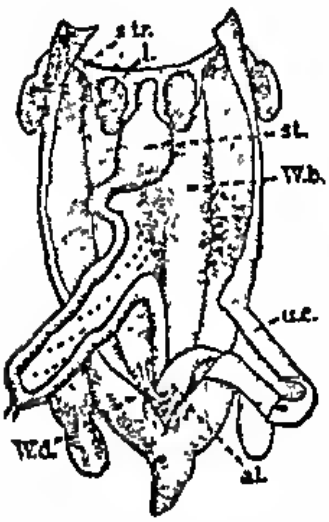


FIG 396.—DISSECTION OF A HUMAN EMBRYO OF THIRTY-FIVE DAYS. (After Coste.)
aL, Bladder, L, lung, st., stomach, s. tr., septum transversum, u. c., umbilical cord, W. b., Wolffian body, W. d., Wolffian duct.

Still another renal organ develops in embryos, anterior to the Wolffian body, and it has been found that the Wolffian duct is primarily the duct of this anterior kidney or *pronephros;* consequently the Wolffian duct is sometimes called the pronephric duct. The pronephros is the functional kidney in only the lowest of vertebrates (myxinoids). Singularly it has been found that 'the human pronephros is by far the best developed within the groups of mammals.'[1] Except for its duct, it entirely disappears in very young embryos (5 mm.). All the renal organs—pronephros, Wolffian body (or *mesonephros*), and kidney (or *metanephros*)—are developed from the nephrotomes. They are all composed of mesodermal tubules, each of which is in close relation with a knot of capillary blood vessels derived from branches of the aorta. Such a knot of vessels is a

[1] FELIX, 1912.

glomerulus, and certain products are eliminated from the glomerulus into the tubules to form the urine.

Excretion in some of the worms is accomplished by means of tubes leading from the cœlom almost directly to the surface of the body in each segment. The inner opening is funnel-shaped and guarded by cilia. Near this opening a glomerulus develops. In the pronephros of vertebrates segmental tubes still drain from the cœlom by a funnel-shaped opening, called the nephrostome, with a glomerulus in relation with each, but they open into two longitudinal ducts, running on each side of the body to the cloaca. In the mesonephros, the nephrotome, or neck of the somite, becomes cut off from the rest of the cœlom; its cavity (often only a slit) represents, however, a specialized portion of the cœlom, and is in definite relation with a glomerulus. One or more tubules develop from each nephrotome and join the pronephric duct. In the metanephros, the cells which form the tubules are thought to have migrated from the posterior nephrotomes. The cavities which develop in them may thus be thought of as isolated portions of the cœlom. Each such cavity comes in relation with a glomerulus, and the tubule which develops opens into a branch of the pronephric duct.

Development of the Wolffian Body and Wolffian Duct. The general relations of the nephrotome to the mesodermic somites and to the cœlomic epithelium have already been briefly discussed (p. 59). A nephrotome from a young rabbit embryo is seen in section in Fig. 397, A, together with its elevation which contributes to the formation of the Wolffian duct. The nephrotome here shown is from one of the anterior segments and belongs with the pronephros.

In human embryos, according to Felix, pronephric tubules are formed from the seventh to the fourteenth segments, and perhaps from those further forward. The elevations to which these nephrotomes give rise turn posteriorly and unite with one another to form the Wolffian duct. This is at first a solid cord of cells which grows posteriorly in the trough between the somites and somatic mesoderm. It lies near the ectoderm, but it is now generally agreed that the ectoderm takes no part in its formation. Finally its growing extremity reaches the ventral portion of the cloaca and fuses with it. Later this ventral part of the cloaca becomes cut off to form the bladder, and the Wolffian ducts then empty into the neck of the bladder. The pronephric tubules meanwhile become detached from the cœlomic epithelium, but they remain rudimentary and degenerate without having any glomeruli formed in connection with them. Occasionally a tubule may persist as a closed cyst.

The mesonephric tubules develop from the more posterior nephrotomes, after the Wolffian duct has formed. They acquire openings into the Wolffian duct, but do not contribute to its development. In producing mesonephric tubules, the nephrotomic tissue becomes detached and separates into masses which form vesicles (Fig. 397, B). Each vesicle elongates and becomes an S-shaped tubule, one end of which fuses with

the Wolffian duct and opens into it; the other end remains blind. A knot of capillaries, derived from a branch of the aorta, develops in the distal concavity of the S and becomes a glomerulus; a glomerulus is formed in connection with every Wolffian tubule. The tubules then elongate and become coiled, and together they produce the rounded swellings on each side of the root of the mesentery, which are the Wolffian bodies (Fig. 397, C). The genital glands arise as mesodermal thickenings on the ventro-medial surface of these bodies.

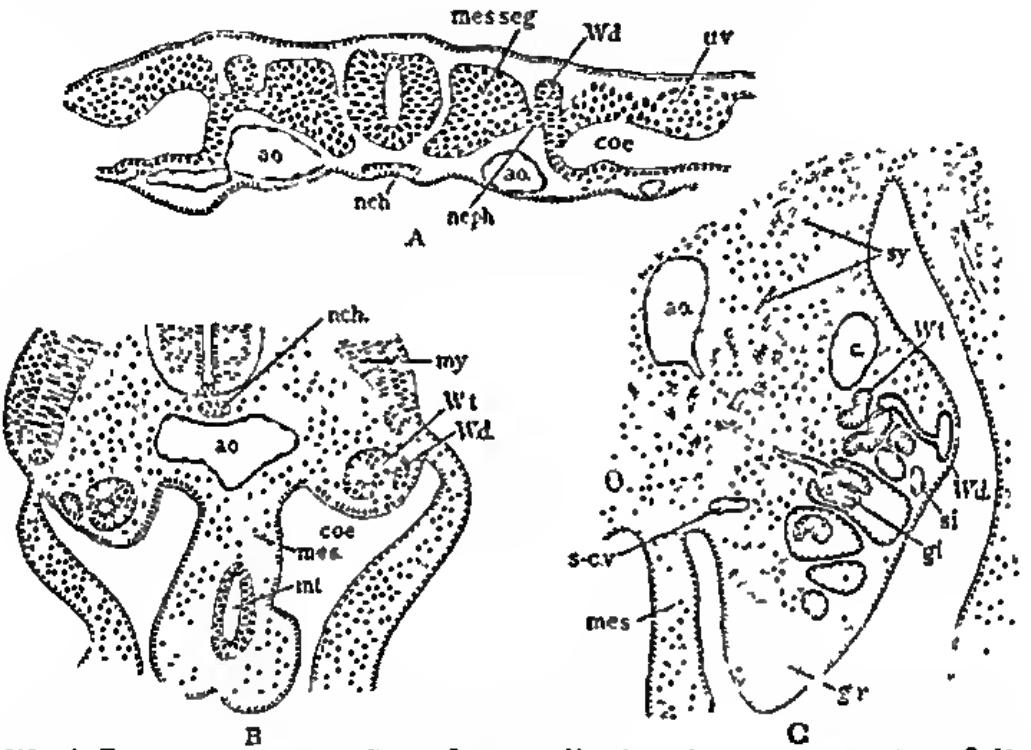


Fig. 397.—A, Transverse Section of a Rabbit Embryo of Nine Days, B, Human Embryo, 4 mm., C, Human Embryo 10 mm.

ao., Aorta, c., posterior cardinal vein, coe., cœlom, gl., glomerulus, g.-r., genital ridge, int., intestine, mes., mesentery; mes. seg., mesodermic somite, my., myotome, nch., notochord, neph., nephrotome, s-c. v., subcardinal vein, si., sinusoid, sy., sympathetic nerves, u. v., umbilical vein, W. d., Wolffian duct, W. t., Wolffian tubule.

A single Wolffian tubule is shown in Fig. 398 and the way in which its distal end envelops the glomerulus is clearly indicated. It is said to form the *capsule* of the glomerulus. By passing through the inner layer of this capsule, fluid from the blood vessels enters the tubule and is conveyed through the Wolffian duct to the bladder. The tubules are generally unbranched, and are lined with simple epithelium. The shape of their coils has been studied by F. T. Lewis.[1] The epithelium is in part glandular, and contributes to the formation of the urine. Finally it may be noted that a nephrotome may divide into several vesicles (sometimes perhaps as many as four), and therefore the number of Wolffian tubules is greater than the number of corresponding segments. In man the

[1] Lewis, F. T., 1920.

maximum number is 83.[1] The mesonephric tubules also extend forward, so that some segments contain both mesonephric and pronephric tubules.

It is generally believed that the Wolffian bodies of mammalian embryos are active renal organs, producing a form of urine which collects in the allantoic sac. In pig embryos this sac and the Wolffian bodies are both unusually large. In the human embryo, however, the allantois is very small and the Wolffian bodies degenerate early, before the kidney becomes functional. Therefore Felix maintained that the Wolffian body 'does not function as an excretory organ.' It has been shown, however, that in those animals in which the allantois and Wolffian bodies degenerate (or never fully develop, as in the mouse) there are indications that fetal excretion takes place through the placenta.[2]

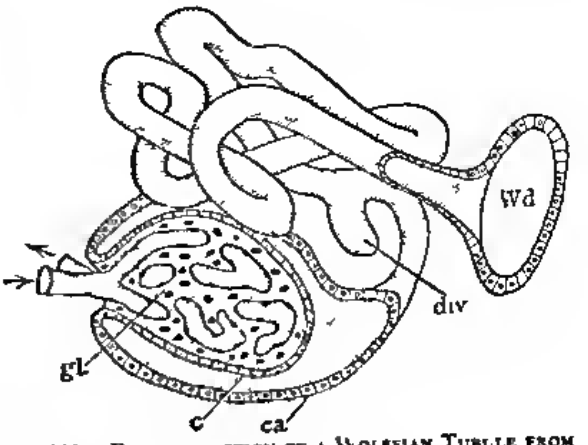


FIG. 398.—RECONSTRUCTION OF A WOLFFIAN TUBULE FROM A HUMAN EMBRYO OF 10.2 MM. (Except the glomerulus, after Kollman.)

c., Inner layer, and c. a., outer layer of the capsule of the glomerulus; div., diverticulum; gl., glomerulus; W. d., Wolffian duct.

Veins of the Wolffian Body. In determining the arrangement of the large veins of the abdomen, the Wolffian bodies are of fundamental importance. They are supplied by the *posterior cardinal veins* which pass from the tail of the embryo, on each side of the aorta, to the heart. Before entering the right atrium of the heart, they are joined by the *anterior cardinal veins* from the head; thus forming the right and left *common cardinal veins*, or 'ducts of Cuvier.' As each posterior cardinal vein extends along the dorsal side of the Wolffian body, it sends branches in among the tubules, and these unite ventrally on each side in the *subcardinal vein* (Fig. 399, A), or in the slightly differently placed *supracardinal vein*, which occurs in some animals.[3] Thus each Wolffian body is lodged in a venous loop formed by the posterior cardinal and subcardinal or supracardinal veins, and such a loop is found in all classes of vertebrates. Venous blood entering the Wolffian body posteriorly circulates among the tubules in lacunar vessels, closely resembling the hepatic sinusoids, and flows out anteriorly. This is the 'renal portal system.' It should be noted, however, that the renal sinusoidal vessels are poorly developed in mammalian embryos.

In sections these veins are readily recognized. The mesonephric arteries pass from the aorta to the glomeruli of the Wolffian body, between the subcardinal vein in front and posterior cardinal vein behind (Fig. 397, C). In places, the subcardinal veins form large anastomoses

[1] TELIN, 1912. [2] BREMER, 1916. [3] MCCLURE AND HUNTINGTON, 1929.

across the mid-ventral line; the posterior cardinal veins are further apart, and receive the intersegmental branches from the dorsal musculature.

As the kidneys grow upward behind the Wolffian bodies, their ureters become encircled by a branch from the posterior cardinal vein (Fig. 399, A). The venous loop around the ureter was described by Hochstetter.[1] The transformation of these veins into the branches of the inferior vena

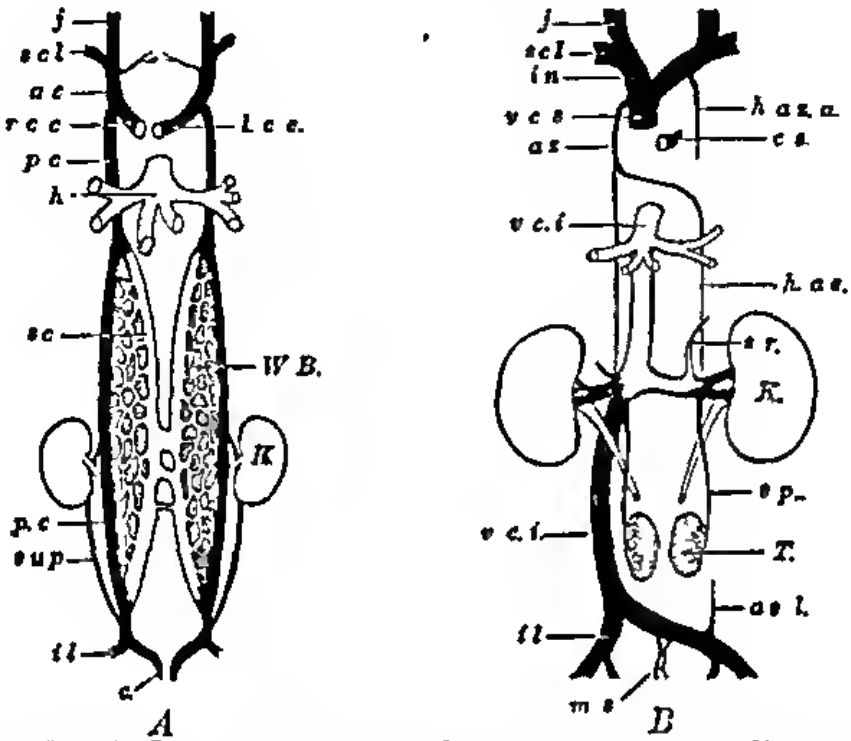


Fig. 399.—The Transformation of the Posterior Cardinal System of Veins.

a. c., Anterior cardinal, as l., ascending lumbar, az., azygos, c., caudal, c. s., coronary sinus, h., hepatic; h. az., hemiazygos, h. az. a., accessory hemiazygos, il., common iliac, in., innominate; j., jugular, K., kidney; l. c. c., left common cardinal, m. s., median sacral, p. c., posterior cardinal, r.c.c., right common cardinal, s. c., subcardinal, scl., subclavian, sp., spermatic, sr., suprarenal, sup., supracardinal, T., testis, v. c. i., vena cava inferior, v. c. s., vena cava superior, W. B., Wolffian body

cava is represented somewhat diagrammatically in Fig. 399, B, and may be briefly described as follows:

The anastomosis between the subcardinal veins becomes a part of the left renal vein. Above this anastomosis the right subcardinal vein connects with the veins of the liver and forms a portion of the vena cava inferior. The left subcardinal vein, above the renal anastomosis, becomes reduced to the left suprarenal vein. The subcardinal veins below the renal anastomosis are associated with lymphatic vessels to which they apparently give rise; otherwise they disappear.

The posterior cardinal veins above the renal anastomosis, after they have been tapped by the formation of the vena cava inferior, are known as the azygos and hemiazygos veins, and the outlet of the left common cardinal becomes cut off as the coronary sinus (Fig. 399, B, which shows also the formation of the superior vena cava). Below the renal anastomosis the posterior cardinal veins give rise to the genital veins (spermatic or ovarian), and the Wolffian body becomes reduced to an appendage of the geni-

[1] Hochstetter, 1893.

tal glands. As the genital glands descend into the pelvis, their veins become elongated; and the corresponding arteries, derived from the mesonephric arteries, are likewise elongated. The supracardinal vein on the right side becomes a part of the vena cava inferior; on the left it is probably represented by the ascending lumbar vein.

The kidneys are supplied by vessels which enter them after they have attained their permanent position. Their arteries and veins consequently pursue a straight course from the aorta and vena cava, respectively, to the hilum of the kidney.

KIDNEY

Development. The kidney develops after the Wolffian body has been formed. It arises in two parts, one of which is an outgrowth of the Wolffian duct; the other is a mass of dense mesenchyma surrounding this outgrowth, and said to be derived from the posterior nephrotomes. Both

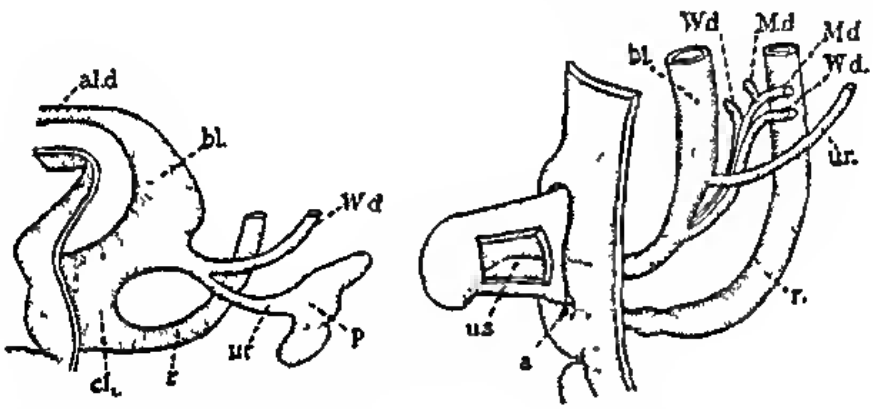


FIG. 400.—THE DEVELOPMENT OF THE RENAL PELVIS AND URETER (Keibel)

A, Human embryo of 11.5 mm (4½ weeks), B, 25 mm (8–9 weeks) a, Anus, al. d., allantoic duct, bl, bladder; cl, cloaca, M. d, Müllerian duct, p, pelvis of the kidney, r, rectum, ur, ureter, u. s., urogenital sinus, W. d, Wolffian duct

parts are mesodermal. The part derived from the Wolffian duct may be considered first.

Each Wolffian duct, near the place where it enters the cloaca, forms a knob-like outpocketing which elongates rapidly, becoming the *ureter*. The distal end of the outpocketing expands and becomes lobular, thus producing the *pelvis* of the kidney. After the ventral part of the cloaca has been split off to form the bladder, the ureter and Wolffian duct, on each side, open into it by a common outlet (Fig. 400, A). Later, the terminal portion of each Wolffian duct is taken up into the wall of the expanding bladder, so that the ureters acquire openings separate from those of the ducts. In this way the dorsal wall of the bladder receives a component of mesoderm. With further growth the orifices of the Wolffian ducts are carried toward the median line and downward toward the outlet of the bladder (Fig. 400, B); later the sphincter forms between them and the ureters, so that while the ureters connect with the bladder, the Wolffian ducts open into the urethra.

Anomalous double ureters and even accessory kidneys are the result of the development of extra ureteral buds from the Wolffian duct. According to their position in relation to the primary ureter they may open into either urethra or bladder.

Fig. 401.—Reconstruction of the Ureter, Renal Pelvis and its Branches in a 20-mm Human Embryo (Huber)

Meanwhile the lobes of the renal pelvis have become deeper and formed pouches known as the *major* and *minor calyces*. In the adult there are usually two major calyces, one at each end of the pelvis, and from these most of the minor calyces grow out; the others spring directly from the main pelvic cavity. There are seven to twenty in all, each representing the single pelvis of a simple kidney, such as is found in the cat or rabbit; the human kidney is thus a multiple organ. From the minor calyces the *collecting tubules* grow out. Each tubule has an enlarged extremity which divides into two branches with a U-shaped crotch, like a tuning-fork. The branches subdivide repeatedly in the same manner, so as to make pyramidal masses of straight tubules radiating from the calyces. Thus the renal outgrowth from the Wolffian duct produces the epithelial lining of the ureter, pelvis, calyces and collecting tubules, including all of their branches.

The second part of the kidney, which consists of dense mesenchyma, called the *renal blastema*, becomes subdivided into masses enveloping the enlarged tips of the branching collecting tubules. Some of its cells become arranged so as to form vesicles (Fig. 402), one of which is shown in the reconstruction, Fig. 403, A. The vesicles are at first entirely separate from the collecting tubules. Each vesicle becomes elongated, making an S-shaped tubule (Fig. 403, B, C), and its outer or upper end unites with the collecting tubule. A glomerulus develops in the lower curve of the S, and is gradually enveloped in the terminal part of the tubule, which thus forms its capsule. Between the capsule and the collecting tubule, the renal tubules become greatly convoluted. One of the loops in the coils thus formed elongates downward, lying close beside and parallel with the collecting tubule; this is the *loop of Henle* (Fig. 403, J).

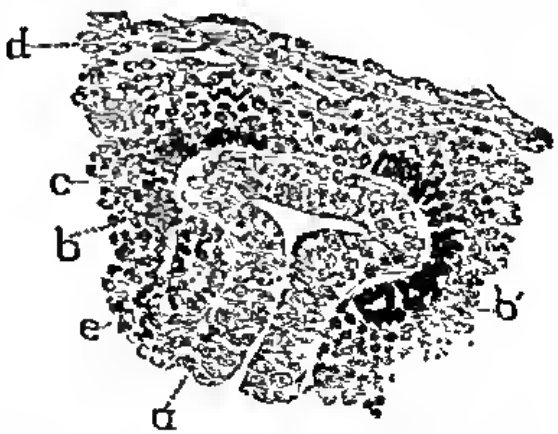


Fig. 402.—From a Section of a Kidney of an 18-mm Human Embryo. × 233 (Huber)

a, Primary collecting tubule, with dilated extremity; b, b', inner layer; and c, outer layer of dense mesenchyma; d, loose mesenchyma, e, vesicle, the beginning of a renal tubule

Three tubules of the adult kidney are shown diagrammatically in Fig. 404. Each *capsule* connects with a ***proximal convoluted tubule***, which, after extending outward toward the surface of the kidney, turns downward as the *descending limb* of Henle's loop. The descending limb is a

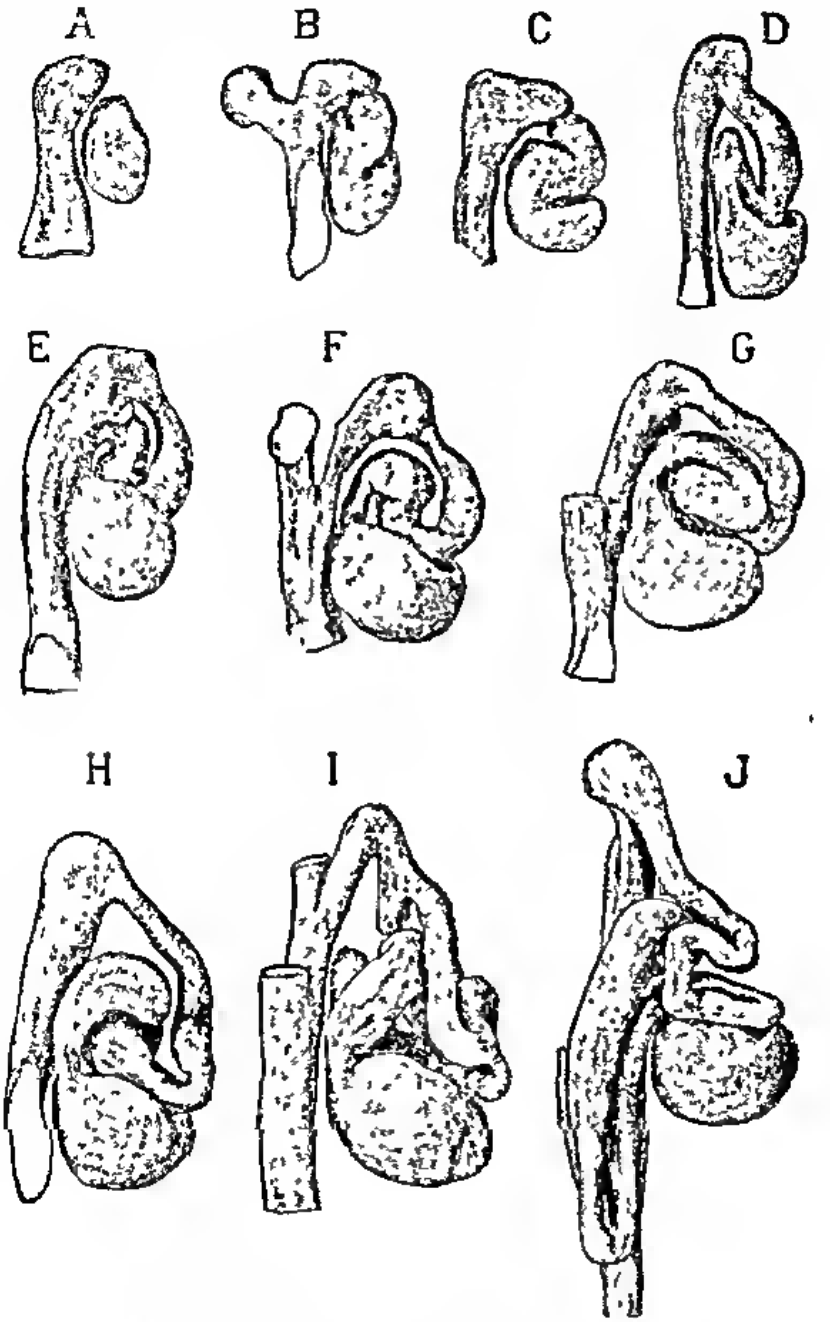


FIG. 403.—MODELS SHOWING SUCCESSIVE STAGES IN THE DEVELOPMENT OF A URINIFEROUS TUBULE INCLUDING THE ASSOCIATED PORTION OF THE COLLECTING TUBULE (*Huber*)
From a human embryo of the seventh month. × 160

straight tubule, the lower portion of which is of small diameter owing to the flatness of the cells in its walls; its lumen is not reduced, and may even be larger than that of the convoluted portion. This 'thin segment,' as shown in the diagram, does not form the entire descending limb, but only its lower part. Frequently it passes around the bend into the ascend-

ing limb, and it may vary considerably in length. The tubule, after turning the bend, forms the *ascending limb* of Henle's loop. It returns to the vicinity of the capsule from which it arose, and makes a few coils, thus constituting the *distal convoluted tubule*. By means of the *junctional tubule* or initial collecting segment it joins the arched collecting tubule and this passes into the straight descending *collecting tubule*. From the capsule to the collecting tubule no branches occur; and this extent of the tubule represents the part derived from mesenchyma. It is known as the *nephron* and forms a renal unit. Huber[1] divides the nephron into five parts: (1) the *renal corpuscle* consisting of the glomerulus and capsule, (2) the *neck segment* including the proximal convoluted segment with the medullary portion, (3) the *thin segment of the medullary loop*, (4) the *thick segment of the medullary loop* with the distal convoluted segment and (5) the *initial collecting segment*. The collecting tubules receive many branches. Traced toward their outlet in the pelvis they become larger, finally forming the *papillary ducts*.

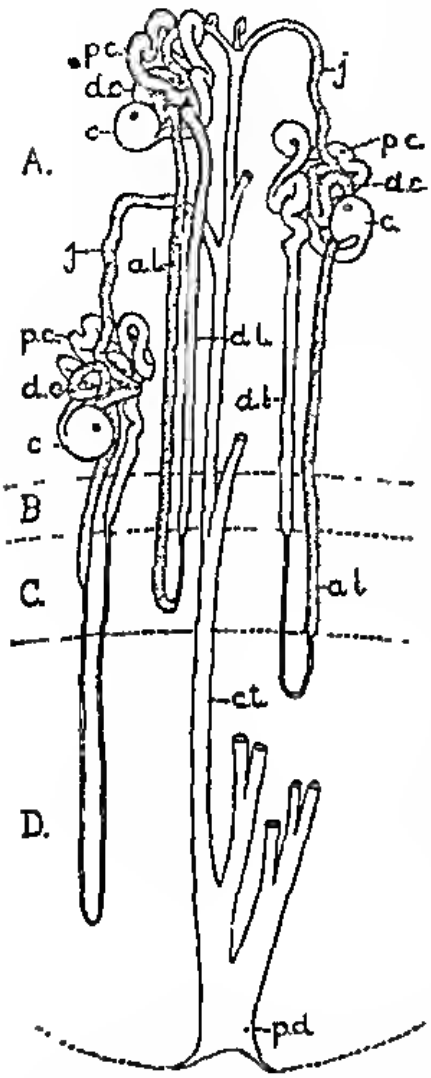


Fig. 404.—Diagram of Three Uriniferous Tubules in Relation with a Collecting Tubule (Modified from Huber)

a. l., Ascending limb of Henle's loop, c., capsule, c. t., collecting tubule, d. c., distal convoluted tubule, d. l., descending limb, j., junctional tubule, p. c., proximal convoluted tubule, p. d., papillary duct

A, cortex, B–D, medulla, subdivided into an inner zone (D) and an outer zone (B–C); the latter includes an inner band or stripe (C), and an outer band (B)

In the diagram (Fig. 404) the tubules are represented as much coarser than is actually the case. Their true proportions in the rabbit's kidney have been shown by Huber, who, with extraordinary success, has isolated individual tubules, keeping them intact from the capsule to the collecting tubule.[2] They are 20–30 mm. in length and less than 0.1 mm. in diameter. Huber's account of the development of the kidney, from which Figs. 401–404 have been taken, is found in the supplement to the American Journal of Anatomy, 1905, vol. 4.

From the shape of the complete urinary tubules as a whole, as shown in Fig. 404, it will be seen that a group of them crowded together will be narrow at the bottom, where the narrow straight tubules are, but broad above, where the glomeruli and convoluted tubules occupy more space. In large numbers the upper mass will bulge so that the straight tubules

[1] Huber, 1932. [2] Huber, 1911.

will be almost surrounded by the combined upper convoluted portions, giving a 'kidney-shaped' figure with the collecting tubules opening at the hilum. The kidney of many animals is composed of such a single group of tubules. The straight tubules, radiating from the hilum, form the medulla; the convoluted tubules and the glomeruli form the cortex. Since the glomeruli are at different levels, representing new additions at different periods of growth, the collecting tubules stretch into the cortex to reach the convoluted tubules, which they join near the glomerulus in each case. The collecting tubules run in groups, which grow smaller and smaller as more and more of the tubules end by joining convoluted tubules. These tapering groups are called rays, sometimes 'medullary rays' because they are of the same substance (straight tubules) as the medulla, sometimes 'cortical rays' because they are found in the cortex. They extend almost to the kidney surface. In the rays are also found the straight tubules of Henle's loop, which can develop more readily among other straight tubules than among the convoluted.

Surface Markings. The cut surface of a human kidney, whether it is divided lengthwise or across (Fig. 405), shows macroscopic markings representing the grouping of the tubules. The kidney is multiple, *i.e.*, it is composed of many single groups of tubules. Two are shown in the figure, each of which is similar to the whole kidney of a cat or a rabbit; from seven to twenty simple kidneys are fused to form the human organ. The ureter opens into the pelvis, an expanded sac, which is prolonged into the cup-like calyces. Every calyx represents the pelvis of a simple kidney, and receives a nipple-like projection of the substance of the kidney, known as a *renal papilla*. Sometimes two of them project into one calyx. They are soft, dark red structures, quite different in gross appearance from the grayish lining of the calyces and pelvis. Toward the apex of each papilla there are from 15 to 20 foramina, which are the orifices of as many papillary ducts; through them the urine enters the calyx. The foramina are barely visible without magnification. Each papilla forms the apex of a *renal* (or Malpighian) *pyramid*, described by Malpighi (1666) in his treatise 'on the structure of the viscera,' which gave the first account of various almost microscopic 'corpuscles' and surface markings. The base of the pyramid is toward the periphery of the kidney, and may be lobular as in the figure. From two to nine embryonic or primary pyramids are said to fuse to form a pyramid of the adult kidney. In favorable specimens the pyramid is seen to be divided into an inner and an outer zone, and the latter is composed of two concentric bands. The significance of these markings will be considered later. The base of each pyramid is surrounded by a lighter zone, the *cortex*, 6–8 mm. wide. Between pyramids this dips down to the renal sinus, forming the renal

columns (of Bertin); one of them is shown in Fig. 405. They represent the fusion of adjacent parts of simple kidneys. The pyramids collectively constitute the *medulla* of the kidney.

The cortex shows radial striations, the tapering rays, which are called the pyramids of Ferrein, and are known collectively as the radiate part of the cortex (*pars radiata*). Between the rays is the convoluted part of the cortex (*pars convoluta*), containing the convoluted tubules and the *renal corpuscles* (Malpighian corpuscles), consisting of the glomeruli and their capsules. They are barely visible without magnification.

Over the outer surface of the kidney, there is a fibrous capsule (*tunica fibrosa*) which may be readily stripped off when normal; and outside of this there is a fatty layer (*capsula adiposa*). The fat surrounds the pelvis and extends into a hollow of the kidney known as the *renal sinus;* this is

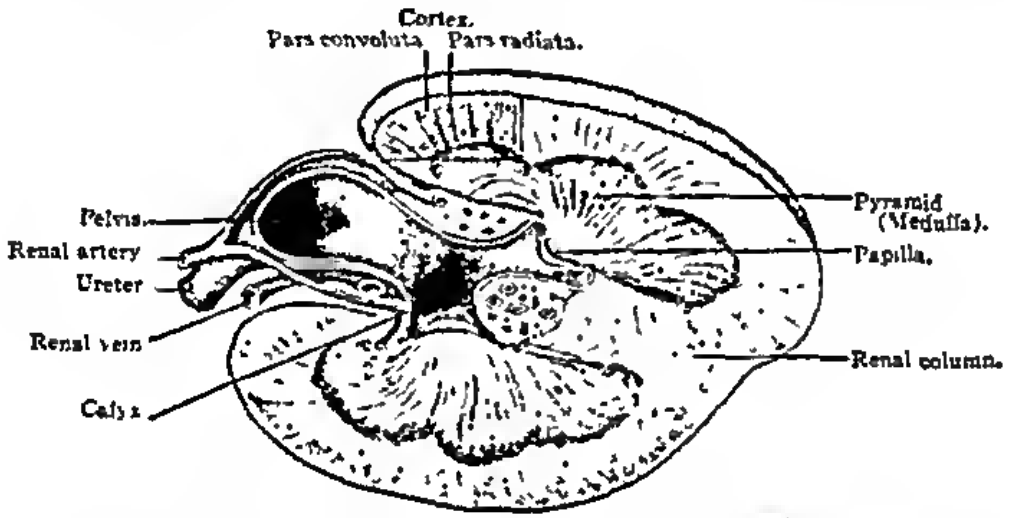


Fig. 405.—The Surface Markings of the Human Kidney. (Brödel.)

the excavation which contains the pelvis and its calyces. In this fatty tissue the large blood vessels enter the kidney, passing chiefly over the anterior or ventral surface of the pelvis; having reached the boundary zone between cortex and medulla at the border of each calyx, they enter it, and pursue an arched course, giving off both cortical and medullary branches.

The arrangement of the renal tubules in relation to the cortex and medulla is as follows. The convoluted part of the cortex contains the capsules, and both proximal and distal convoluted tubules. The rays contain the collecting tubules, together with the outer portions of Henle's loops. The medulla contains the larger collecting tubules and the deeper portions of Henle's loops; since these are all straight tubules, the medulla resembles the radiate part of the cortex. According to Peter[1] the tubules which are connected with capsules deep in the cortex, near the boundary zone, send their Henle's loops much further into the medulla than those

[1] Peter, 1927.

from the outer capsules; and in the deeply-placed tubules the thin segment of Henle's loop is not limited to the descending limb but extends well up into the ascending limb. Thus it happens that a broad *inner zone* of the medulla (*i.e.*, toward the papilla) contains only thin segments of renal tubules in addition to the large collecting tubules (Fig. 404, D); and the zone so characterized may be distinguished macroscopically. The papilla contains only collecting tubules, but the loops of Henle turn

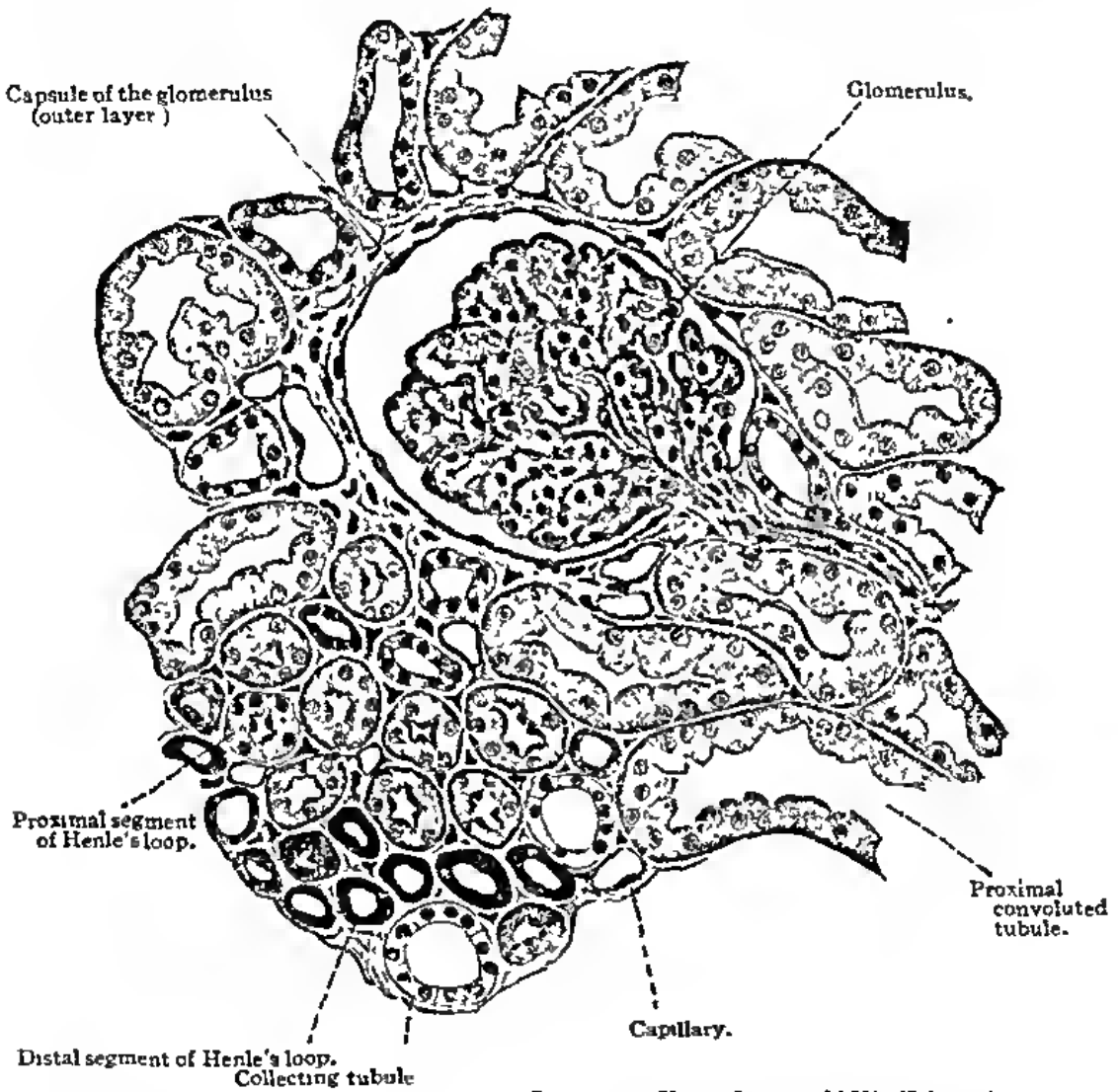


FIG. 406.—TANGENTIAL SECTION OF THE CORTEX OF A HUMAN KIDNEY. × 200 (Schaper)
The pars radiata is seen in the lower left corner. The line from 'capsule of the glomerulus' passes between two distal convoluted tubules.

back at different levels, and therefore the papillary zone entirely free from loops is not well defined. The *outer zone* of the medulla contains both thick and thin segments of Henle's loops, in addition to the collecting tubules. In the descending limbs the change to thin segments occurs at a more or less definite level within this outer zone, thus subdividing it into a narrow outer band, with few thin segments, and an inner band containing many of both sorts.

Sections of the Kidney. Since a radial section of the kidney shows both cortex and medulla, it is the form usually made for pathological examinations. The tubules may be studied to better advantage, however, in tangential sections, one through the cortex and the other through the medulla. The tubules are then seen in cross section. The rays of the cortex appear as islands of circular sections surrounded by the irregular convoluted tubules, among which are the scattered renal corpuscles. The greater part of such an island is shown in the lower portion of Fig. 406. The renal tubules are lined throughout with simple epithelium and their characteristic features will now be considered, beginning with the glomerular capsule.

The renal corpuscle develops as the invagination of one side of the blind end of the embryonic S-shaped tubule by the intrusion of a glomerulus. It is essentially a knot of arterial capillaries covered by one wall of the tubule, the inner layer of the capsule (of Bowman), while the outer wall forms the outer layer of the capsule. The two layers are continuous where the arteries enter the glomerulus. The space between the two layers is continuous with the lumen of a convoluted tubule. The function of the inner layer is to pass water and certain salts in solution from the blood to the lumen. This is provided for by the presence of very thin epithelial plates which merge with the capillary endothelium to form a thin membrane.

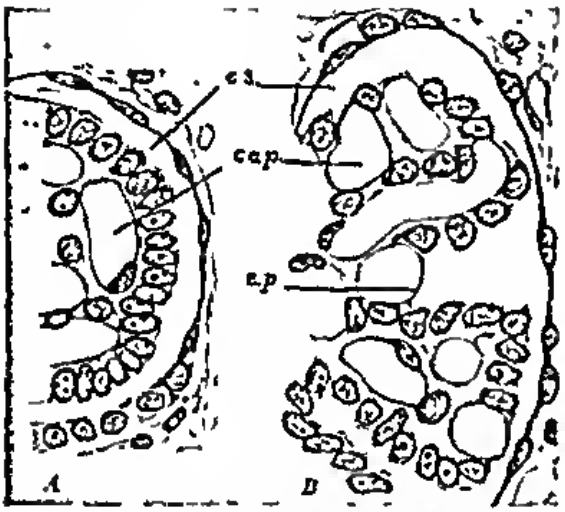


FIG. 407.—PARTS OF TWO GLOMERULI FROM KIDNEY OF HUMAN FETUS OF 4 MONTHS.

A, younger form the slit-like capsular space, c. s., is bounded by the flat epithelium of the capsule and the low columnar cells covering the glomerular capillaries. cap B, nearer medulla the growing glomerular vessels have stretched the overlying epithelium, making several thin epithelial plates, e. p., in relation with the endothelium.

The formation of this membrane can be studied in radial sections of the kidney of a four months' fetus. The renal tubules which have their capsules close to the medulla are the first to develop; others are formed successively outward as the collecting tubules continue to grow, the youngest being immediately beneath the kidney capsule. They represent in section the stages shown in Fig. 403. In the youngest corpuscles the inner layer is simple columnar epithelium, the nuclei very crowded. In older corpuscles this epithelium becomes cuboidal, and the blood vessels of the glomerulus press close to the base of the cells (Fig. 407, A); in later stages, (B), the capillaries push apart the cell bases of the inner layer and approach the surface, until only a thin plate-like layer of protoplasm

covers the endothelium. In the adult the endothelium of the capillaries and the surface epithelium are separated by a structureless basement membrane, connecting with a central connective tissue or reticular core of the glomerulus, in which are a few fibroblasts (Fig. 408). As in the alveolar wall of the lung, the continuity of the epithelium has been doubted; some authors consider it a continuous, though very thin sheet,[1,2] in which the spherical nuclei occupy thicker, almost cuboidal areas, while others recognize separate, individual cells (Deckzellen), of stellate form more or less like the pericytes of blood vessels,[3] which leave a great part of the capillary wall 'naked.' The endothelium of the capillaries is also often extremely thin except near the nuclei, so that its continuity has

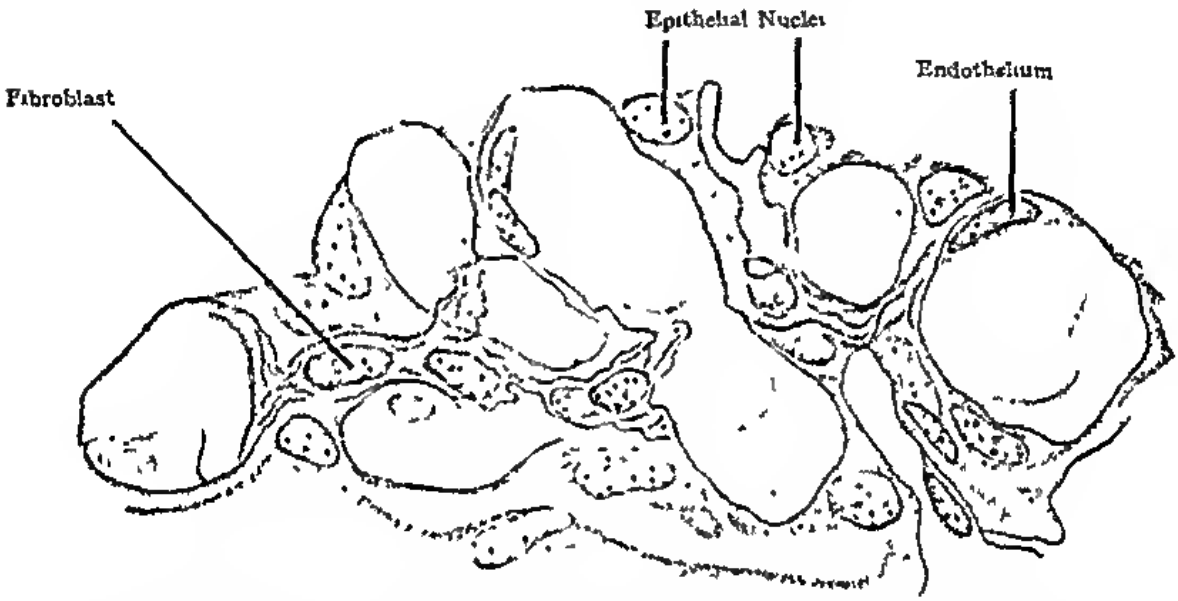


FIG. 408.—A LOBULE OF A GLOMERULUS, HUMAN KIDNEY, 3 μ SECTION, FLEMMING'S FIXATION STAINED WITH HÆMATOXYLIN EOSIN, PHOSPHOMOLYBDIC ACID, METHYL BLUE. THE CONNECTIVE TISSUE ELEMENTS STAIN BLUE. (Copied from von Möllendorff, 1930.)

also been questioned. Cell boundaries are hard to detect by silver nitrate methods, and it may be a syncytium. The activity taking place at this barrier is probably purely physical, through an inert membrane, instead of physiological or secretory, due to cell action.

The capillary net in the glomerulus is divided into lobules (usually five) by deep clefts (Fig. 414), plainly visible when the glomerulus is favorably oriented. The epithelial layer closely follows the lobulations, and thus presents a greatly increased surface. The entire glomerulus grows to a diameter of 0.13 to 0.20 mm.

The epithelium of the outer or parietal layer of the capsule is flat and rests on a basement membrane of two layers, one structureless, the other reticular. At the 'neck of the capsule' in man the epithelium

[1] BENSLEY AND BENSLEY, 1930. [2] ZIMMERMANN, K. W., 1933.
[3] VON MÖLLENDORFF, 1930.

changes to the type found in the proximal convoluted tubule. In some animals the latter extends well into the capsule.

The proximal convoluted tubules are large (40 to 60 μ in diameter) with irregular lumens, sometimes star-shaped or slit-like, because of the height and irregular surfaces of the epithelial cells. With the usual staining methods no cell boundaries are shown, but by special treatment they appear curiously vermiform and interlocking (Fig. 409) on surface view.

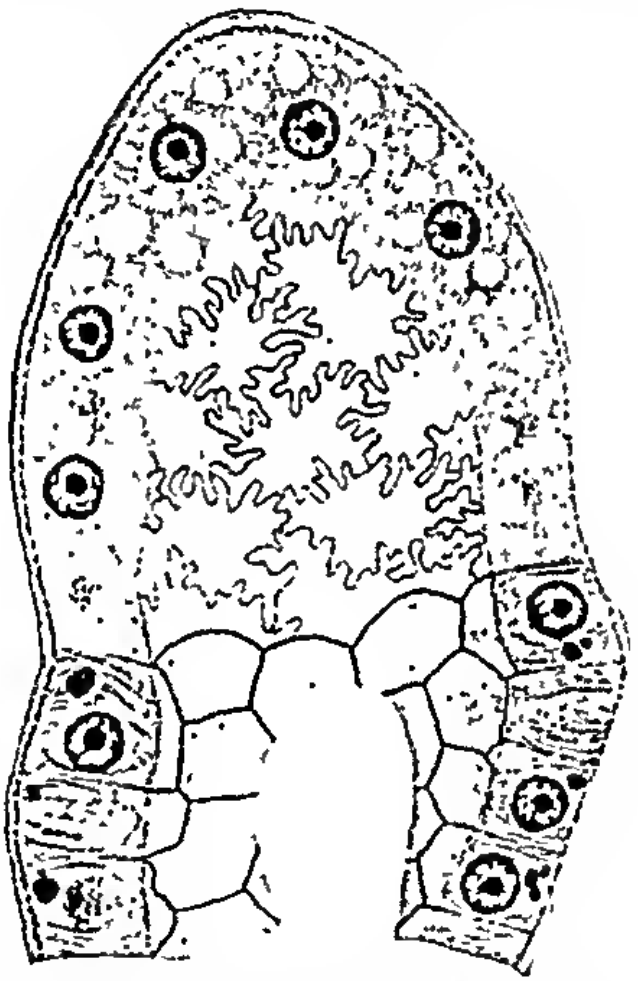
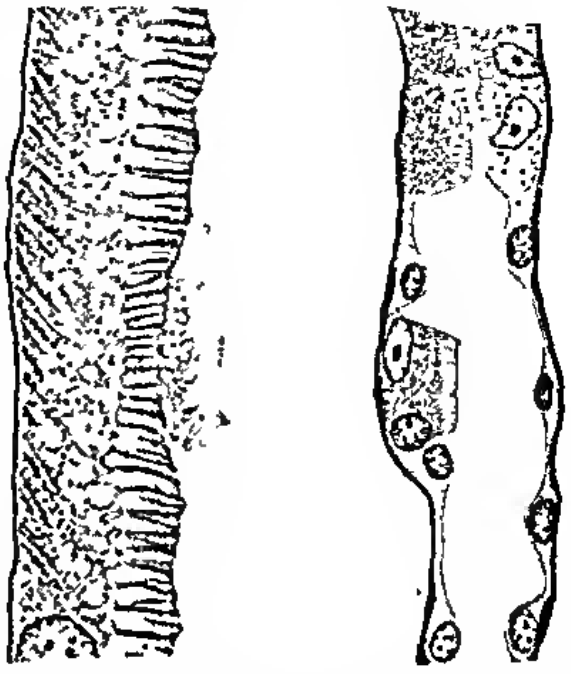


Fig. 409.—Pars Radiata, Cat. Sudden Change of Epithelium in Descending Limb of Henle's Loop. Iron Hæmatoxylin. (Copied from Zimmermann, 1911.)

The cells show granules, basal striations, mitochondria and a Golgi apparatus, situated in the apical pole. The differentiation at the free surface takes the form of a cuticula, which under certain circumstances appears vertically striated or provided with short cilia-like processes, the 'brush border.' This is typical of this part of the nephron. Von Möllendorff considers this border as a cuticle provided with minute pores, through which secretions may pass, and offers surface views to show these tiny holes (Fig. 410, A). The 'brush border' may show wide variations in height and appearance. The cells of this segment are lower and the lumen wider during the copious production of urine.

The upper segment of the descending limb of Henle's loop is similar in general to the proximal convoluted tubule. It is, however, a straight or slightly spiral tubule found in the radiate part of the cortex; and in some animals (*e.g.*, cat) the epithelium differs in that the fat content of the cells ceases abruptly and at the same spot the wavy cell outlines change to the usual hexagonal form (Fig. 409). These appearances are not constant in all animals, however, and have not been recognized in man, so that it is common to think of these two portions of the nephron as having the same functional significance.



A B

FIG 410.—A, PROXIMAL CONVOLUTED TUBULE (GUINEA-PIG), SHOWING BASAL STRIATIONS AND 'BRUSH BORDER,' APPEARING AS PORES ON SURFACE VIEW. B, DESCENDING LIMB, SHOWING 'BRUSH BORDER' AND SUDDEN CHANGE TO THIN SEGMENT. (Copied from von Möllendorff, 1930.)

The change to the thin segment of the loop is abrupt, though occasional groups or individual cells may overlap (Fig. 410, B). The thin segments are slender but have large lumens. Cell membranes are indistinct, and the cells are so flat that their nuclei may occasionally cause elevations; the cytoplasm is never so thin as that of the endothelium of the capillaries. As is shown in Fig. 404, the thin segment of the loop may occur on the descending or the ascending limb of the loop, or on both. It is not profitable, therefore, to attempt to distinguish its position.

The change to the thick ascending portion of the loop is also abrupt. Here the cells become low cuboidal, the nuclei either spherical or oval, with long axis parallel to the surface. The cytoplasm is darker, often showing basal striations. The 'brush border' is lacking and the lumen wide. The ascending portion comes in close proximity with the vas afferens of its own glomerulus and there the epithelial cells next to the vessel become closely packed, the *macula densa*. The significance of this feature is unknown. Beyond this point the few distal convolutions occur, usually on the surface of the group of proximal convolutions. The epithelium continues as in the ascending portion. The tubules become gradually larger, and may have irregular bulges. They are typically shown in Fig. 406 (there being one on each side of the label line to the 'capsule of the glomerulus'). Huber describes these tubules as showing 'an outer dark zone which is finely striated, and an inner zone which is lighter, the nuclei being placed at the junction of the two zones.' It is

probable, from their position, that the distal convoluted tubules in Fig. 406 are parts of the tubule which connects with the glomerulus shown in the figure. The distal convoluted tubules in man open directly into the collecting tubules, with no recognizable connecting piece of different epithelial type, such as is found in some animals.

The arched collecting tubules, into which the distal convoluted tubules empty, pass into the collecting tubules of the rays, which are readily identified. They have round and clear-cut lumens; cell walls are distinct (in all but the smallest), and the nuclei are regularly arranged. Thus the collecting tubule resembles an excretory duct.

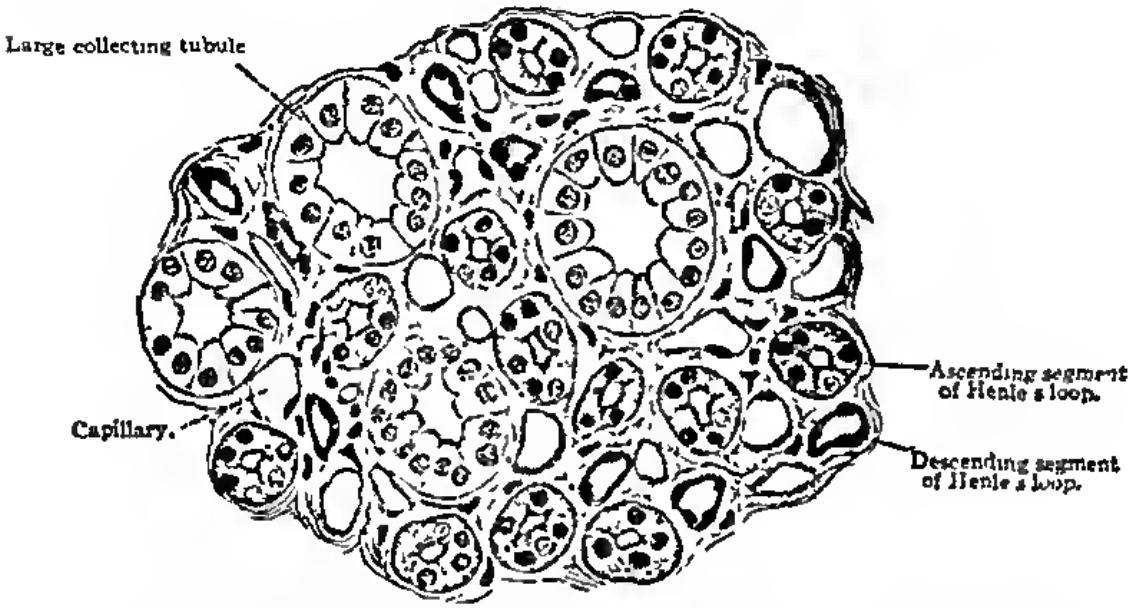


Fig. 411.—Transverse Section through the Medulla of a Human Kidney. × 320. (Schaper.)

The medulla (Fig. 411) contains the same elements as the rays. The collecting tubules are larger, and their walls are more distinct. Among their columnar cells a few are decidedly darker than the others. The thick segments of Henle's loops are easily distinguished from the thin segments. The loops are found only in the outer part of the medulla, as they do not reach the papilla. The larger papillary ducts are lined with tall columnar epithelium which continues on to the surface of the papillæ, where it changes gradually to the stratified epithelium of the calyces and pelvis.

The function of the different portions of the renal tubule has long been the subject of investigation. At the present time there is general agreement that the glomerulus acts as a physical filter for all of the non-colloid constituents of the blood plasma; and histologically it seems admirably fitted for this function. In this connection the student should consult the brilliant technical studies of Richards and his co-workers.[1] This group has shown that when glomerular filtrate is collected from a single glomerulus (frog) by puncture of its capsule, all of its constituents are present in essentially the

[1] Richards, 1929.

same concentration as in an ultrafiltrate of blood. The large cells of the proximal convoluted tubule probably serve a double function, reabsorbing certain substances from, and perhaps adding others to, the glomerular filtrate in its passage along the lumen. The problem is further complicated by the finding that in certain species of fish the kidney (mesonephros) is normally composed of tubules only, without glomeruli,[1] yet the urine is essentially the same as that from fish with glomerular kidneys. 'Glomerular filtration, tubular reabsorption, and tubular secretion all probably occur to some extent in all vertebrate kidneys (except the aglomerular kidney). The relative importance of the filtration-reabsorption mechanism and of tubular secretion of any given substance depends on the substance in question, the particular species of animal under consideration, and the conditions obtaining at the time of observation '[2] The thin segments and distal convoluted tubules seem to be concerned chiefly with reabsorption, primarily of water. The collecting tubules presumably act merely as ducts.

Connective Tissue. Between the renal tubules there is a small amount of interstitial connective tissue. It is more abundant toward the papillæ and around the vessels and glomeruli than elsewhere. Beneath the epithelium of the tubules it forms basement membranes, apparently homogeneous, but actually composed of fine fibrils. The normal amount of interstitial tissue should be carefully studied, since its increase is indicative of an important pathological condition. This tissue is continuous with that of the fibrous capsule. The latter contains elastic fibers, which increase in abundance with age, and also smooth muscle fibers.

FIG. 412.—KIDNEY OF A CHILD AT BIRTH (Hertwig)

Lobes and Lobules. In embryonic life the kidney is divided into *lobes*, bounded by the renal columns, and indicated by grooves upon the outer surface (Fig. 412). The grooves become obliterated during the first year. In the ox similar grooves are permanent; in many mammals, as in the cat and rabbit, they never exist, since the kidney has but one lobe, papilla and pyramid. The *lobules* or *structural units* of the kidney are the areas centering around each radiate division of the cortex, by which they are drained. They are not bounded by connective tissue septa.[3]

Blood Vessels. The kidney has a capillary circulation. The renal artery passes from the aorta to the *hilum*, or notch on the medial border of the kidney. It divides into several branches, most of which pass over the ventral surface of the pelvis into the fat around the calyces (Fig. 405). Thence, as *interlobar arteries*, they run in the renal columns between the cortical and medullary substance, and in the same position turn as *arciform* or arcuate *arteries* to run parallel to the capsule. Their course is usually in the long axis of the kidney as a whole, and, as they do not anastomose with each other, the occlusion of one of them may cause an

[1] MARSHALL AND GRAFFLIN, 1928. [2] MARSHALL, 1934. [3] TRAUT, 1923

infarct of a large part of a kidney lobe. They give off branches, the *interlobular arteries*, which pass toward the capsule through the convoluted part of the cortex, and their terminal branches enter the fibrous

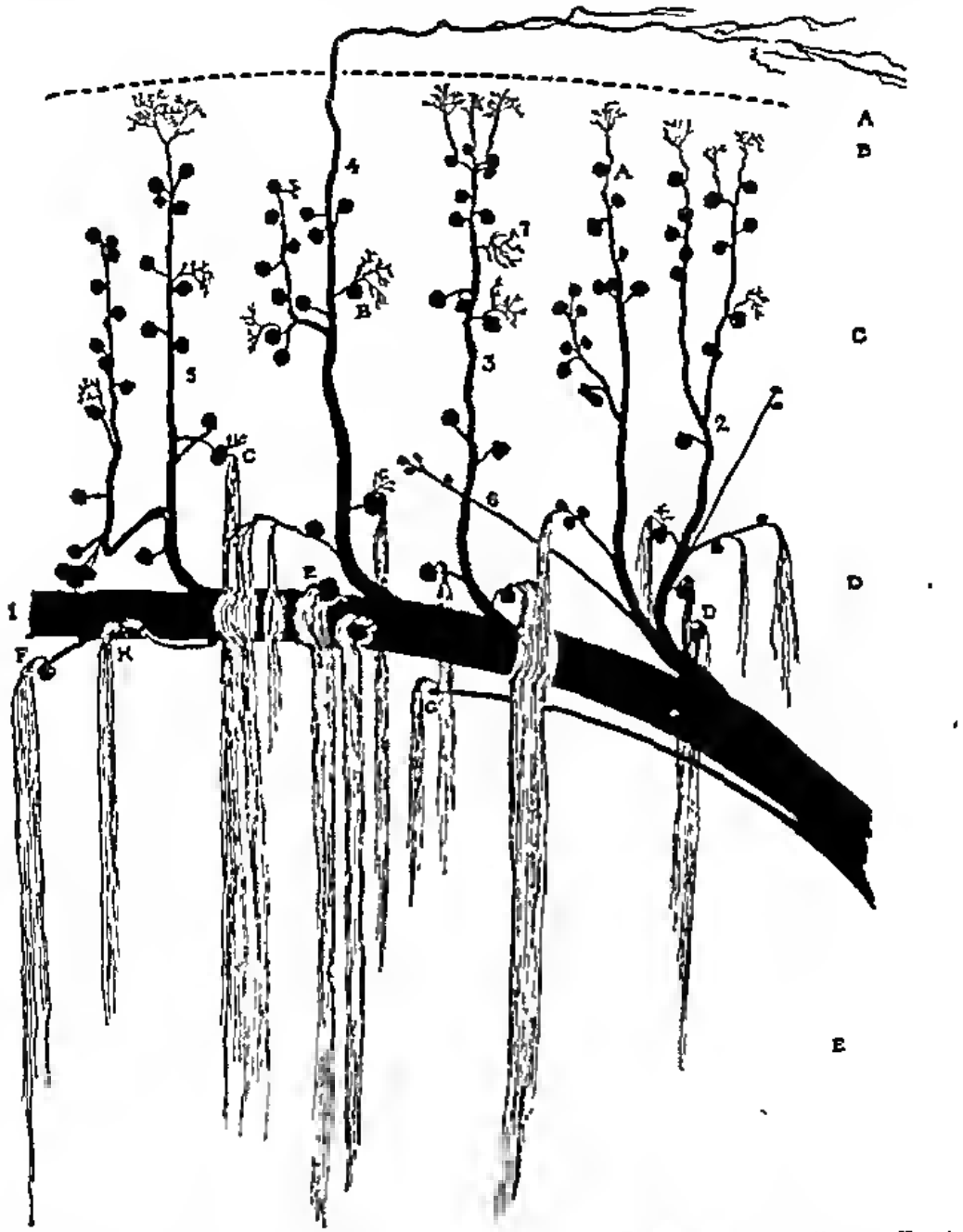


FIG. 413.—A SEMI DIAGRAMMATIC REPRESENTATION OF THE FINER ARTERIAL DISTRIBUTION IN THE KIDNEY. (D. M. Morison—courtesy of the Wistar Institute.)

A, capsule, B, subcapsular zone of cortex, C, cortex proper, D, corticomedullary zone, E, medulla, 1, arcuate artery giving off numerous interlobular arteries of various types, the long straight bundles are arteriæ rectæ passing down into the medulla

capsule. The interlobular arteries give off numerous small branches, each one passing as an afferent artery (vas afferens) to a single *glomerulus*. Since the number of glomeruli in the human kidney up to about forty years of age is approximately one million, the numbers of these branches can be appreciated. Very old persons lose from about one-third to one-

half of the glomeruli. Around the afferent vessel near the glomerulus, the smooth muscle cells become thickened and have in section an 'epithelioid' appearance. This feature of a thickening of muscle cells at the afferent pole is called by Zimmermann[1] the '*Polkissen*' or polar cushion. Some authors regard the epithelioid cells as 'glandular,' the secretion controlling local blood pressure. The afferent vessel breaks up into

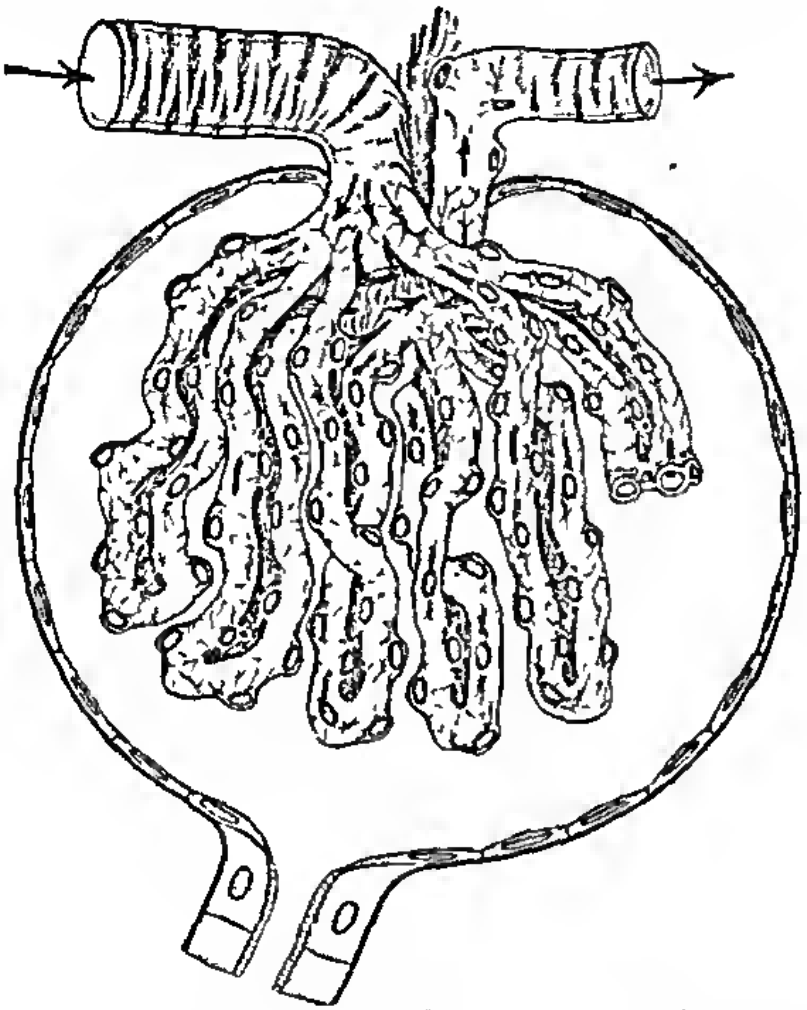


FIG. 414—A SCHEME OF A RENAL CORPUSCLE. THE NUMBER OF LOOPS ARE REDUCED FOR ILLUSTRATION (von Möllendorff)

several primary branches and these in turn to secondary branches which are resolved into a knot of up to fifty separate capillary loops in a glomerulus. Vimtrup[2] estimates from the total length of capillaries in each glomerulus (about 25 mm.) a capillary surface area for the whole kidney of 0.78 sq. meters. The glomerular capillaries seldom make anastomoses, and unite to form a single efferent artery (vas efferens) which is usually smaller in diameter than the afferent vessel. The entire glomerulus then is regarded as arterial.[3] There is evidence that the indi-

[1] ZIMMERMANN, K. W., 1933. [2] VIMTRUP, 1928.

[3] MCGREGOR (*Amer. Jour. Path.*, vol. 5, pp. 545–557, 1929) discusses many details on the finer structure of the normal glomerulus with a review of the literature since Malpighi.

vidual glomeruli may be active intermittently. Soon after leaving the glomerulus the efferent vessel divides into small branches. These spread as a true capillary plexus among the convoluted tubules in the neighborhood of each glomerulus and pass on to the cortical rays, where the capillary net assumes a longer mesh. The blood is returned to *interlobular veins*, which course with the interlobular arteries, and lead to *arciform* or *arcuate veins* and thence to *interlobar veins*. The interlobular

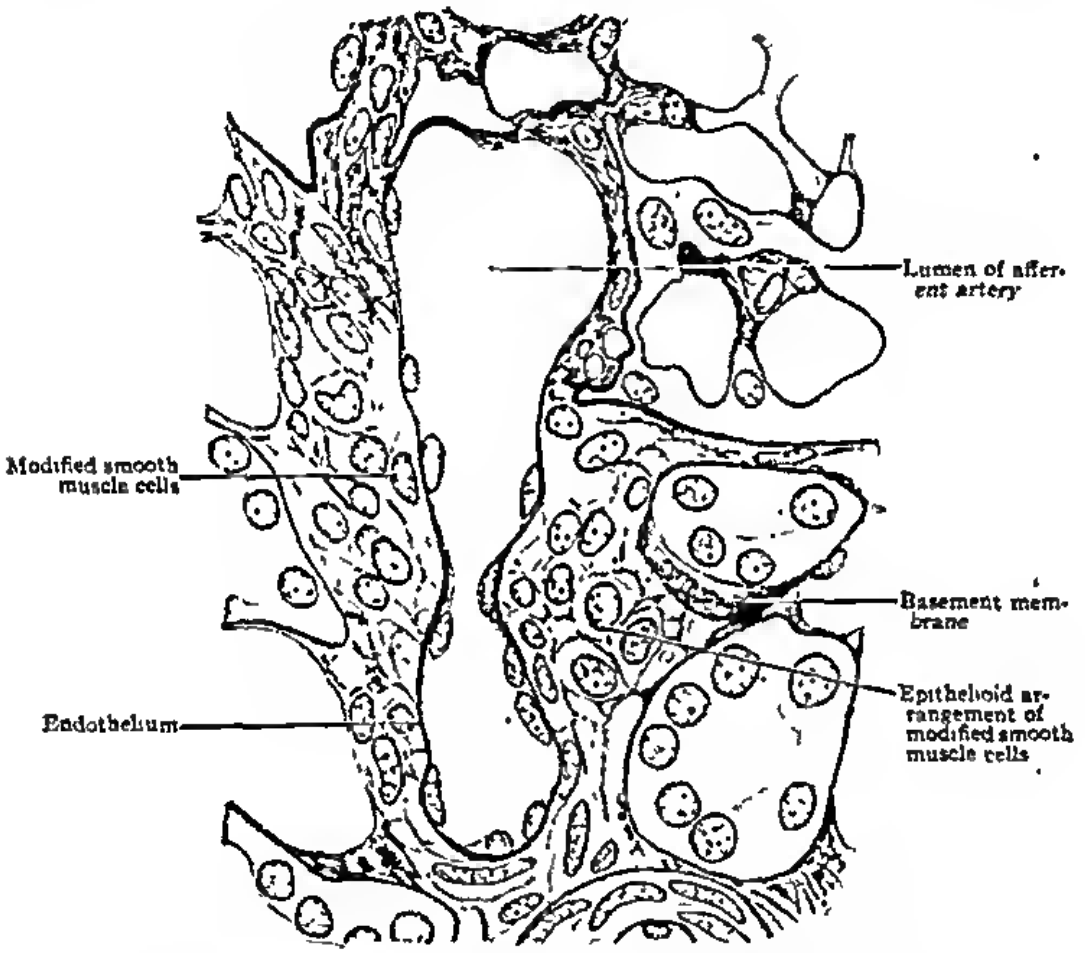


FIG. 415.—SECTION THROUGH THE AFFERENT ARTERY OF A GLOMERULUS OF A HUMAN KIDNEY.
The musculature in the vessel wall has become modified and forms a polar cushion or "Polkissen." Methyl blue and eosin. (Zimmermann.)

veins arise from converging veins in the renal capsule, which on surface view form stellate figures (*venæ stellatæ*). The interlobular veins drain the capillaries of the cortex, but have no direct relation with the glomeruli. It will be noted that the kidney is exceptional in having both its terminal arteries and veins at the periphery of its lobules.

The medulla is supplied by straight arteries (*arteriolæ rectæ spuriæ*). These were formerly described as (1) continuations of the elongated net in the cortical rays, or as branches (2) of the arciform arteries, (3) of the interlobular arteries, or (4) of the efferent arteries. All these vessels may be present, but those derived directly from the arteries are apparently

insignificant; in the normal mammalian kidney essentially all of the blood which reaches the tubules has first passed through some glomerulus. The arteriolæ rectæ spuriæ run in groups of ten or twenty vessels, certain areas of the medulla being comparatively avascular. The medullary veins empty into the arciform veins. *The interlobar veins* follow the arteries, passing out from the hilum of the kidney over the ventral surface of the renal pelvis.

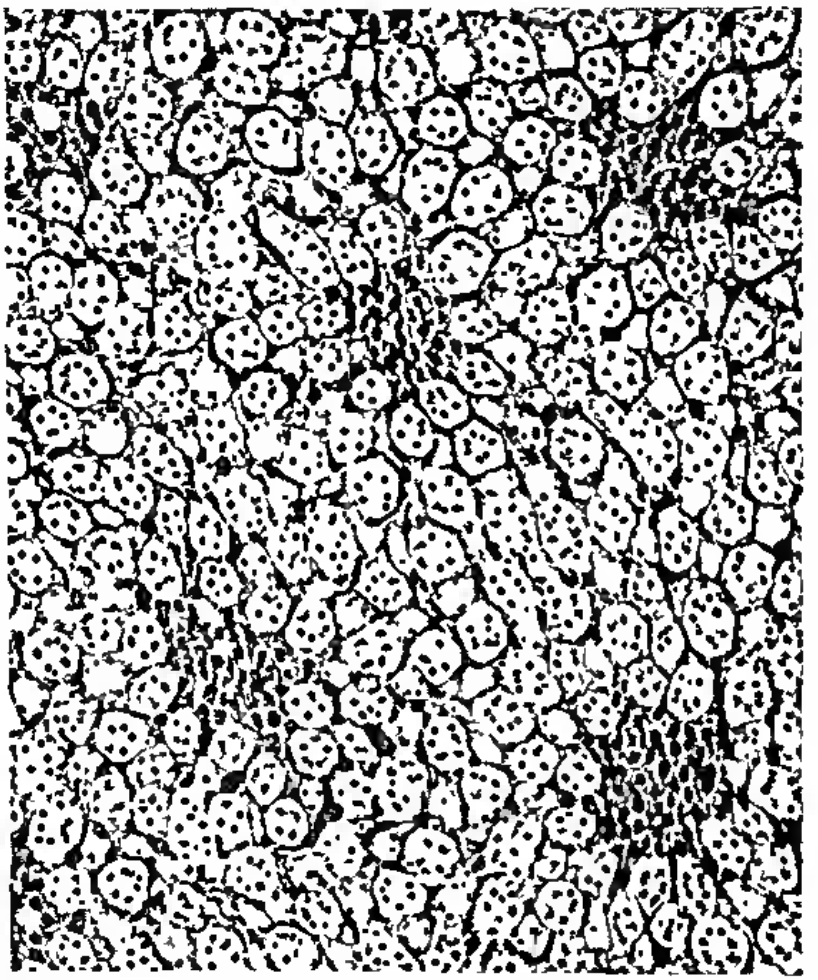


FIG. 416.—A TANGENTIAL SECTION OF THE KIDNEY OF A CAT

The arteriolæ rectæ spuriæ are shown in the dark areas injected with carmine-gelatin. Formaldehyde fixation, hæmatoxylin.

The lymphatics form in the cortex of the kidney wide plexuses around the renal corpuscles and convoluted tubules which pass into long-meshed networks accompanying the straight and looped tubules in the medulla. Some of these leave at the hilum along with the blood vessels, while others pass through the tunica fibrosa to connect with a network in the adipose capsule. Both sets of vessels proceed to neighboring lymph glands.

The *nerves* are medullated and non-medullated. There is a sympathetic plexus at the hilum associated with small ganglia, and from it interlacing nerves extend into the kidney around the vessels. Fine

branches supply the epithelial cells, especially those of the convoluted tubules. They form plexuses beneath and above the basement membrane, and have free intercellular endings.

RENAL PELVIS AND URETER

The renal pelvis and ureter both consist of a mucosa (and submucosa), muscularis and adventitia. The mucosa includes the epithelium and lamina propria and is ordinarily thrown into longitudinal

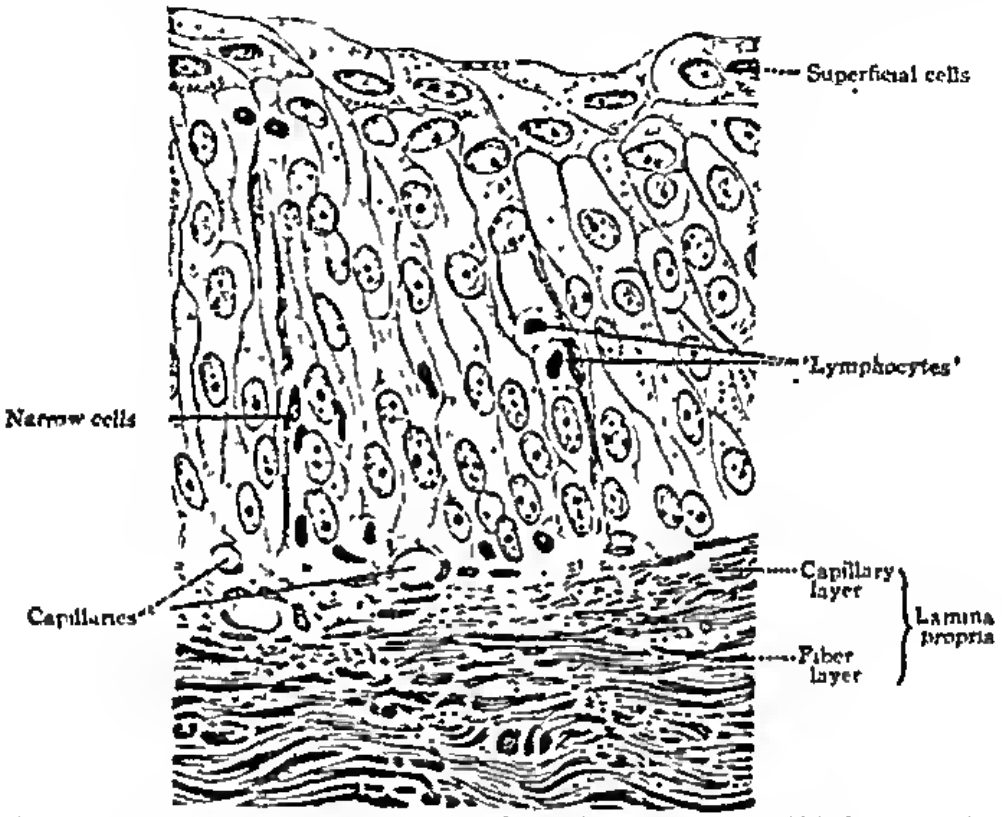


FIG. 417.—TRANSVERSE SECTION THROUGH THE WALL OF A RENAL CALYX. Zenker-formaldehyde fixation; Azan (von Mallendorff.)

folds. A submucosa is sometimes recognized, characterized by a looser texture and containing a vascular plexus, but there is no sharp line of demarcation, such as is offered in the intestine by the muscularis mucosæ. In sections the epithelium resembles that of the moderately contracted bladder, and its cells when found detached in urine are not distinguishable from bladder cells. The epithelium is stratified but consists of a few layers, 'transitional epithelium.' The basal cells are rounded, those of the middle layer are club-shaped or conical with rounded ends, and the outer cells are columnar, cuboidal, or somewhat flattened. Their lower surface may be indented by the rounded ends of several underlying cells, as is particularly the case in the contracted bladder. Near and over the renal papillæ the epithelium is simple columnar. Two nuclei are often found in a superficial cell, and in some

animals they are said to arise by amitosis. Leucocytes frequently enter the epithelium. In some animals mucous glands have been found extending into the lamina propria, and there are gland-like pockets in

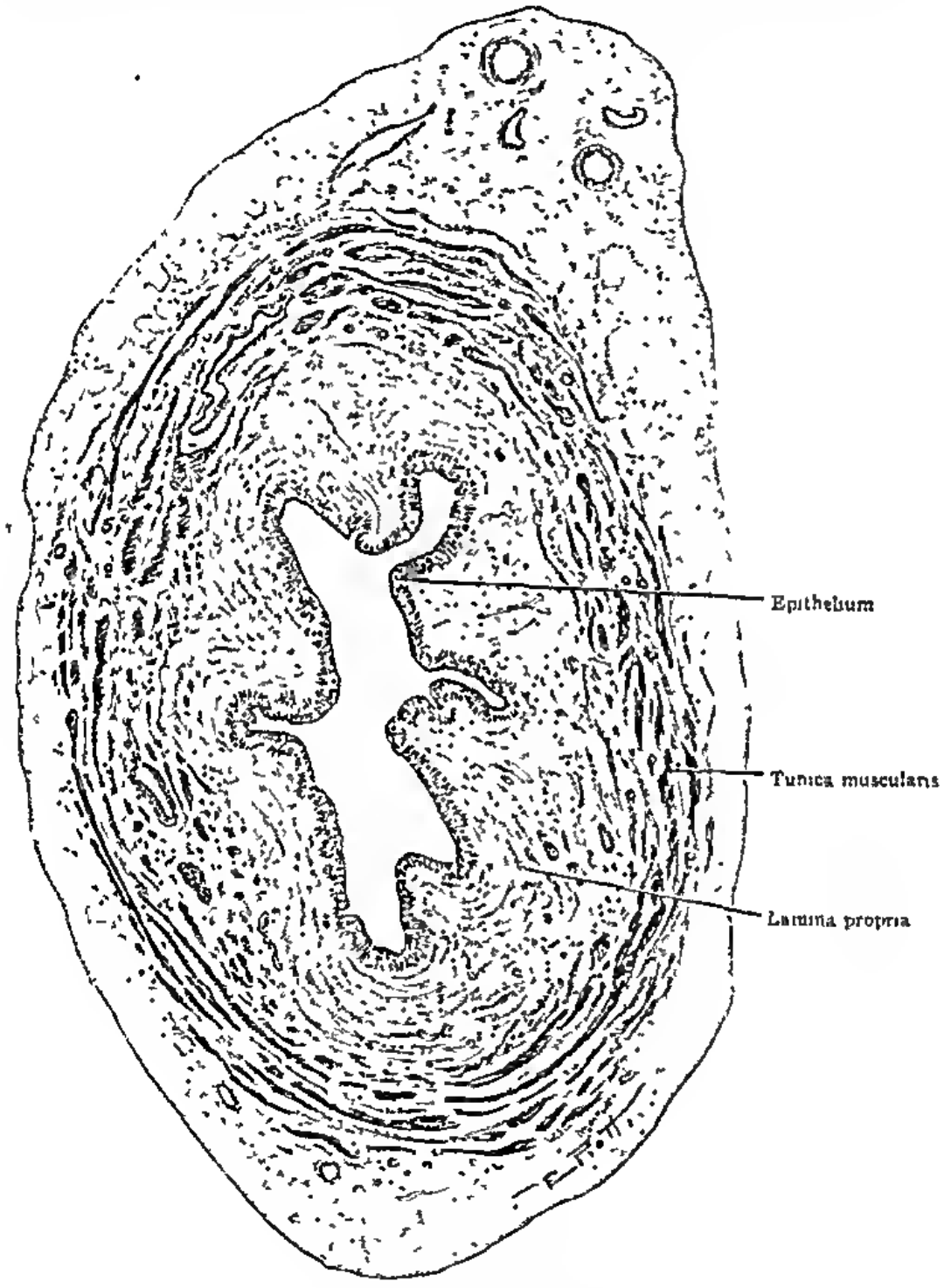


FIG. 418.—A CROSS-SECTION OF A HUMAN URETER
Formaldehyde fixation, hæmatoxylin and eosin

man. Some of these have no lumen and it is said that none are true glands. Capillary blood vessels, which are abundant in the mucosa, are found directly beneath the epithelium and present the deceptive appear-

ance of becoming intra-epithelial. The lamina propria consists of fine connective or reticular tissue, with few elastic fibers. It contains many cellular elements and some lymphocytes, and passes without a definite boundary into the loose connective tissue of the submucosa.

The tunica muscularis consists, in the upper half of the ureter, of an inner longitudinal layer and a second circular layer of smooth muscle. In the lower half a third outer layer of longitudinal fibers is added, specially thickened along the last 5 cm. All these layers are composed of coarse bundles of fibers, with considerable connective tissue among them.

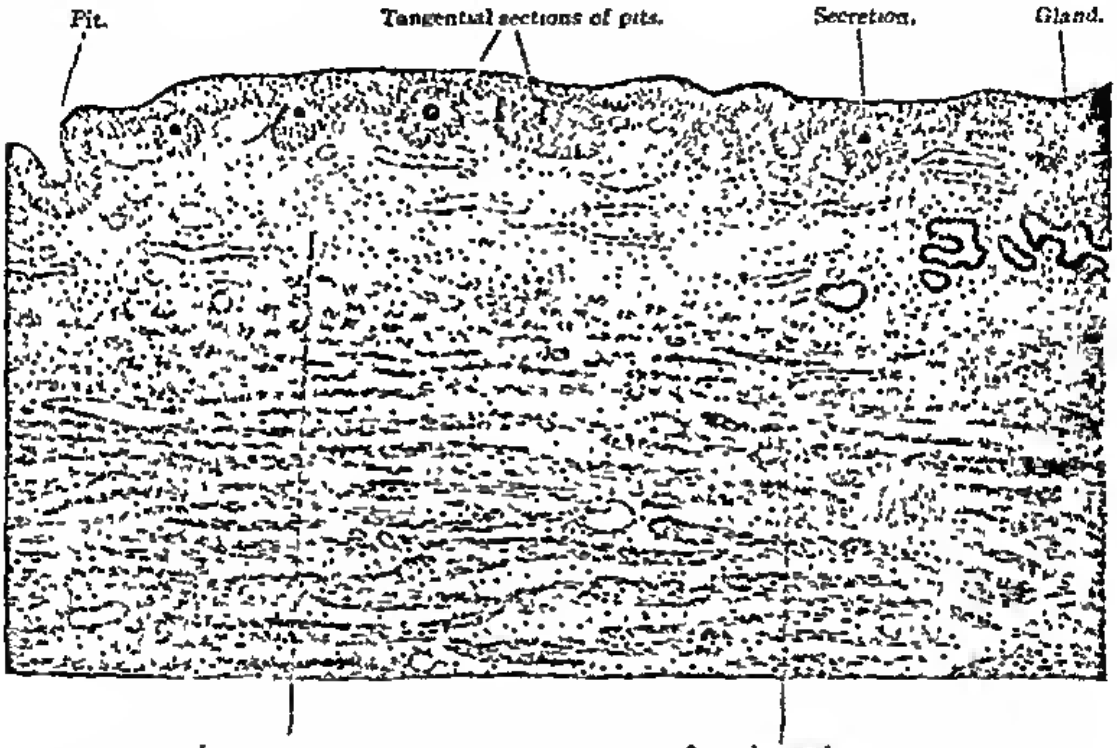


FIG. 419.—SECTION THROUGH THE FUNDUS OF THE URINARY BLADDER OF AN ADULT MAN. × 48

Around the papillæ of the kidney the circular fibers of the calyces form a sort of sphincter. The part of the ureter which passes obliquely through the wall of the bladder has only longitudinal fibers, ending in the lamina propria of the bladder. By contracting they open the outlet of the ureter. The adventitia consists of loose fibro-elastic connective tissue.

Lymphatics and blood vessels are numerous. There are sympathetic nerves to the muscles, and free sensory endings in the lamina propria and epithelium.

BLADDER

The development of the bladder from the ventral part of the cloaca has been described on page 455. Its epithelium is entodermal whereas that of the ureters opening into it is mesodermal. There is, however, no demarcation between the layers in the adult, since both produce the same

sort of 'transitional epithelium.' This term, introduced by Henle (Allg. Anat., 1841) as a designation for epithelia which are intermediate between stratified squamous and simple columnar, is now generally restricted to the peculiar epithelium of the bladder, ureter and renal pelvis.

The bladder wall consists of a mucosa, an ill-defined submucosa, a muscularis, and a serosa or adventitial layer. The epithelium has been described as two-layered in the distended bladder, the outer cells having terminal bars; in the contracted condition it becomes several-layered, and the bars form a net extending into the epithelium. Thus it is not believed that during distention the layers are merely flattened; they are thought to 'slip by each other.' The columnar cells may, however, become extremely flat. The appearances of the epithelium in the bladder and ureter of the dog under various conditions of distention and contraction have been figured by Harvey[1] (Figs. 47 and 48). The superficial cells have a cuticular border; they often contain two nuclei, and their darkly granular protoplasm has been considered suggestive of secretory activity. Round or oval pockets extend into the lamina propria (Fig. 419). Some of them have no lumen, or are detached from the epithelium, but others are pits containing a colloid substance. The pits are rudimentary glands. In the adult, branched tubules lined with cylindrical epithelium may sprout from the bottom of the pits, thus forming true glands. Their occurrence is limited to the fundus, which is the dorsally bulging lowest part of the bladder, and to the neighborhood of the urethral outlet. In the latter position they have been regarded as rudimentary prostatic glands.

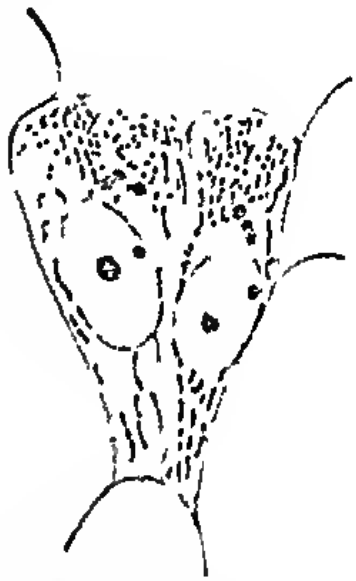


Fig. 420.—Superficial Epithelial Cells from the Urinary Bladder Showing Mitochondria. Human.

Levi fixation, Heidenhain iron-hæmatoxylin (Takahashi.)

The lamina propria is of connective and reticular tissue, with many cellular elements, and little elastic tissue. It contains a rich superficial plexus of capillaries directly beneath the epithelium. Because of the absence of a definite basement membrane, the capillaries often indent the under surface of the epithelium and may in sections appear to lie within it. The reason for this close relation between epithelium and blood vessels, such as might be expected in an active gland, is not clear, since the bladder epithelium is supposed to be merely an inactive lining with neither secretive nor absorptive functions. The lamina propria may

[1] Harvey, 1909.

contain infrequent solitary nodules. Peripherally it blends with the submucosa, as in the ureter.

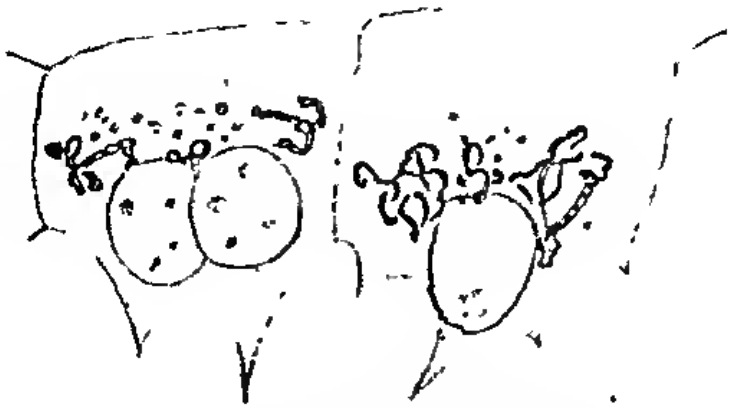


Fig. 421.—Superficial Epithelial Cells from the Urinary Bladder Showing a Golgi Apparatus. Human Kolatchev technique (Takahashi)

The muscularis consists of smooth muscle fibers arranged in three interwoven layers, which are seldom separable in sections. They are an inner longitudinal, middle circular, and outer longitudinal layer. The circular fibers are strengthened at the beginning of the urethra to form the 'internal sphincter' of the bladder, a muscle not always distinct.

Fig. 422.—Section through a Small Sympathetic Ganglion in the Wall of the Urinary Bladder. Bielschowsky Method. (P. Stohr Jr.)

The serosa is a connective tissue layer covered with mesothelium. In the non-peritoneal part of the bladder it is replaced by an adventitia or fibrous layer.

Non-medullated nerves, with scattered groups of ganglion cells, are found outside the muscles and also among them. Medullated fibers terminate around the ganglion cells; others pass through the ganglia to intra-epithelial sensory endings.

URETHRA (IN THE FEMALE)

The urethra is much shorter in the female than in the male—the average being between 40 and 50 mm., the extremes 25 and 60 mm. It is exclusively the outlet of the urinary tract and is homologous with that part of the male urethra which lies between the internal orifice and the

opening of the prostatic utricle. The shallow vestibule is equivalent to the part of the male urethra between the prostatic utricle and the external urethral orifice. According to F. P. Johnson,[1] the urogenital sinus in the male grows chiefly in length and in the female in breadth (dorso-ventrally). In human embryos of about 60 mm. in length he saw

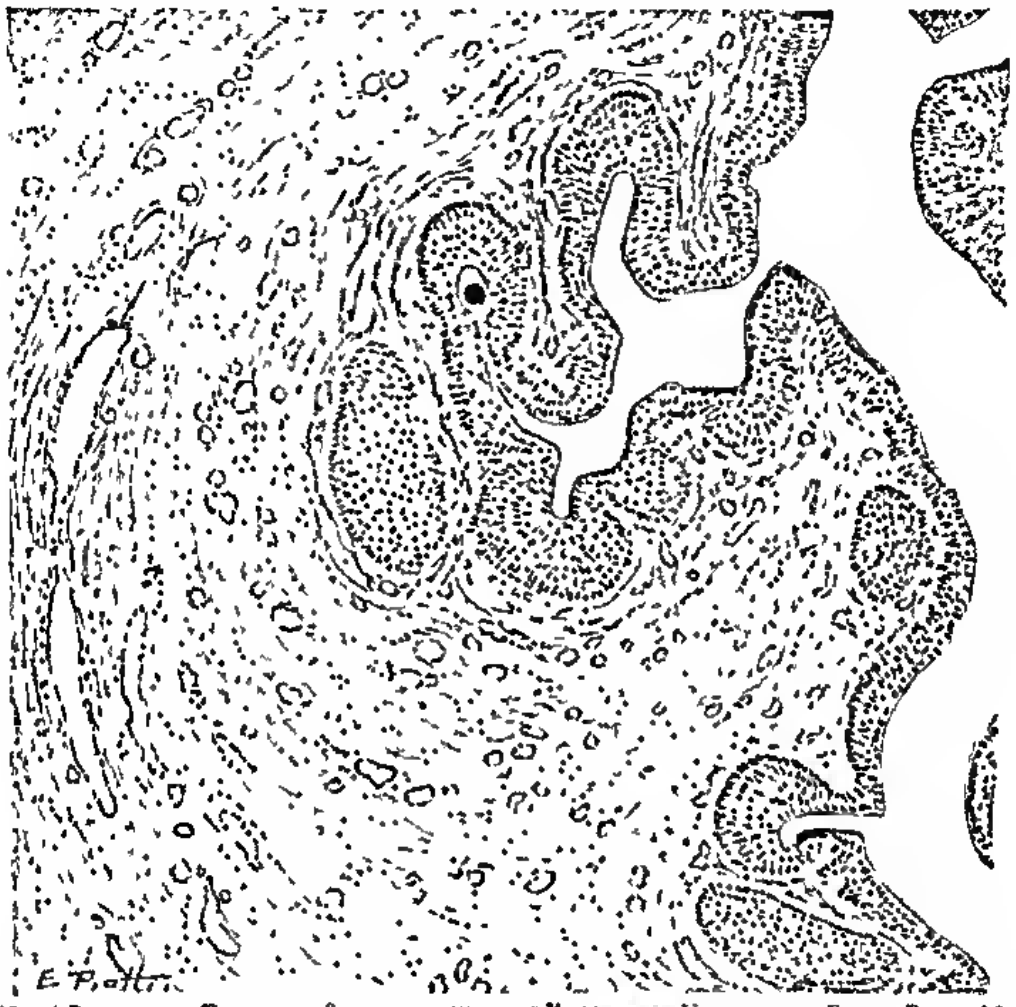


Fig 423.—A Portion of a Transverse Section through the Wall of the Urethra of a Female Rhesus Monkey. One of the glandular pits shows a rounded inclusion similar to the concretions seen in the prostate gland. *Zenker-formaldehyde fixation, Masson's trichrome stain.*

the formation of glands, similar to the prostatic glands, along the walls of the whole female urethra. These glands are said to be fewer and are more retarded in development than the prostatic glands in the male. He divides them into two groups—the true urethral glands homologues of the prostatic glands above the opening of the prostatic utricle and the sinus glands below. The sinus glands are represented in the adult by the para-urethral glands or Skene's ducts.[2] The secretion of these urethral

[1] Johnson, 1922.

[2] Skene, Alex, J. C., 1880. Described two tubes from the urinary meatus, near the floor of the urethra—$\frac{1}{8}$" to $\frac{3}{4}$" deep, which may become involved in inflammation of the vulva, urethra or vagina (Amer. Jour. Obst., vol. 13, pp. 265-270).

glands is mucoid and accumulations of it may form concretions[1] (Fig. 423).

The lumen of the urethra in the adult is irregularly crescentic, with longitudinal folds (Fig. 424). The epithelium varies in different parts and in different individuals. It has been described as pseudostratified columnar, but partly on the surface and in the numerous bays and pits, *urethral lacunæ*, which dip into the under-lying lamina propria, the epithelium is stratified squamous. In places this stratified epithelium may reach a thickness of 100μ. 'Transitional' epithelium is observed

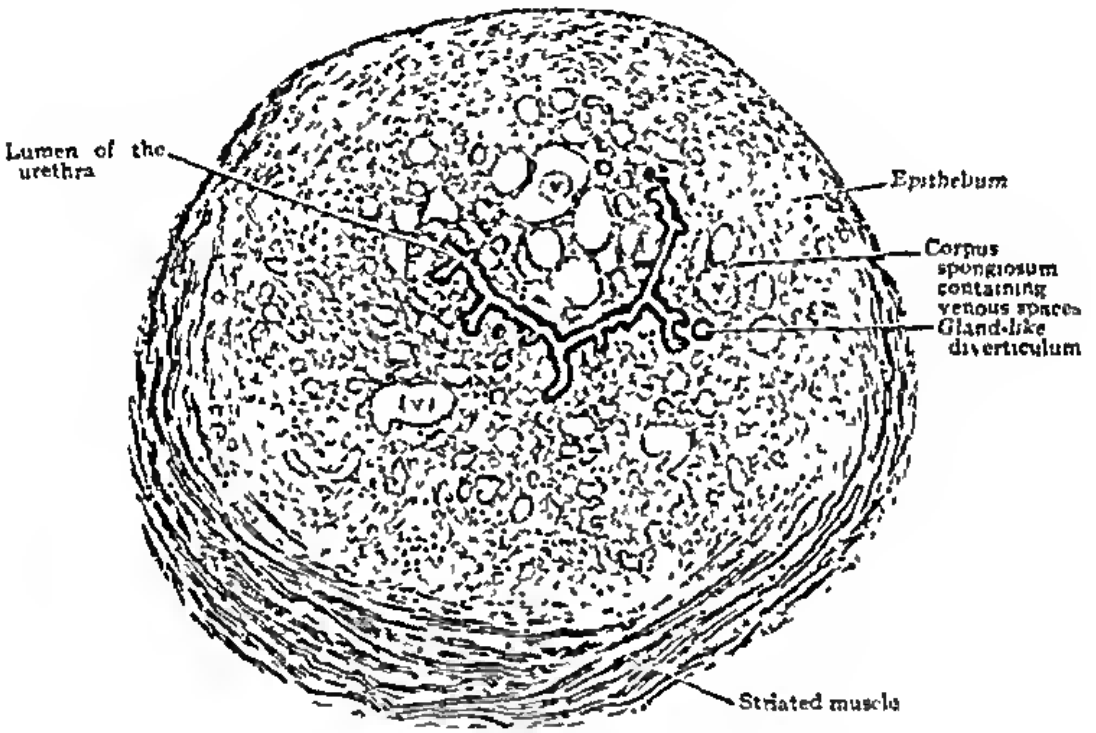


FIG. 424.—A TRANSVERSE SECTION OF THE FEMALE URETHRA. HUMAN. Picric acid sublimate fixation × 10 (von Ebner).

near the bladder, while the terminal part of the urethra is lined with stratified epithelium. The epithelium of Skene's ducts is columnar. The sub-epithelial connective tissue stratum, rich in elastic fibers, is not clearly marked out into a mucosa and a submucosa. It contains extensive networks of thin-walled veins, the largest having diameters up to 260μ (v. Ebner) and constituting a *corpus spongiosum*. This is comparable with the upper part of the more highly developed corpus cavernosum urethræ of the male. 'This tissue would in the main be described as erectile, also as compressible' (Henle). The arteries are smaller and less numerous than the veins. The lymphatics drain toward the bladder. The muscularis is a thick layer, consisting of inner longitudinal and outer

[1] VIRCHOW, R., 1853. Observed concretions in the urethral glands of old women and discussed the possibility that these glands in the female were homologous with the prostatic glands of the male (Arch. f pathol Anat, vol. 5, pp. 403–404).

circular smooth muscle fibers, among which the veins extend, and connective tissue with many elastic fibers is abundant. The two layers are continuous with the musculature of the bladder, the outer circular muscle being condensed at the neck. Outside the layers of smooth muscle there are found bundles of striated muscle of the *M. constrictor urethræ*, best developed above where they form a complete ring-like sphincter. This *M. sphincter urethræ* is smaller in the female than in the male. Below, the striated muscle is absent from the posterior surface of the urethra and passes from in front backwards to the vagina; both structures are enclosed in a common muscular sheath forming a urogenital sphincter. Nerves are very numerous and groups of two, three or four Vater-Pacinian corpuscles have been seen in single sections in the loose connective tissue outside the muscular layers.

MALE GENITAL ORGANS

Development and General Features. The discovery that the Wolffian bodies become a part of the genital system was made by Oken, through dissections and injections of dog embryos [1] Rathke studied these 'Oken's bodies' further, and found more accurately their relation to the epididymis and ductus deferens. Müller[2] wrongly declared that they do *not* form the epididymis, but he discovered that *'at the time* when *the Wolffian bodies* are most highly developed, the germ of the ovary or testis lies on their inner side; and on their outer side, extending even to their upper end, there is a duct which does not connect with the Wolffian bodies—it appears to have arisen from their short and much stouter excretory duct.' He saw that this second duct, now known as the *Mullerian duct*, formed a part of the uterine tubes In fact it forms the entire tubes, together with the uterus and the upper part of the vagina. In the male some of the Wolffian tubules and the Wolffian *ducts are utilized as the efferent passages for the sperm cells, the Mullerian ducts* degenerating except for some interesting vestigial structures; in the female the Müllerian ducts persist (see p. 514), while the Wolffian bodies and duets are reduced to vestigial remnants.

The *genital glands* in either sex begin as a thickening on the ventromedial border of each Wolffian body (Fig. 425). A section of this *genital ridge* is shown in Fig. 397, C. The ridge is a dense mass covered by the peritoneal epithelium, which here consists of a syncytium, and a closely packed inner mass, or 'epithelial nucleus,' derived from the peritoneum. In this nucleus two types of cells appear, the sex cells and the indifferent cells. In the male they are arranged in cords, which later become tubules and connect with some of the tubules of the Wolffian body. Thus the spermatogonia find a pathway to the exterior through a Wolffian duct. In the female the nucleus is invaded by connective tissue and divided into small separate groups of cells, which enclose oögonia. There is no open connection with the Wolffian tubules and Wolffian duct, both of which degenerate. The oögonia are imprisoned within the ovary.

[1] Oken, 1807. [2] Müller, 1830.

There is a difference of opinion as to whether the tubules of the testis are formed directly from the 'epithelial nucleus' within the genital ridge, or as invaginations from the peritoneal epithelium. According to Felix[1] *everything* that is later developed within the genital ridge has a common origin from the peritoneal epithelium. The ridge consists of an epithelial mass which soon separates from the peritoneal layer. Beneath the peritoneum this mass produces the dense connective tissue capsule which surrounds the testis, called, from its whiteness, the *tunica albuginea;* within

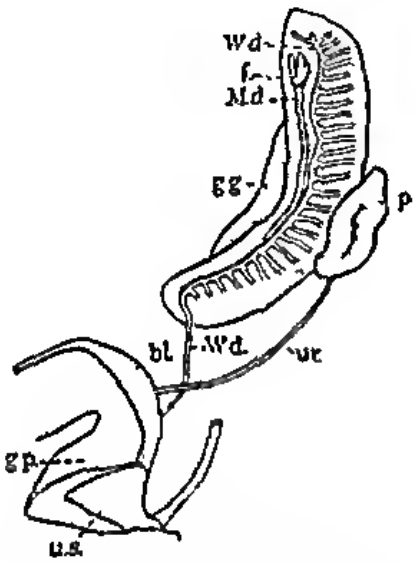


FIG. 425.—FROM A RECONSTRUCTION OF A 13 6-MM HUMAN EMBRYO (Thyng)

bl., Bladder, f., fimbriæ, g g., genital ridge, g p., genital papilla, M d., Müllerian duct, p., renal pelvis r., rectum, ur., ureter, u s., urogenital sinus, W. d., Wolffian duct.

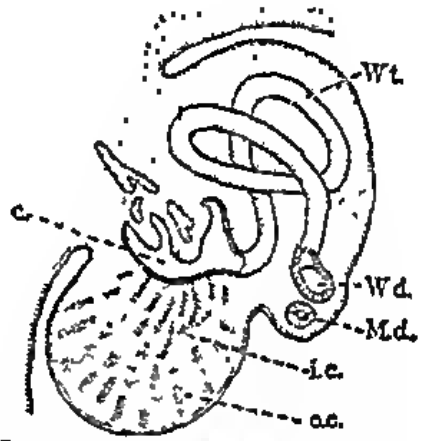


FIG. 426.—DIAGRAM OF THE DEVELOPMENT OF THE TESTIS, BASED UPON FIGURES BY MACCALLUM AND ALLEN

c., Glomerular capsule; i. c., inner or zeta cords, M. d., Müllerian duct; o. c., outer or sex cords, W. d., W. t., Wolffian duct and tubule

the genital ridge it is 'quite suddenly' resolved into anastomosing cords with looser tissue between them, and the cords become the tubules of the testis. Allen, in an earlier account,[2] likewise finds that the cells of the peritoneum and the underlying mesenchyma appear to form a continuous protoplasmic network, and 'the stroma cells are practically identical with the peritoneal cells from which they are originating.' But Allen concludes that 'the tubules of the testis are formed as solid invaginations of the peritoneum, which later become separated from it, and grow by the activity of their component cells.' *Such a condition is shown* diagrammatically in Fig. 426.

As the cords become detached from the peritoneum, they form arching anastomoses, convex toward the periphery of the ridge; and with further growth they become greatly convoluted. *They acquire lumens,* and become the *tubuli contorti,* in the walls of which spermatogenesis takes

[1] FELIX, 1912. [2] ALLEN, B. M., 1904.

place. The shapes presented by these tubules in the embryo have been modeled by Bremer.[1]

Toward the interior of the genital ridge the cords become more slender and converge toward the Wolffian body. There they are embedded in a considerable mass of tissue, which in the adult becomes the *mediastinum testis*. The inner ends of the contorted tubules, toward the mediastinum, remain straight, forming the *tubuli recti;* and these,

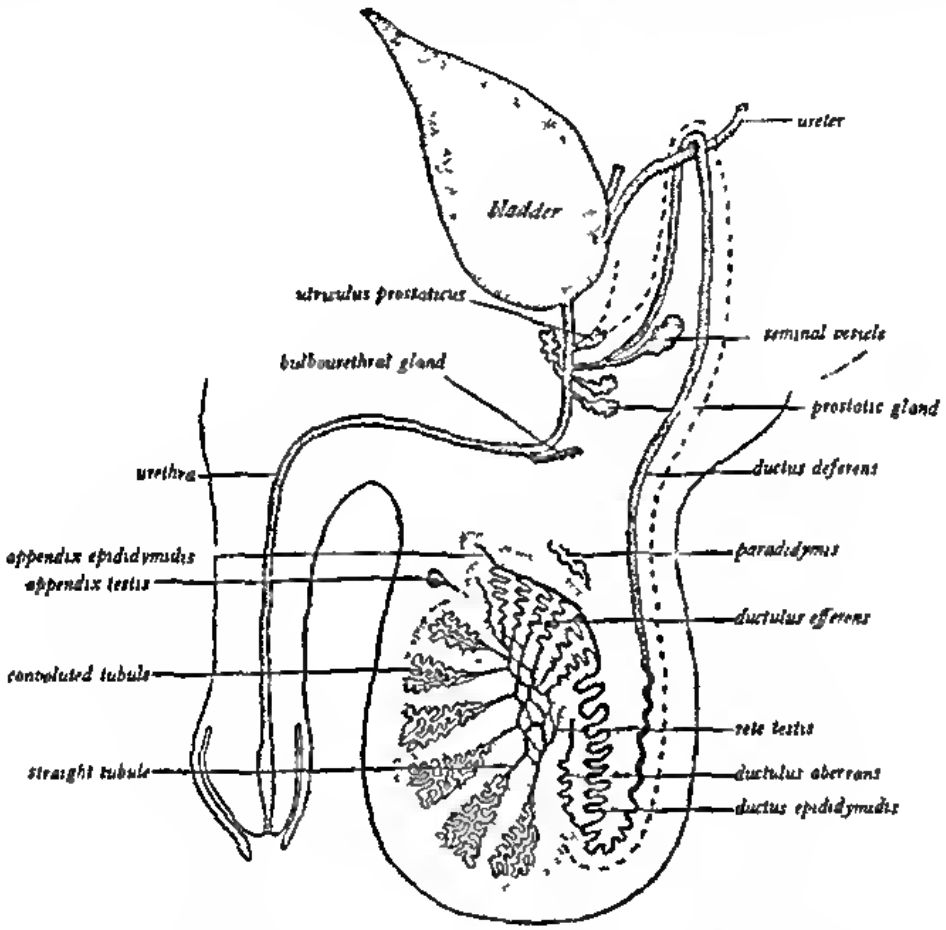


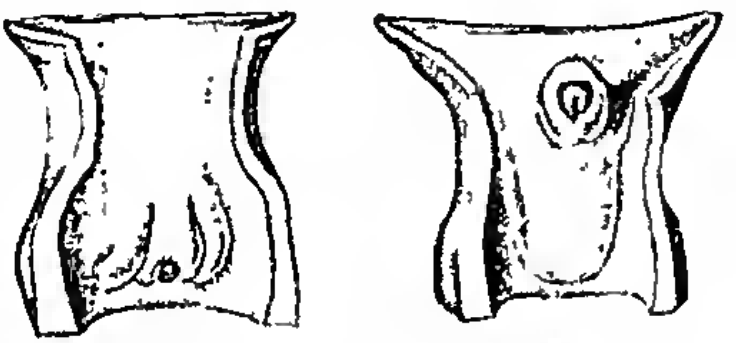
FIG. 427.—DIAGRAM OF THE MALE SEXUAL ORGANS (Modified from Eberth, after Waldeyer)
(The course of the Müllerian duct is indicated by dashes)

further inward, become thin-walled and anastomose freely, thus constituting *the rete testis* (Fig. 427). According to Allen the tubuli contorti are developed from the middle third of the genital ridge only; the cords of the anterior third grow down between these and the Wolffian glomeruli, in the mediastinum, acquire connections with the tubuli contorti and become the tubuli recti and the rete.

All the tubules thus far considered are produced by the genital ridge. Their inner ends, which form the rete, acquire openings into the capsules of the degenerating Wolffian glomeruli, or sometimes directly into a Wolffian tubule. From ten to fifteen Wolffian tubules thus become con-

[1] BREMER, 1911.

nected with the rete testis, and serve to convey the genital products to the Wolffian duct; these tubules are known as the *ductuli efferentes*. In the adult each of them is a greatly convoluted tube which if straightened measures 8 inches (20 cm.) When coiled, it forms a conical mass or *lobule of the epididymis*, with its apex toward the rete, and its base toward the Wolffian duct which it enters. The Wolffian duct, which passes along the dorsal surface of the testis, is also greatly convoluted so that it measures about 20 feet when straight (6–7 meters). Together with the efferent ducts this coiled mass constitutes the *epididymis* (Gr. ἐπί, upon; δίδυμος, testis). Along the testis the Wolffian duct is called the *ductus epididymidis*, and from the testis toward the urogenital sinus it is named the *ductus deferens*. Near its termination a saccular outgrowth,



A B

FIG. 428.—RECONSTRUCTIONS OF THE EXTERNAL GENITALIA OF TWO HUMAN EMBRYOS 100 MM., CROWN-RUMP LENGTH. A, female, B, male (Mosskowicz)

like a distended gland, develops from each Wolffian duct. It is called the *seminal vesicle*, and that portion of the Wolffian duct between the duct of the vesicle and the urethra is named the *ejaculatory duct*. Thus the Wolffian duct is arbitrarily divided in the adult into three parts, the ductus epididymidis, ductus deferens, and ductus ejaculatorius.

The External Genital Organs. After the cloaca has been divided into ventral and dorsal portions by the downward growth of the perineal septum, the ventral portion below the outlets of the Wolffian ducts is called the *urogenital sinus*. It receives both urinary and genital products, and in the male it forms all of the urethra below the orifices of the ejaculatory ducts. In the young embryo, the distal part of the urogenital sinus becomes laterally compressed so that it forms an epithelial plate. This plate reaches the external surface of the body along the mid-ventral line of an elevation known as the *genital papilla* (or tubercle). The genital papilla (Figs. 425 and 428) becomes very prominent in embryos of both sexes. In the male it continues its development and forms the penis, along the under side of which the urogenital sinus acquires a cleft-like opening (Fig. 429, A). This elongated aperture closes from behind forward, along the line permanently marked by a *raphé* (or seam). A rounded terminal

glans is early differentiated at the apex of the genital papilla. The epidermis is adherent to it, but later becomes separated by the formation and splitting of an epithelial plate, thus producing the reflection of skin called the *prepuce*. The urogenital sinus becomes secondarily prolonged through the glans so as to form the terminal part and external orifice of the urethra. The entire urethra is divided into three parts: (1) the prostatic portion (*pars prostatica*), which includes the outlet of the bladder, together with the upper end of the urogenital sinus, and receives the ejaculatory and prostatic ducts; (2) the membranous part (*pars membranacea*), which is the short dilatable portion traversing the 'pelvic diaphragm'; and (3) the long cavernous portion (*pars cavernosa*), which is surrounded by the cavernous vascular tissue.

The claim has been made[1] that minute differences in the configuration of the genital papilla give the earliest indication of the sex of the embryo, but Wilson,[2] after careful comparison of the papillæ and the developing genital glands in a series of human

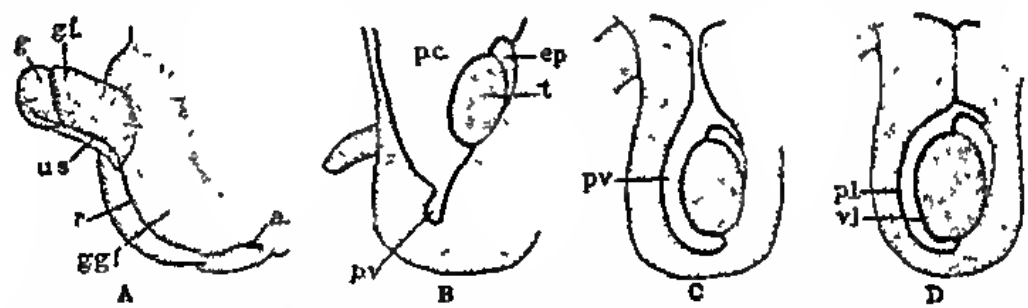


FIG. 429.—A, DIAGRAM OF THE EMBRYONIC EXTERNAL GENITAL ORGANS IN THE MALE, B, C, D, DIAGRAMS OF THE DESCENT OF THE TESTIS (*Eberth*)

a, Anus; ep, epididymis; g, glans penis, g f, lesser genital folds, g g f, greater genital folds; p. c, peritoneal cavity, p v, processus vaginalis, r, raphe, t, testis, p. l, parietal layer of the tunica vaginalis, u. s, urogenital sinus, v. l., visceral layer of the tunica vaginalis.

embryos, warns that the distinctive male characteristics may be delayed and are untrustworthy.

The papilla in the male is subject to abnormalities. If the urogenital sinus remains open on the under side of the penis, along the raphé, the condition is known as *hypospadias*.[3] The epithelial plate may extend further than usual, to the ventral surface of the papilla and even up along the ventral body wall to the umbilicus, the whole bladder (see Fig. 290, D) thus being connected with the epidermal ectoderm in the mid-line. A more or less extensive opening along this line would lead to the conditions of *epispadias* (urethral opening on the dorsum or ventral surface of the penis) or *exstrophy* of the bladder (in which the ventral bladder wall is lacking, the whole mucous surface with the entrance of the ureters being widely exposed)

The *scrotum* develops as a median pouch at the dorsal end of the urogenital raphé. It is continuous above with the pair of large genital folds which tend to encircle the base of the genital papilla, being deficient only below (Fig. 429, A). At the stage when the testis and Wolffian body are still within the abdomen, lying behind the peritoneum, the peritoneal cavity sends a prolongation, the *processus vaginalis*, over the

[1] SPAULDING, 1921. [2] WILSON, K. M., 1926. [3] BREMER, 1932b.

pubic bone into each half of the scrotum (B). A large retroperitoneal column of connective tissue, the *gubernaculum testis*, extends from the posterior end of each testis into the depth of the scrotum. For reasons still obscure, such as unequal growth or the shortening of this cord, the testes pass down in front of the pubic bones, into the scrotum (C). The Wolffian duct becomes bent over the ureter as shown in Fig. 427, and this important relation is found in the adult. Except on its dorsal border, the testis is closely invested by the peritoneum of the processus vaginalis. Later the distal part of the processus becomes separated from the abdominal cavity by the obliteration of its stalk. The part remaining about the testis is the *tunica vaginalis*, having a parietal and a visceral layer as shown in Fig. 429, D. The descent of the testes is completed shortly before birth, except in the occasional cases of 'undescended testis.'

In certain animals the testis remains normally within the abdominal cavity. In others it becomes functionless if so retained or if placed there experimentally. Such testes are called cryptorchid. Moore[1] suggests that in these cases a difference in the temperature between peritoneal and scrotal cavities may cause the degeneration, and would consider the scrotum as an organ for regulating temperature. Why it is useful in some animals and not in others is not clear.[2]

TESTIS

The general arrangement of the parts of the testis, as they appear in cross section, is shown in Fig. 430. From the tunica albuginea, small connective tissue septa (*septula testis*) pass to the mediastinum, dividing the testis into '100–200' pyramidal lobules with their apices toward the rete. The tunica albuginea is a dense connective tissue layer, containing numerous elastic fibers which increase in abundance with age. Its outer surface is covered with the visceral layer of the tunica vaginalis. The inner portion of the albuginea is very vascular, forming a distinct layer at birth (the tunica vasculosa). Connective tissue extends from the septula among the convoluted tubules. Immediately surrounding them there is a delicate basement membrane, followed by a layer of closely interwoven elastic fibers and flat cells.

The *convoluted tubules* are very long, slender loops, anastomosing or opening at each end into the rete, and may be branched or 'double arched.'[3] They are coiled or bent in stiff, short curves, each tubule forming several small, compact convoluted areas, which enclose terminal arterioles, and may thus be regarded as secretory units. Anastomoses between tubules of neighboring lobules have been reported.[4]

The tubules are lined with a highly specialized stratified epithelium (Fig. 433). The cells divide and differentiate as they pass from the basal

[1] Moore, 1924. [2] Wislocki, 1933. [3] Curtis, 1918. [4] Johnson, 1934.

layer outward. Finally each outer cell produces a single large cilium, or flagellum, projecting from the free surface, and becomes detached as a spermatozoon. The process of transformation of the basal cells, or spermatogonia, into spermatozoa is known as *spermatogenesis*. Its cyto-

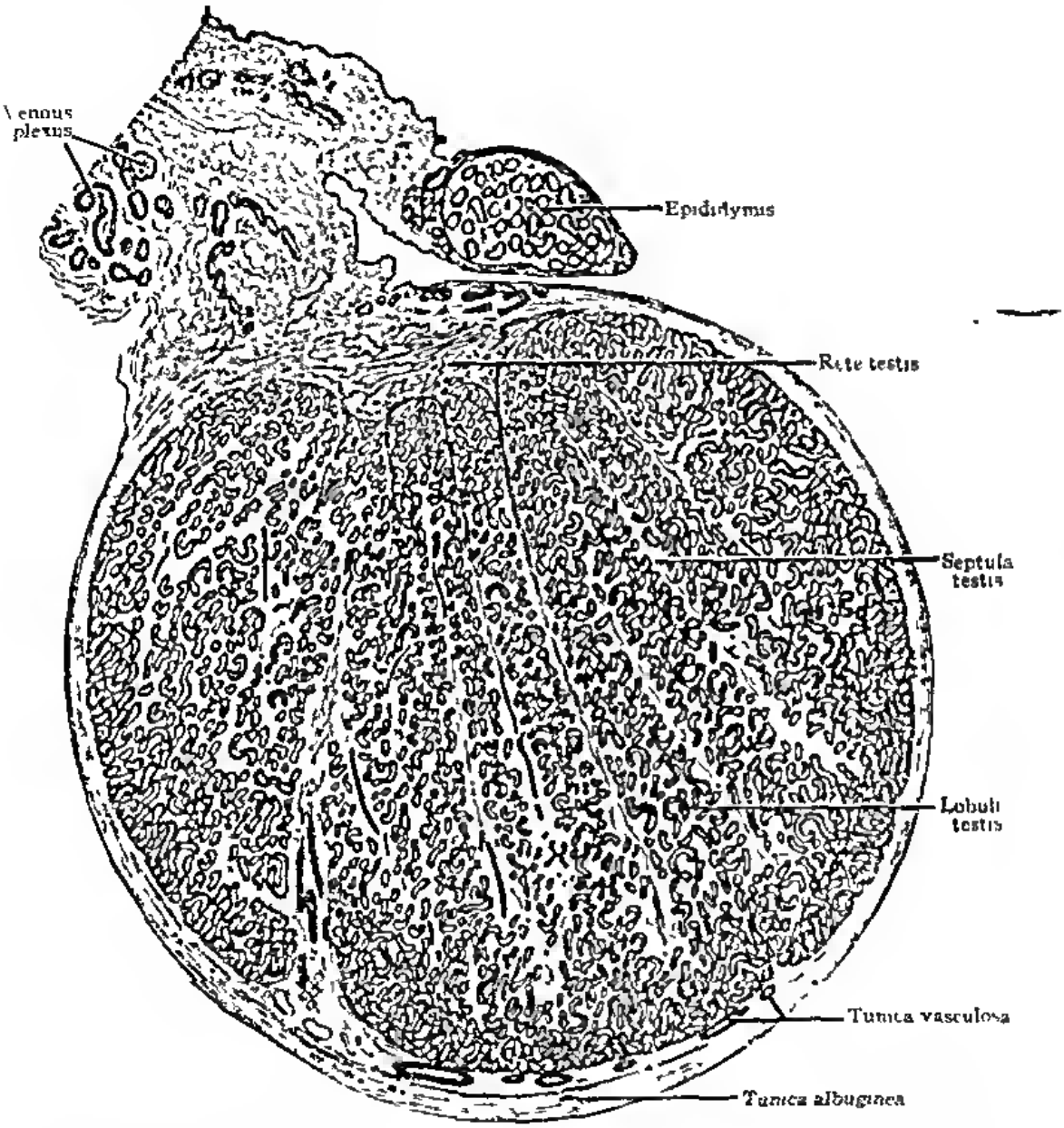


FIG. 430.—TRANSVERSE SECTION OF THE TESTIS OF AN ADULT MAN. × 4. Hansen's hæmatoxylin. (von Mollendorff.)

logical features, as observed in the testis of the grasshopper, have already been described (p. 41). Ordinary sections of the human testis present the following characteristics.

Each tubule is composed of cells of two sorts—*sexual cells* and *sustentacular cells*. At birth the cords and developing tubules contain relatively few sexual cells. These are characterized by their large size, clear cytoplasm, and round vesicular nuclei. It is said that they retain a

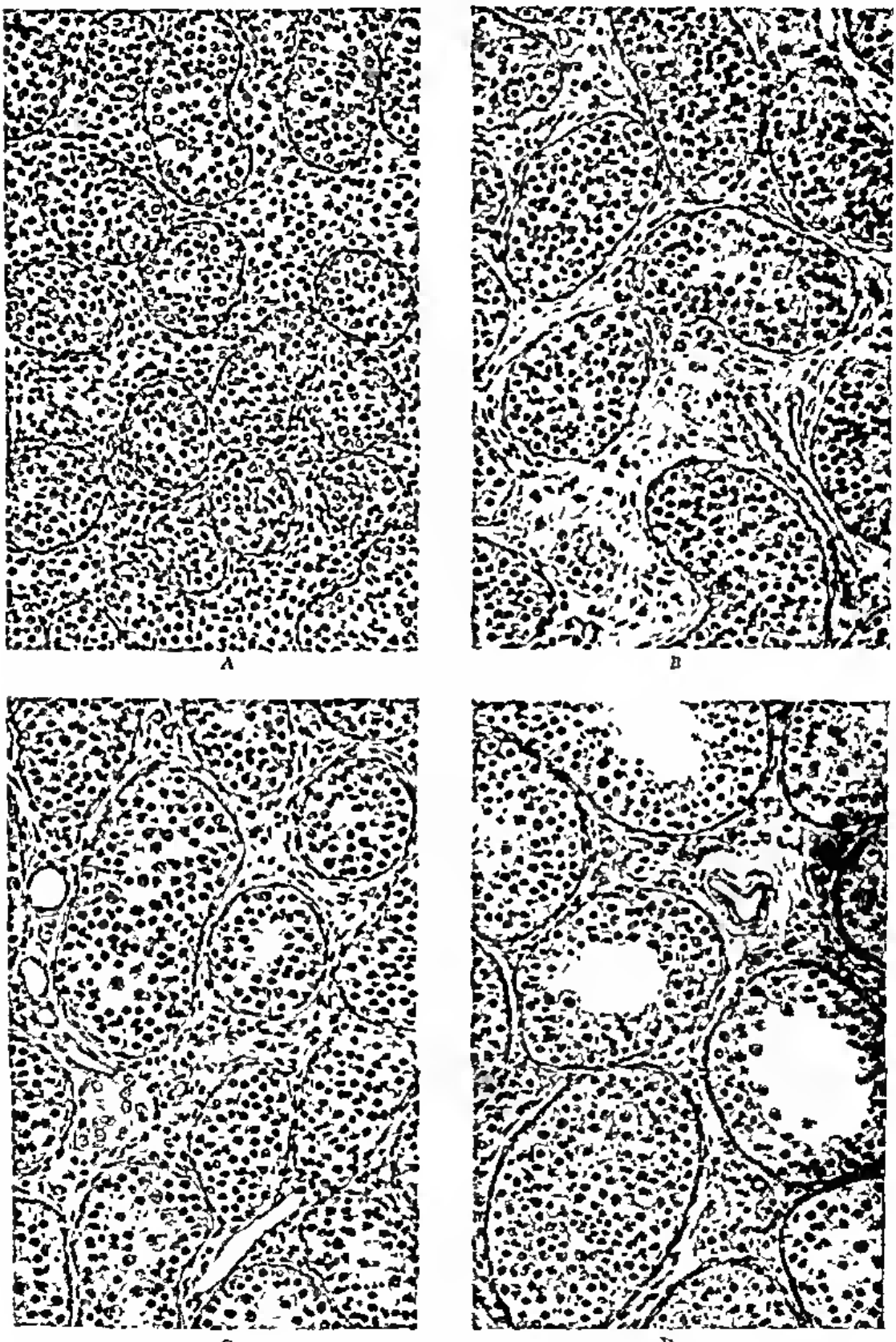


Fig. 431.—Sections through the Human Testis, in the Embryo and in Youth

A, 140 mm. crown-rump length embryo, B, a 12 yr. old boy, C, a 14 yr. old boy—spermatocytes in most tubules, D, 16 yr. old youth—most tubules show a canaliculation and well formed interstitial cells. (Shreve.)

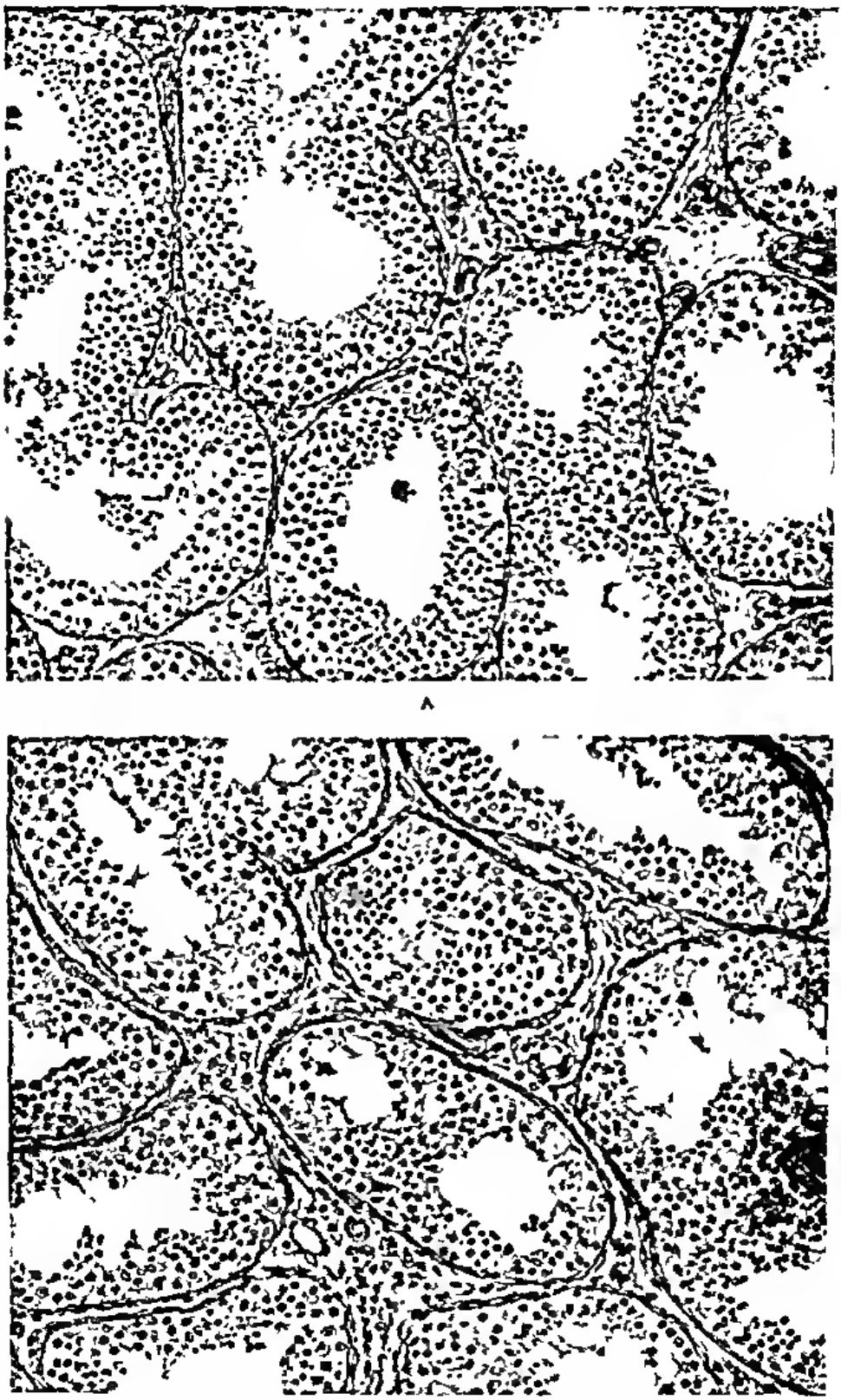
primitive granular arrangement of their mitochondria. These cells multiply by ordinary mitosis, producing the spermatogonia. Thus the sexual cells in various forms eventually far outnumber the sustentacular cells.

The sexual or genital cells are apparently produced from the cords in the testis, relatively late in embryonic development. It was suggested by Nussbaum, however, that the sexual cells are set apart much earlier—'they do not come from any cells that have given up their embryonic character or gone into building any part of the body.' In accordance with this idea, it is considered by some authorities that in the segmentation stages, a line of undifferentiated cells is set apart to become the sexual cells, and that from the beginning they are distinct from the somatic cells which form the rest of the body. Various authors have attempted to trace the genital cells before their appearance in the testis or ovary; large round cells have been noted in the different germ layers and in various supposed pathways toward the genital ridge. They are said to be recognizable by a difference in mitochondrial content. By some these are considered primordial germ cells, which will later be incorporated in testis tubule or ovarian follicle; for others they are merely degenerating cells of the tissue. In the fowl, Goldsmith[1] finds that the germ cells migrate from the pro-amniotic region through the blood stream to the future gonad. In a 4 5 mm. human embryo Hamlett[2] traces them from the mesenchymal tissue of the gut wall to the germinal ridge by way of the mesentery.

The sustentacular or supporting cells, often called *Sertoli's cells*,[3] are at first indifferent cells forming a syncytium. With the increase in the number of spermatogonia, their cytoplasm is resolved into a network of strands, moulded by the surrounding cells (Fig. 433). Their nuclei are radially compressed into ovoid shapes, and lie in columns of cytoplasm extending from the periphery of the tubule toward its lumen. Each nucleus has a distinct nucleolus, apart from which its chromatic material is very scanty. In man the nuclei are in the lower half of the branching protoplasmic columns, the polygonal bases of which are in contact with one another beneath the spermatogonia; in some animals (*e.g.*, mouse) the nuclei are flattened against the basement membrane. Within the cytoplasm fat droplets occur, together with brown granules; crystalloid bodies in pairs may also be found. The heads of the spermatozoa may appear attached to, or embedded in, the cytoplasm of the sustentacular cells, which are supposed to nourish them. The spermatozoa may be gathered in characteristic clumps at their upper ends.

In ordinary sections of the testis, the sustentacular cells may be recognized by their distinctive nuclei. The sexual cells in the basal row are presumably spermatogonia. Above them are the spermatocytes, which are larger; their nuclei usually show spiremes or other indications of cell division. Secondary spermatocytes are further out than the primary spermatocytes; and above them are the spermatids in various stages of transformation into spermatozoa. Since spermatogenesis occurs in

[1] Goldsmith, 1928. [2] Hamlett, 1935. [3] Sertoli, 1865.



A

B

FIG. 432

A, section through a testis of a 34 yr old healthy man, B, of a 74 yr old man (senile testis) (Steve)

'waves,' the surface cells in a tubule cut lengthwise form a succession of zones, each of which shows gradations from young spermatids to mature spermatozoa.[1] In transverse sections all the superficial cells may be of one stage, which differs from that in the adjoining tubule.

Stages in the transformation of a spermatid into a spermatozoon are shown in the diagram Fig. 435. The chromosomes of the spermatid disappear in a dense chromatic network which becomes apparently homogeneous. This deeply-staining nucleus passes to one end of the

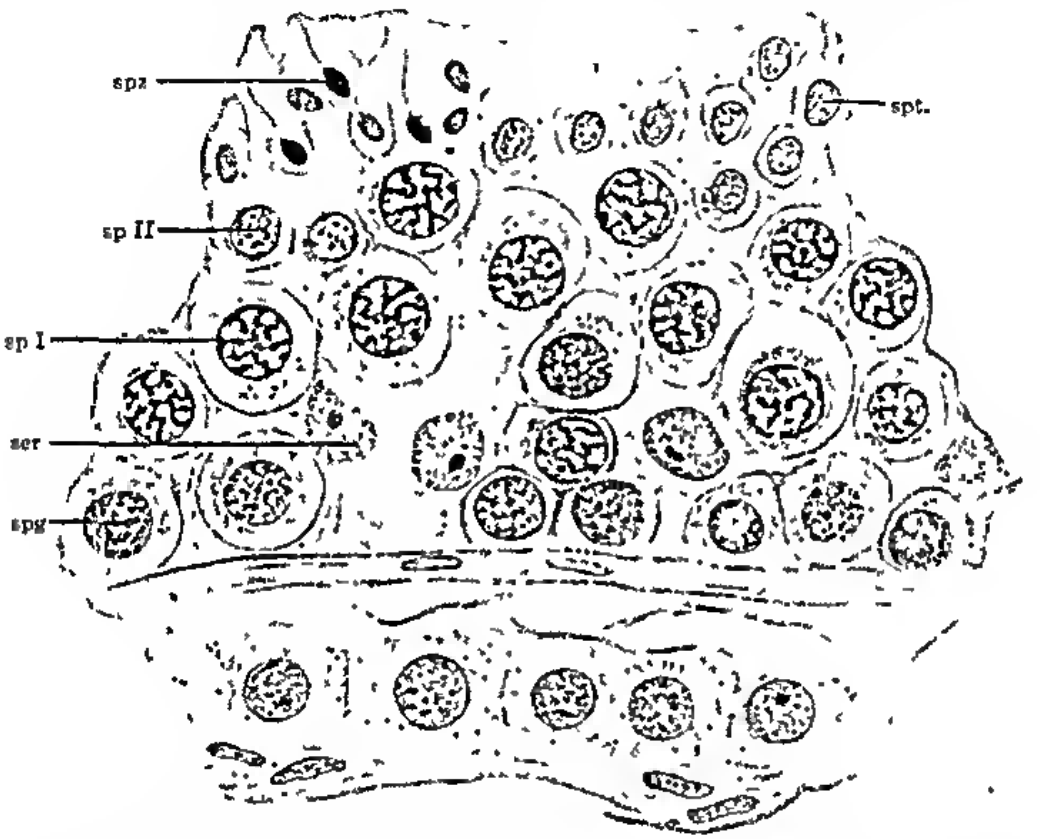


FIG 433.—HUMAN TESTIS, LONGITUDINAL SECTION OF SEMINIFEROUS TUBULE, TO SHOW WAVE OF SPERMATOGENESIS. INTERSTITIAL CELLS BELOW.

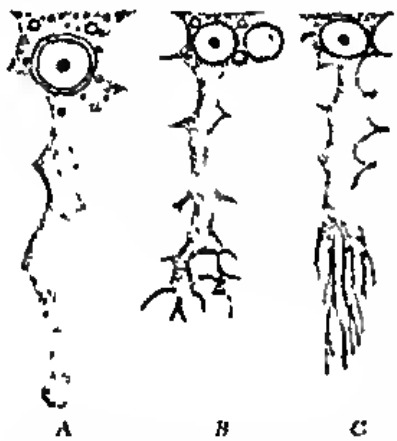
Ser., sustentacular cell nucleus, spg., spermatogonia; sp. I and II, spermatocytes, spt., spermatid; spz., spermatozoon.

cytoplasm of the spermatid. It becomes the essential part of the *head* of the spermatozoon, which in man is a flattened structure, oval on surface view, and pyriform with its apex forward when seen on edge. The head is at the anterior end of the spermatozoon, which during its development is directed toward the basal layers of the convoluted tubule. The anterior end of the head is probably covered by a thin layer of cytoplasm, known as the *galea capitis*. The archoplasm of the spermatid (known as the *idiozome*) is said to leave the centrosome and to enter the protoplasm of the galea capitis, where it forms the *perforatorium*. If this exists in man, it is in the form of a cutting edge following the outline of

[1] CURTIS, 1918.

the front of the head; in other animals the perforatorium may be a slender spiral or barbed projection, which enables the spermatozoon to penetrate the ovum.

The cytoplasm of the spermatid forms an elongated mass at the posterior end of the nucleus. It contains the centrosome which soon divides in two. Of these the anterior forms a disc which becomes adherent to the nuclear membrane. The posterior centrosome also becomes a disc after giving rise to a motile *axial filament*, which grows out from it like a cilium. The disc-like centrosome attached to the anterior end of the filament becomes thin in such a way that its peripheral portion is detached, and as a ring surrounding the filament it passes to the posterior limit of the cytoplasm. The cytoplasm between the two parts of the posterior centrosome is reduced to a thin layer in which a spiral filament develops, winding about the axial filament. Distal to the centrosome ring, the axial filament, which consists of fine fibrils, is surrounded by a thin membrane, which terminates or becomes very thin near the extremity of the filament. This membrane in salamanders forms a conspicuous undulating frill, in man it is inconspicuous. The preceding account is based on studies of the Guinea-pig;[1] for descriptions of human spermatozoa the student is referred to Arey[2] or to Gatenby and Beams.[3]



A B C

FIG. 434.—LIVING SPERMATOGENIC CELLS FROM THE SEMINIFEROUS TUBULES OF A RAT

A, isolated from fresh tissue × 600. B and C, Müller's fixation × 480. (Redrawn from original figures by Sertoli: Taf IV, fig. 1, Arch. per le Sci. Med. Vol. 2, 1878.)

Mature spermatozoa are divided into three parts—the *head*, *neck*, and *tail*. The head (3–5 μ long and 2–3 μ wide) includes the nucleus, galea capitis, and perforatorium. The neck consists of the anterior centrosome and the substance, not traversed by the axial filament, between it and the posterior centrosome. The neck in man is not constricted as in some forms, yet it is a place where the head may become detached. The tail includes three parts, the *connecting piece*, *chief piece* and *end piece*. The connecting piece (6 μ long and scarcely 1 μ wide) consists of cytoplasm, axial and spiral filaments, and the two parts of the posterior centrosome. The chief piece (40–60 μ long) is the axial filament with its surrounding membrane; and the end piece (10 μ) is a prolongation of the filament. When the spermatozoa become free, they float in the albuminous fluid secreted in small quantity by the tubules of the testis. They pass through the straight tubules and rete to the epididymis, in which they accumulate, and where they first become motile. Their motility is greater, however, in

[1] MEVES, 1899. [2] AREY, 1930. [3] GATENBY AND BEAMS, 1935.

the seminal fluid, which is a mixture of the products of the epididymis, seminal vesicles, prostate, and bulbo-urethral glands. By an undulating movement of the tail, the head is propelled forward, always being directed *against* such a current as is made by cilia, at a rate of $\frac{1}{8}$ of an inch in a minute. Water inhibits the motion, which is favored by alkaline fluids; it occurs also in those faintly acid. For three days after death spermatozoa may retain their activity in the seminal passages; in the female urogenital tract they may live a week or more. In addition to normal spermatozoa, giant forms, and some with two heads or two tails occur, but these are probably functionless abnormalities. The production of spermatozoa, beginning at puberty, continues throughout life, but with advancing age the rate diminishes. Since about 60,000 spermatozoa occur in a cubic millimeter of seminal fluid, it has been estimated that 340 billions are produced in a lifetime.

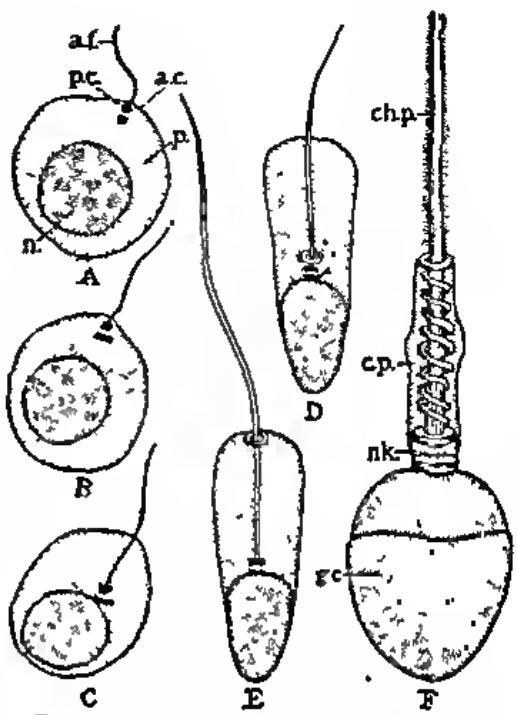


FIG 435.—DIAGRAMS OF THE DEVELOPMENT OF SPERMATOZOA (Meves)

a, c, anterior centrosome, a. f, axial filament, c. p., connecting piece, ch. p, chief piece g. c, galea capitis, n, nucleus, nk, neck, p, protoplasm, p. c., posterior centrosome

The discovery of spermatozoa was reported to the Royal Society of London, in 1677, by Leeuwenhoek. They were first seen by Dr Ham, 'a man of singular modesty,' to whom Leeuwenhoek gives full credit for the discovery in his letters to the Royal Society. He wrote as follows:

'This discerning youth visited me and brought with him, in a small glass vial, seminal fluid from a man who had cohabited with a diseased woman; and he stated that after some minutes when the fluid had become so attenuate that it could be put in a slender glass tube, he had seen living animalcules in it, which he thought were produced by some putrefaction He added that those animalcules seemed to him to be provided with tails, and that they did not survive the space of twenty-four hours. Moreover he declared that when terebinth had been given to the patient internally, the animalcules appeared to be dead.

'I poured this material in a glass tube and examined it in the presence of Dr. Ham, and saw some live animalcules in it. But when after two or three hours, I examined the material more carefully, by myself, I saw that all the animalcules were dead.'

Leeuwenhoek diligently pursued the study of these animalcules, and found them in enormous numbers in the semen of insects, fishes, birds and quadrupeds. He estimated that there were *150,000,000,000* in the *milt of one fish*, or more than ten times the number of men then living (13,385,000,000 homines in orbe terrarum). Leeuwenhoek believed that the animalcules were of two sexes, and that the egg consisted of a fluid in which they swam about and developed To some it seemed not unreasonable that new

individuals should be enclosed in the spermatozoa, like an insect in its chrysalis, and Dalenpatius (1699) thought that he could observe them. As quoted by Vallisneri, he wrote as follows, illustrating his account with a figure.

'We have seen some animalcules having just the form of tadpoles such as are found in brooks and muddy bogs in the month of May. The tail is four or five times as long as the body. They move with wonderful rapidity and by the strokes of their tails produce little waves in the substance in which they swim. But who would believe that in these a

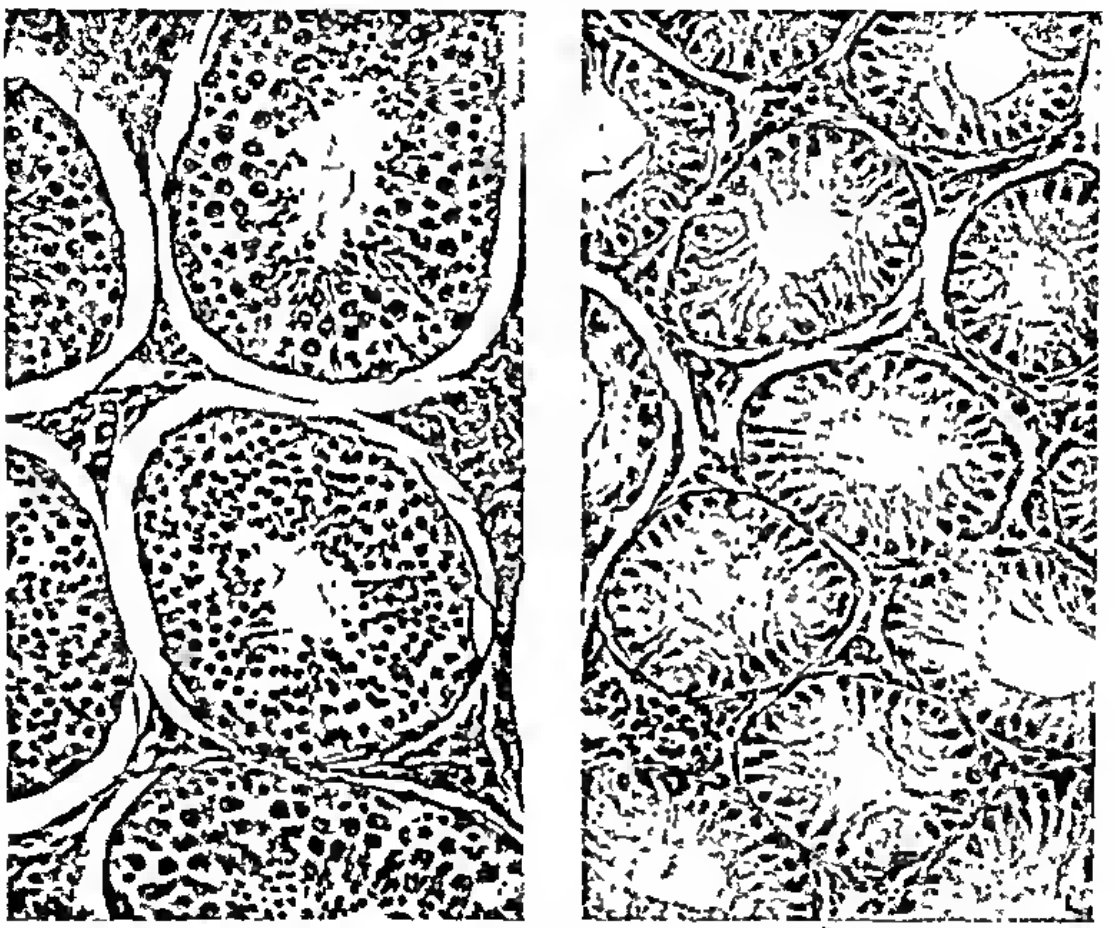
FIG. 436.—HUMAN SEMINIFEROUS TUBULES.
Formaldehyde fixation, 75% hydrochloric acid maceration and teased. (F. P. Johnson.)

human body was hidden? Yet we have seen such with our own eyes. For while we were observing them attentively, a large one threw off its surrounding membrane and appeared naked, showing distinctly two legs, thighs, breasts and arms. The cast-off skin, drawn upward, covered the head like a cap, and it was a delightful and incredible sight. Because of the minuteness of the object, the sex could not be distinguished. After the little creature had lost its membrane it soon died.'

This is a gross presentation of the *preformation theory*, according to which the various parts of the adult are represented in the very young embryo. It was held by many who could not verify such observations. An alternative theory is that of *epigenesis*, according to which the body and its parts arise out of formless substance. Descartes (1664) wrote that the source of a new individual 'seems to be only a confused mixture of liquors, which, serving to leaven one another, become heated; some of their agitated particles dilate, and press upon the others, gradually disposing them in the way necessary to form organs.' Such physico-chemical speculations, however, are quite as imaginative as any

views of the preformationists and Descartes's epigenesis was early characterized as 'a very lame account of the forming of an animal.' Nevertheless, the doctrine of epigenesis, as advocated by Harvey (1651) and Wolff (1759), prevailed over the cruder ideas of preformation. If, however, the spermatozoon can contribute to the production of only one of the myriad forms of animals, even the sex of which is apparently predetermined, it is evident that the spermatozoon must possess a very definite chemical composition, and perhaps a corresponding ultra-microscopic structure. Doubtless there is a preformation no less remarkable than that expressed through the active imagination of Dalenpatius.

In the connective tissue between the seminiferous tubules are found scattered groups of *interstitial cells* or cells of Leydig[1] (Fig. 11). They



A B

FIG. 437.—SECTIONS OF DEER TESTIS SHOWING SEASONAL DIFFERENCES.

A, October, approaching the rutting season, B, June, during the period of sexual quiescence × 135 Formaldehyde fixation, Hæmatoxylin and Eosin. (Wislocki.)

probably arise from the cells of the 'epithelial nucleus,' not utilized in the formation of the testis tubules, though some authors consider them as mesenchymal in origin, migrating from the mediastinum testis. They are large, rounded or polygonal structures, in close contact, and without distinct cell boundaries. Their nuclei may be either dark and granular, or pale with distinct nucleolus, very similar to the nuclei of the Sertoli

[1] LEYDIG, FRANZ, 1850. 'Zur Anatomie der männlichen Geschlechtsorgane und Analdrüsen der Säugethiere.' Zeitschr. f. wiss. Zool, Bd. 2, p. 1–57. Description of interstitial cells on p. 9.

cells. These two appearances may indicate secretory phases, but the usual secretory granules and other evidences of secretion are not found in their cytoplasm, though fat droplets, pigment granules, especially in old age, and rod-shaped crystalloids of unknown significance may be present.[1,2] They are not phagocytic to injected dyes and so differ from the macrophages which are also present in small numbers. The interstitial cells, although not always intimately related with the vessels, are thought to produce an internal secretion, the testicular hormone, and certain observations favor this concept. Others consider the hormone as the product of the testis as a whole.

The hormone, known as the male sex hormone, is produced apparently only on stimulation of the testis by the gonadotropic fraction of the anterior lobe of the pituitary

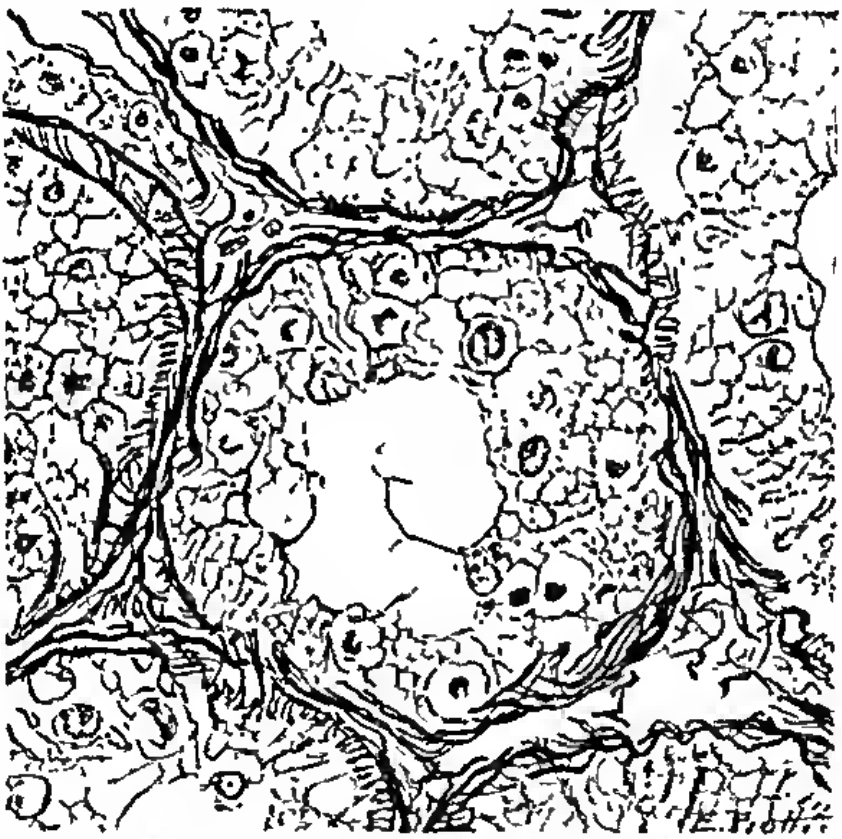


FIG. 438.—ARGYOPHIL RETICULUM IN THE TESTIS OF A VIRGINIA DEER
Formaldehyde fixation, silver impregnation according to Pap; hematoxylin.

gland, and governs the development of the accessory male genitalia and the secondary male characteristics which appear at puberty, and thereafter is necessary for their regulation. It is thought to be self-regulatory,[3] in that too great a concentration in the blood will inhibit the function of the anterior lobe. Like the female sex hormone (p. 535) the testis hormone can be isolated from urine, even from that of women, and from some plants. It has been produced in crystalline form. Its presence and potency are usually tested by the action on immature animals in producing pubertal changes. The interstitial cells are considered to be the source of this hormone, for in cryptorchid testes, or in those exposed to X-ray or many other injurious agents, the sex cells only are

[1] RETINKE, 1896. [2] RASMUSSEN, 1932. [3] MOORE, 1932.

destroyed, the cells of Sertoli and of Leydig remaining apparently intact; such animals according to their age, either undergo normal pubertal changes or suffer no degeneration of the accessory genital organs. In the senile atrophy of the testis the interstitial cells at first increase but later degenerate, and in this case the accessory glands also suffer, notably the prostate.

Tubuli Recti and the Rete. Large convoluted tubules may be 140 μ in diameter. As they pass toward the epididymis they decrease in size;

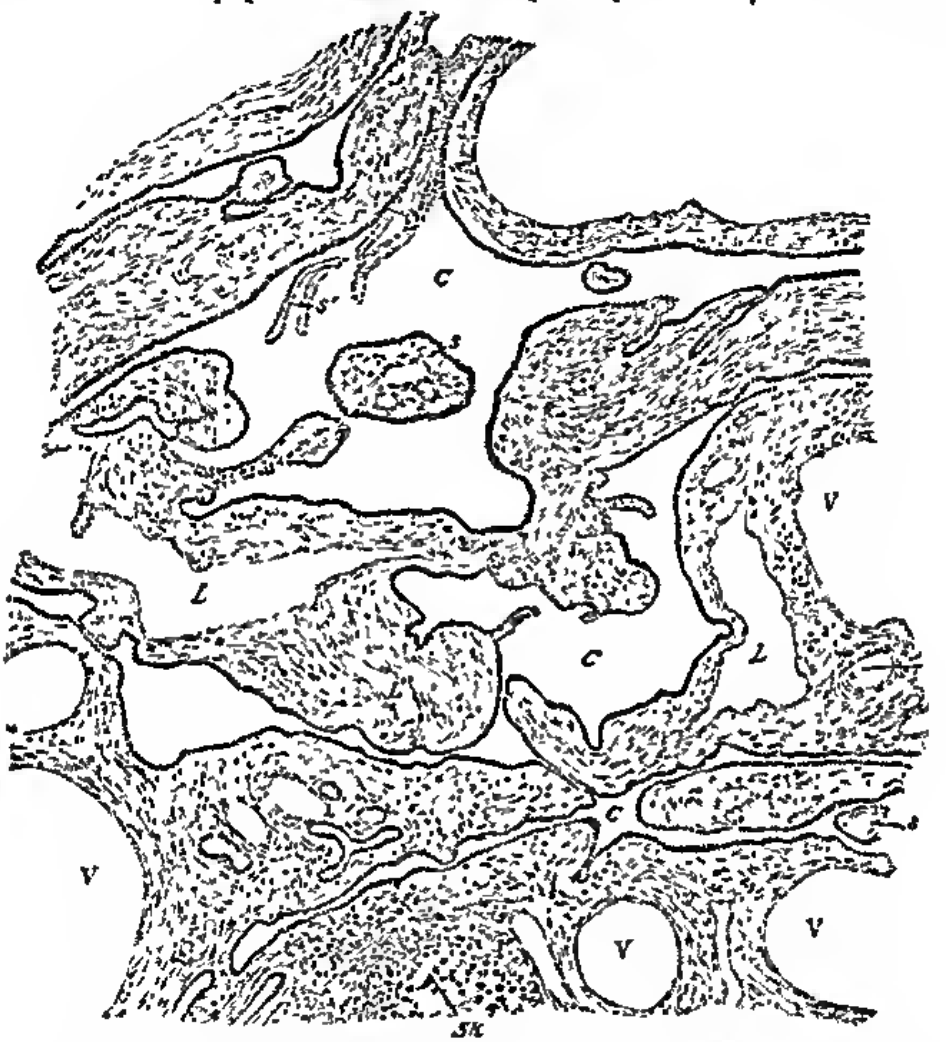


FIG. 439.—SECTION OF THE HUMAN RETE TESTIS. × 96 (Kölliker)

A, Artery, C, rete tubules, L, lymphatic vessels, s, connective tissue partly surrounded by rete tubules, Sk, part of a convoluted tubule, to the left of which are sections, probably of straight tubules, V, vein

they receive branches at acute angles and their windings diminish. Sexual cells disappear, leaving only the sustentacular cells in the form of a simple columnar epithelium. This flattens abruptly to form the lining of the straight tubules. These may enter the septula at any point from near the mediastinum to quite near the tunica albuginea. Both the straight tubules and the rete are lined with a simple epithelium of low cells. In some places these are very flat, suggesting endothelium; in others they are columnar. The characteristic dilatations of the rete tubules are shown in Fig. 439. They contain spermatozoa and immature sexual cells together with pigment granules and broken down cells.

The *arteries* of the testis are branches of the internal spermatic artery, which descends through the spermatic cord, beside the ductus deferens. The branches enter the testis in part through the mediastinum, and in part through the tunica vasculosa. They thus enter the septula both from the mediastinum and from the periphery. Branches leave the septula and form capillary plexuses around the convoluted tubules. There is some indication of vascular units within the lobules, but they are not

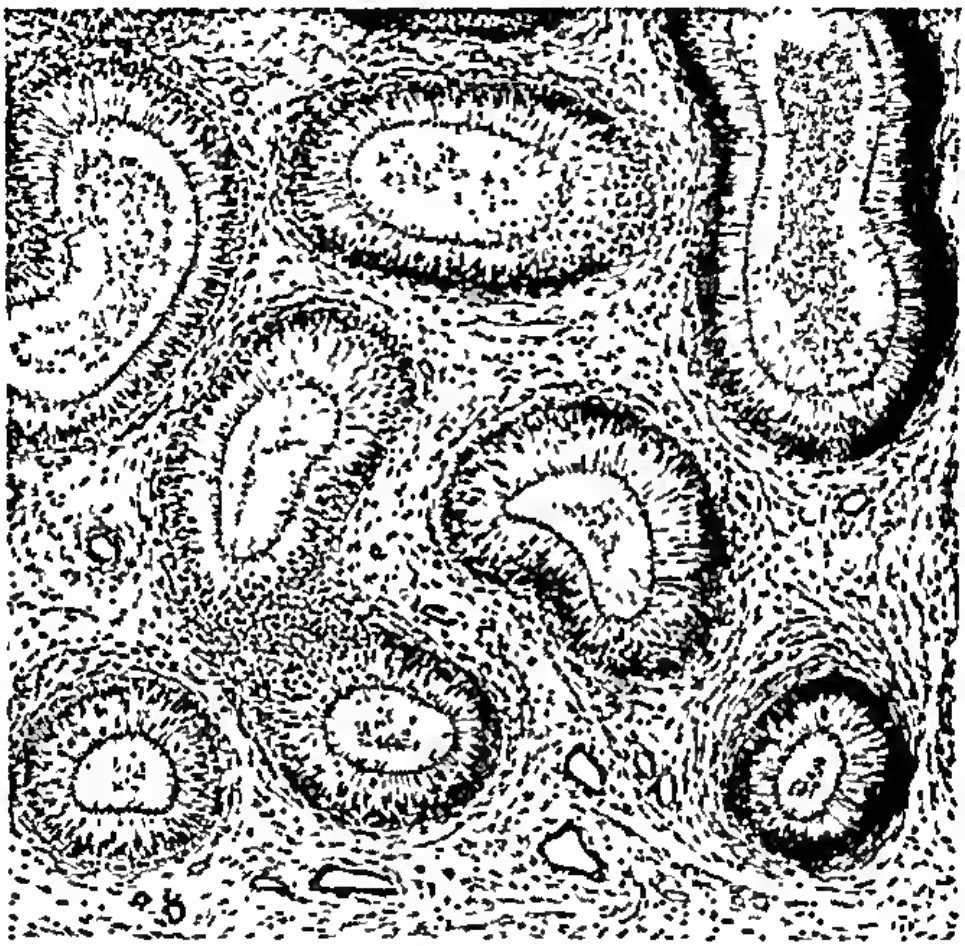


Fig. 440.—A Section from the Head of the Epididymis of a 32 Yr. Old Man. Sublimate-formaldehyde acetic acid fixation. Heidenhain's iron hæmatoxylin and chromotrope 2 R. × 90. (Sieve.)

well-marked. The veins accompany the arteries. Lymphatic vessels are numerous in the tunica albuginea and extend among the tubules. Nerves from the spermatic plexus form a net in the deeper part of the tunica albuginea, and from there surround the vessels and tubules; the presence of intra-epithelial endings has not been established with certainty.

EPIDIDYMIS

The *efferent ducts*, which pass from the rete to the duct of the epididymis, are lined with an epithelium in which groups of columnar cells alternate with those which are cuboidal (*Figs.* 442 *and* 443). Thus the inner surface of the epithelium has depressions suggesting glands, but the basal

surface is free from outpocketings. The epithelium is generally simple, although in the tall parts it may appear two or three-layered. The cells

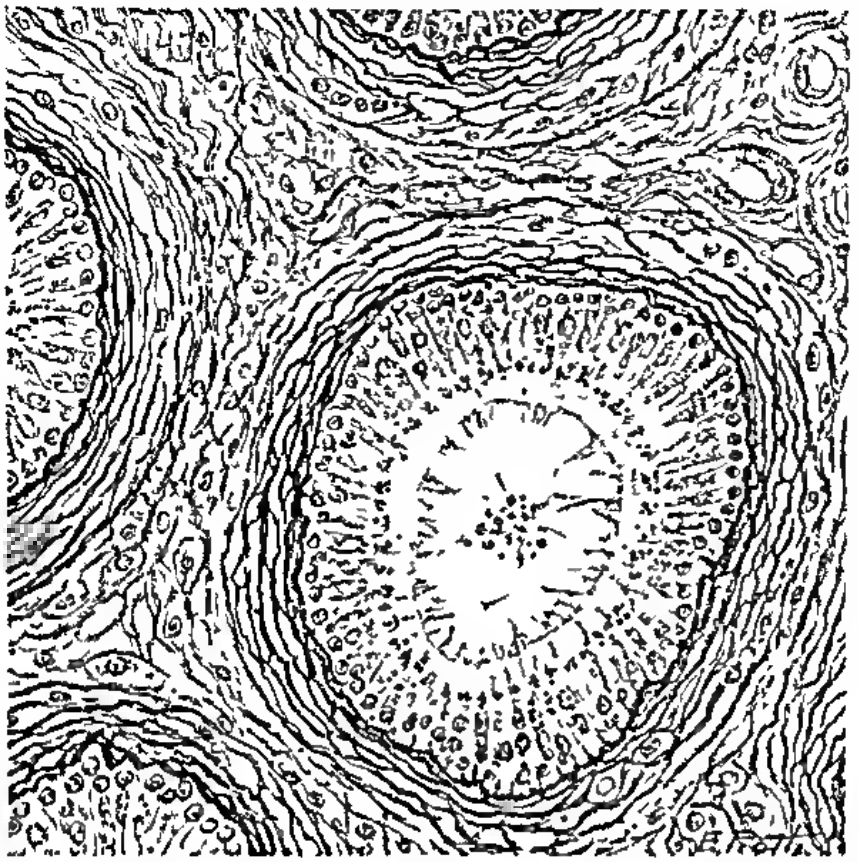


Fig. 441.—Argyrophil Reticulum in the Epididymis of a Virginia Deer
Note the stereocilia and the impregnation of the Golgi apparatus. Formaldehyde fixation, silver impregnation according to Pap, hæmatoxylin

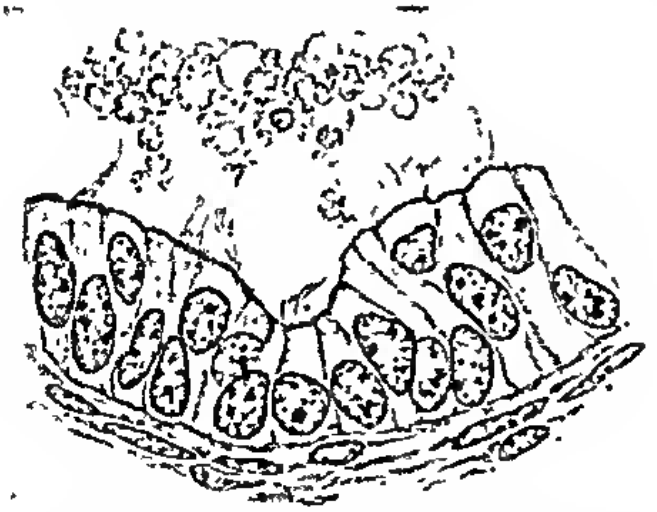


Fig. 442.—Portion of the Epithelium of a Ductus Efferens of a New-born Child
Sublimate-formaldehyde-acetic acid fixation, Heidenhain iron hæmatoxylin and chromotrope 2R. × 800. (Pfeiffer.)

contain fat, pigment, and other granules, and produce a secretion which may appear in vesicular masses on the surface of the cells. Often the tall cells, and occasionally the short ones, are ciliated.[1] The cilia vibrate so as

[1] Benoit, 1926.

to produce a current toward the ductus epididymidis. The cilia and secreting vacuoles are said to represent different phases of secretory activity in similar cells; but Young[1] presents experimental evidence that the function of the vasa efferentia is mainly resorptive. Beneath the cilia basal bodies are found, said to be derived from the centrosomes and not genetically related to mitochondria. During the ciliated phase the cells divide only amitotically.[2] The epithelium rests on a striated

FIG. 443.—TRANSVERSE SECTION OF A DUCTUS EFFERENS FROM A TESTIS OF A 25 YR. OLD BOY
Note the very tall epithelial cells. Zenker fixation, Delafield's hæmatoxylin and eosin. × 400 (Pfeiffer)

basement membrane which is surrounded by a layer of circular smooth muscle fibers, several cells thick. The muscle layer is thickest toward the ductus epididymidis. Among the muscle cells there are elastic fibers, which, like those of the ductus epididymidis and ductus deferens, first appear at puberty. There are no glands in the efferent ducts, but the irregularities in the epithelium are thought to be due to glandular activity. Before puberty and in old age these irregularities are slight.

The *ductus epididymidis* is lined by a two-rowed epithelium, with rounded basal cells and tall outer columnar cells. In some animals and

[1] YOUNG, 1933. [2] JORDAN AND HELVESTINE, 1923.

occasionally in man the basal cells are scattered or absent, the epithelium being of the pseudostratified type. The surface cells contain secretory granules and sometimes pigment, and have in the middle of their free surfaces long non-motile hairs, without basal bodies, which in sections are usually matted in conical processes. These are said not to be discernible in fresh preparations; this would indicate that they represent coagulated secretions. According to Jordan and Helvestine, however, these cilia are the result of the disappearance of the hyaloplasmic constituents along the distal border of the cells during secretory activity. The epithelium may contain round cavities opening into the lumen or forming closed cysts. The delicate membrana

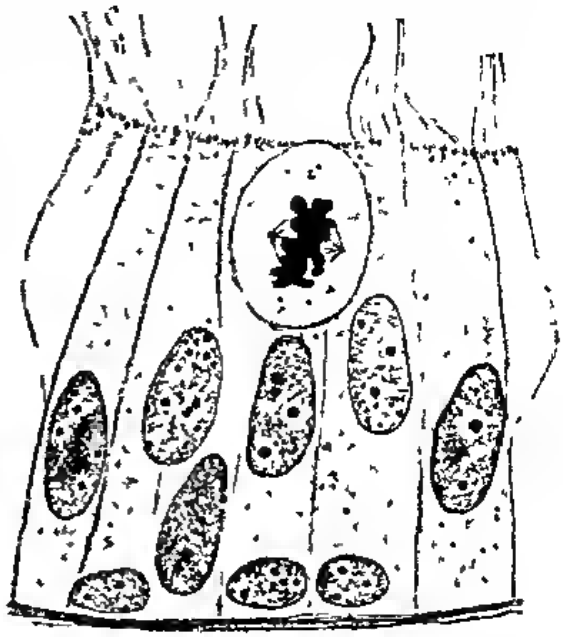


FIG. 444.—SECTION OF EPITHELIUM FROM THE DUCTUS EPIDIDYMIDIS OF A RABBIT

Observe the centrosomes, the stereocilia and one cell in mitosis. Bouin fixation, Heidenhain iron-hæmatoxylin (Nassonov)

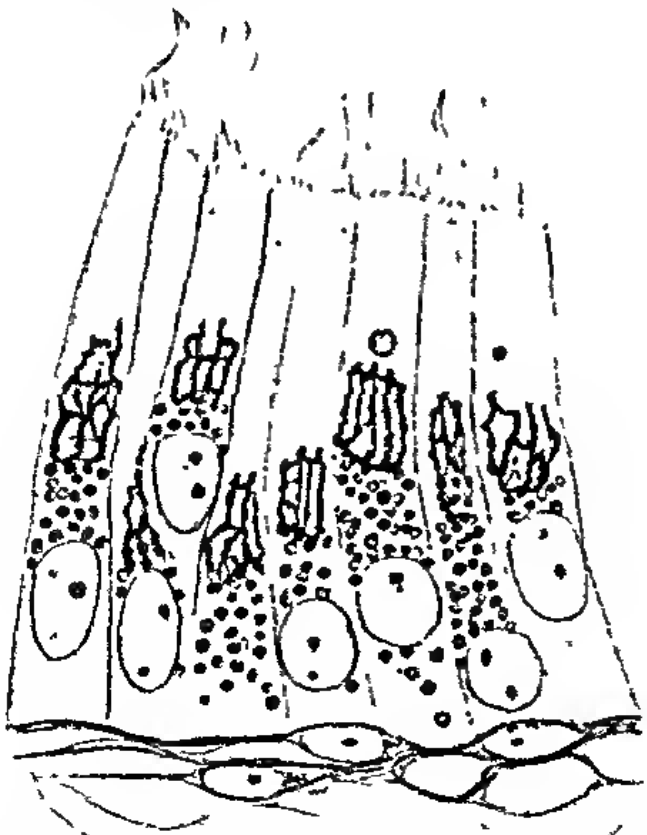


FIG. 445.—A GROUP OF EPITHELIAL CELLS FROM THE BEGINNING OF THE DUCTUS EPIDIDYMIDIS OF A RABBIT SHOWING AN IMPREGNATION OF THE GOLGI APPARATUS. KOLATCHEV-OSMIUM METHOD. (NASSONOV)

propria and thick circular muscle layer complete the wall of the ductus, the convolutions of which occur in a loose connective tissue. Toward the ductus deferens the muscle layer thickens. There are no glands in the ductus epididymidis, but its cells produce considerable secretion in which the spermatozoa become active.

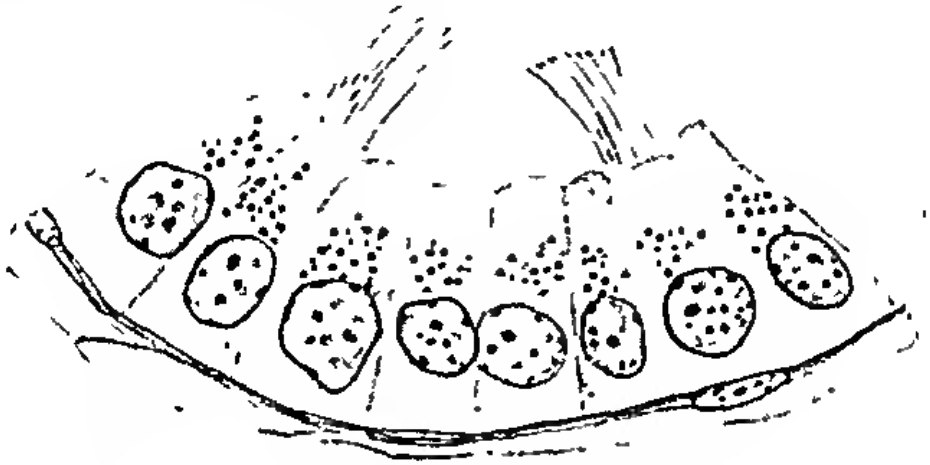


Fig. 446.—The Storage of Trypan Blue in the Epithelial Cells of the Ductus Efferens of a Rabbit. (Nassonov.)

The blood vessels of the epididymis, which are few in comparison with those of the testis, lie in part so close to the efferent ducts as to cause the membrana propria to bulge toward the epithelium. The nerves, beside perivascular nets, form a thick *plexus myospermaticus* provided with sympathetic ganglia. It is found in the muscle layer, which it supplies, sending fibers also into the mucosa. In the ductus deferens and seminal vesicles this plexus is said to be more highly developed than in the epididymis.

APPENDICES, PARADIDYMIS, AND OTHER PERSISTENT EMBRYONIC REMNANTS

It has been noted that only 10–15 of the Wolffian tubules persist as efferent ducts; in man, according to Felix, these are the fifty-eighth to seventieth out of a series of eighty-three which develop. Thus a great many degenerate, and certain appendages of the epididymis are explained as persistent remnants. The *appendix epididymidis* may represent a part of the Wolffian duct or an anterior tubule (Fig. 427); its history is still obscure. Other anterior tubules may be retained as *appendages of the rete*. Still other remains of the Wolffian body, apparently derived from the tubules below those which become efferent ducts, are known as *aberrant ducts* (*ductuli aberrantes*). There may be two or three of them; usually there is said to be but one. It proceeds from the duct of the epididymis, or rarely from the ductus deferens at its junction with the duct of the epididymis, and terminates in a coiled mass, sometimes

having branches. The length of the aberrant duct is '4–36 cm., generally 5–8 cm.' It has the same type of epithelium as the epididymal duct.

The Müllerian duct, related with the female genital system (see p. 515) and vestigial in the male, arises as an outpocketing of the cœlomic epithelium near the anterior end of the Wolffian body. The orifice into the peritoneal cavity becomes surrounded by irregular folds known as *fimbriæ*. As the Müllerian duct grows posteriorly by the elongation of its blind end, it lies in contact with the Wolffian duct as seen in Fig. 425, but the Wolffian duct does not contribute toward its formation. The two Müllerian ducts reach the neck of the bladder side by side, and acquire openings into it between those of the Wolffian ducts. Near the bladder the two Müllerian ducts fuse with one another so that their distal part is represented by a single median tube, on either side of which is a Wolffian duct (Fig. 400, B, page 455). In the female the united portion becomes the *vagina* and *uterus*, and the separate parts are the *uterine* (or Fallopian) *tubes*. In the male the united portion becomes a small blind pocket, the *prostatic utricle*, opening into the prostatic urethra. Each fimbriated extremity becomes transformed into the *appendix testis*, and the remaining portion of the duct, except for occasional fragments, becomes obliterated. Thus only the two extremities of the Müllerian ducts are ordinarily permanent in the male.

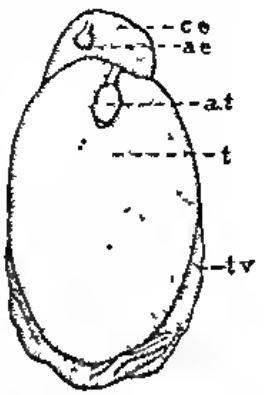


Fig. 447.—Front View of a Testis, Somewhat Reduced (Eberth).

a. e., Appendix epididymidis, a t, appendix testis c e, caput epididymidis t, testis, t. v., tunica vaginalis.

The appendices are frequently called *hydatids*, which is a general term for watery cysts. The *appendix testis* projects from the groove between the head of the epididymis and the *testis* (Fig. 447). It is quite constant, having been reported in 90% of the testes examined. The projection is covered with the peritoneum of the tunica vaginalis, which may be thickened around it, or corrugated, suggesting the fimbriated orifice of the uterine tube. The appendix consists of vascular connective tissue and encloses a canal, or fragments of canals, lined with simple columnar epithelium which is sometimes ciliated. It is generally not cystic, and it may be pedunculated, so that the terms 'hydatid of Morgagni' and 'sessile hydatid,' formerly applied to it, are inappropriate. Although its canal has been reported as connecting with the seminal ducts, this is not now believed to be the case; the structure is regarded as the degenerated end of the Müllerian duct.

The *appendix epididymidis* (stalked hydatid) is not always present. Among 105 cases examined by Toldt it was found twenty-nine times. It consists of loose vascular connective tissue covered by the vaginal s, and contains a dilated canal lined with columnar epithelium, sometimes ciliated. The canal generally has no connection with the tubules of the epididymis. It is regarded as a persistence of detached degenerating Wolffian tubules, or possibly of the terminal portion of the Wolffian duct.

The *paradidymis* is 'a functionless remnant of the Wolffian body,' situated behind the head or upper end of the epididymis and in front of the veins which accompany the

ductus deferens. Giraldès first described it[1] and Kölliker named it the 'organ of Giraldès;' Henle called it the *paraepididymis* (*i.e.*, the organ beside the epididymis), and Waldeyer later shortened the term and changed its meaning. Felix,[2] contrary to the earlier descriptions, places the paradidymis 'between the epididymis and the testis, slightly below the head of the epididymis.' Toldt[3] recognized two forms of paradidymis, but both are behind the epididymis and in front of the veins of the spermatic cord.

The first is found frequently, but by no means regularly, in older embryos and in children. It is a round or elongated structure, conspicuous because of its white color, found on the ventral side of the spermatic cord, either behind the head of the epididymis or higher up. Microscopically it is seen to be a thin, coiled, blind canal, expanded in places, and lined with a simple columnar epithelium. Occasionally there are two to four such structures at varying distances from one another. In later years they all disappear. They never contain spermatozoa.

The second form of paradidymis was found by Toldt in late childhood and in adults, but it does not occur regularly. It is always immediately behind the head of the epididymis and in front of the pampiniform plexus. It consists of a canal, sometimes with saccular dilatations, which is easily followed with the naked eye. The tubule may be closed at both ends, or one end may connect with the epididymis or testis; sometimes one end connects with the testis and the other with the epididymis. These tubules may contain spermatozoa, and they have been said to resemble the efferent ducts in structure. They may be ciliated.

Toldt regards the first form of paradidymis as due to persistent Wolffian tubules, and the second as a late separation of an efferent duct from its connection with the epididymis. He notes that the second form may give rise to cysts of varying size. Other cysts in the vicinity of the epididymis are said to arise from inpocketings of the tunica vaginalis.

DUCTUS DEFERENS

The ductus deferens begins as a convoluted tube continuous with the ductus epididymidis; it becomes straight and passes to its termination in the ductus ejaculatorius. Shortly before reaching the prostate it exhibits a spindle-shaped enlargement or *ampulla* about $\frac{1}{2}$ inch long and $\frac{3}{8}$ inch wide (Fig 450) The ductus deferens consists of a mucosa, muscularis and adventitia. Near the epididymis the epithelium is like that of the ductus epididymidis, but during its course in the spermatic cord this changes to a simple columnar layer, without basal cells; the non-motile cilia may disappear. Toward the ampulla there may be several rows, resembling the epithelium of the bladder. It rests on a connective tissue lamina propria, which is surrounded by the three layers of the muscularis. The inner and outer layers are longitudinal and generally less developed than the middle circular layer. The adventitia is a loose elastic connective tissue, blending with that which forms the *spermatic cord*. The latter contains numerous arteries, veins, lymphatics and nerves, together with the striated muscle fibers of the cremaster muscle, and the rudiment of the processus vaginalis. The veins are very numerous and constitute

[1] Giraldès, 1857. [2] Felix, 1912. [3] Toldt, 1892.

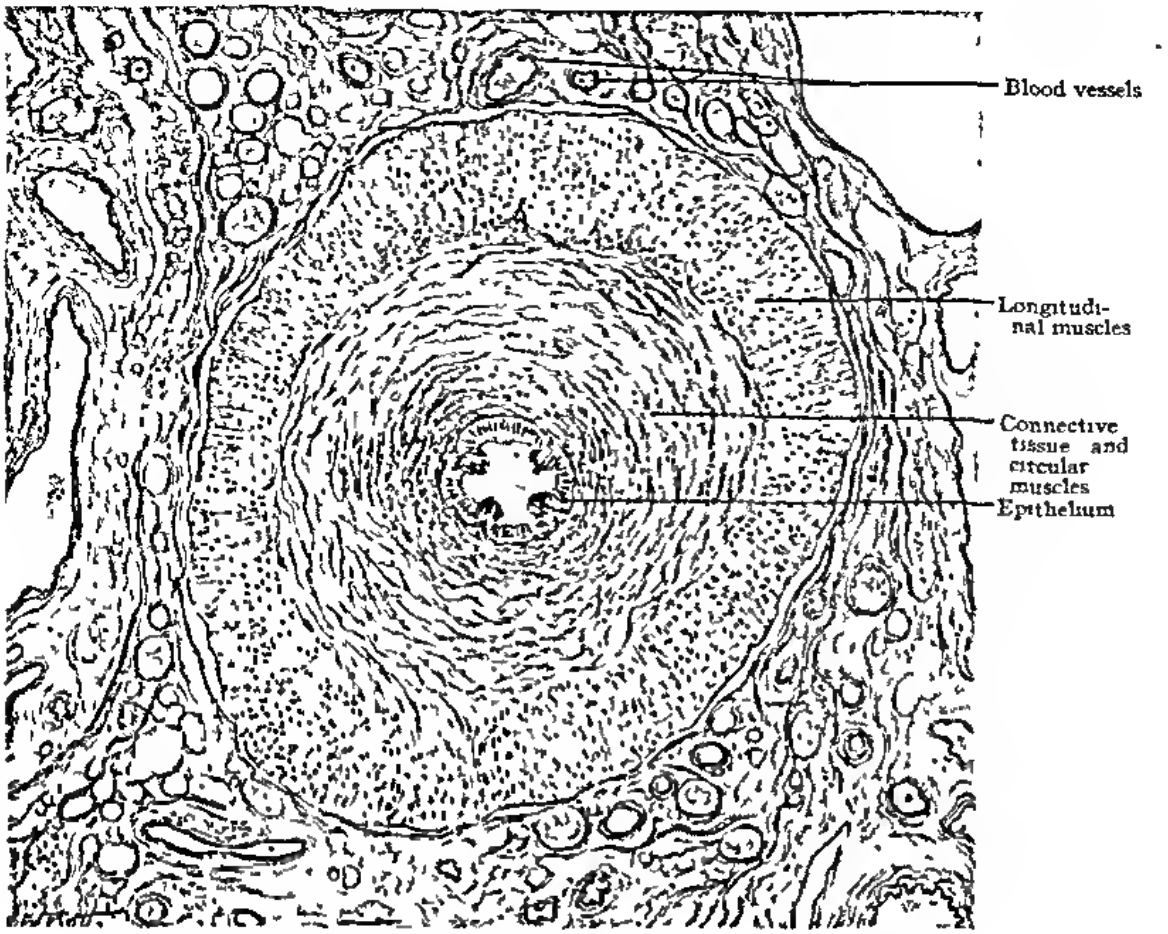


FIG. 448.—CROSS SECTION OF THE HUMAN DUCTUS DEFERENS
Zenker fixation hæmatoxylin and eosin

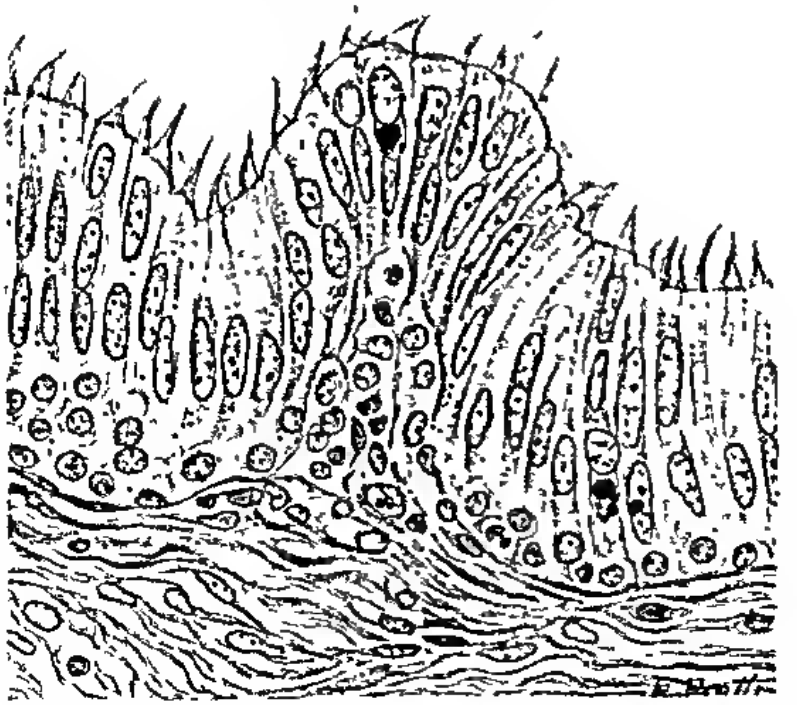


FIG. 449. A PORTION OF THE EPITHELIUM SHOWN IN FIG. 448 AT A HIGHER MAGNIFICATION.

the pampiniform plexus (*i.e.*, tendril-like). Their walls are usually provided with a very thick musculature including both circular and longitudinal fibers.

In the ampulla the longitudinal folds, which are low in the ductus deferens, become tall and branched, so that they partly enclose irregular spaces (*diverticula*). Similar folds occur in the seminal vesicles. It is doubtful whether in either place any of the spaces should be considered glands. Around the ampulla the musculature is irregularly arranged; the longitudinal layers separate into strands which terminate toward the ejaculatory ducts.

SEMINAL VESICLES AND EJACULATORY DUCTS

The seminal vesicles grow out from the ductus deferentes at the prostatic ends of their ampullæ. Their development has been studied by

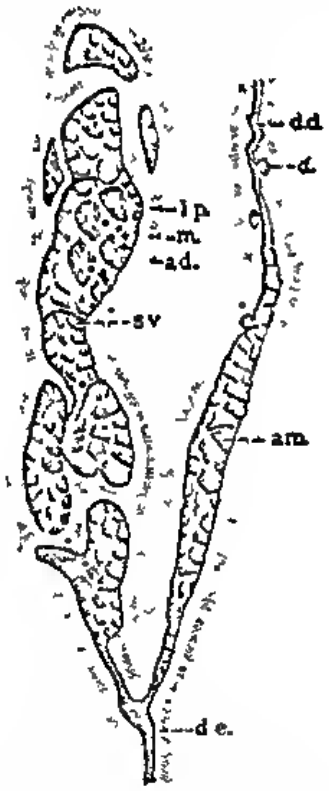


Fig. 450.—Seminal Vesicle and Ductus Deferens (This is natural size) (Eberth)

ad., Adventitia; am., ampulla; d, diverticulum; d. d., ductus deferens; d. e., ductus ejaculatorius; m., muscularis; s v, seminal vesicle; l. p, lamina propria

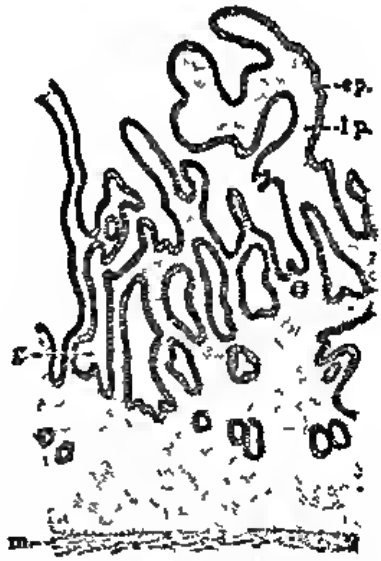


Fig. 451.—Vertical Section of the Wall of a Seminal Vesicle (Kölliker)

ep, Simple epithelium; g, gland-like depression; m., muscularis; l. p, lamina propria

Watson.[1] Each consists of a number of saccular expansions arranged along the main outgrowth, which is irregularly coiled. The lining of the sacs is honeycombed with folds as shown in Fig. 451. There are no true glands, the irregularity serving merely to increase the surface. The epithelium may consist of two layers, for between the bases of the

[1] Watson, 1918.

low columnar cells a varying number of small cells may be found, sometimes forming an almost complete basal layer. The height of the columnar cells varies with the distention of the vesicles. Granules occur in the cells, lying in colorless vacuoles at certain phases of secretion, and after puberty they are said to contain an iron-free lipochrome, giving a brownish color to the epithelium. The secretion is a clear gelatinous material, sometimes brownish. In specimens stained with eosin it appears as a stringy, dull-red mass, partly filling the larger lumens. Spermatozoa are generally found in the human vesicles, but except during sexual

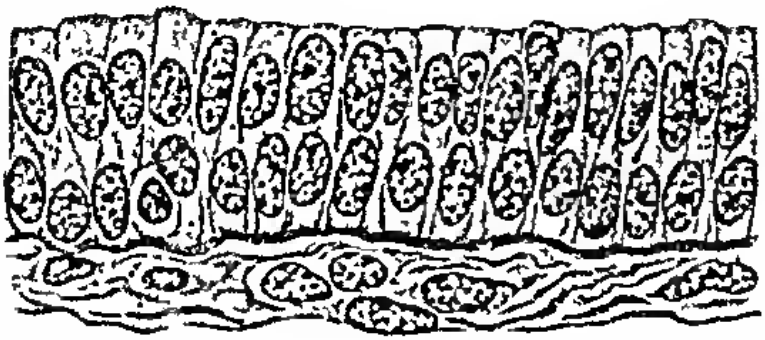


FIG 452.—PSEUDOSTRATIFIED COLUMNAR EPITHELIUM FROM THE EJACULATORY DUCT OF A 34 YR OLD MAN Sublimate-formaldehyde-acetic acid fixation, Heidenhain iron hæmatoxylin and chromotrope 2R × 800 (Stieve)

excitement they are absent from the vesicles of rodents; this and other facts indicate that the function of the organ is primarily glandular.

The maintenance of the seminal vesicles seems to depend on the presence of a testicular hormone.[1] After castration in rats the epithelium shows no signs of secretion, and the cells become low cuboidal. Within a few days after the subcutaneous injection of repeated doses of the purified lipoid fraction of fresh bull testes dissolved in olive oil the epithelium regenerates, and secretion granules again appear.

The lumens of the various sexual glands are generally of very large caliber, associated with the storing of secretions. During ejaculation the spermatozoa are forced from the ductus epididymidis and ductus deferens by the action of their muscular walls, and the contents of the seminal vesicles and prostate are added. According to Akutsu,[2] the stimulation for the entire complex is carried by the hypogastric nerves.

The *ductus ejaculatorii*, along their dorso-median sides, are beset with a series of appendages, which do not project externally but are wholly enclosed in the connective tissue of the duct. Some of these appendages show the same structure as the seminal vesicles and therefore might be described as accessory seminal vesicles; others are simply convolutions of tubulo-alveolar glands which may be compared with prostate glands. The mucous membrane of the ductus ejaculatorii is like that of the seminal vesicles, except that its folds are not so complicated. Muscle

[1] MOORE, HUGHES AND GALLAGHER, 1930. [2] AKUTSU, 1903.

fibers occur only around the appendages. The wall of the duct itself consists of an inner dense layer of connective tissue with circular strands, and an outer loose layer (adventitia).

PROSTATE

The prostate is a group of branched tubulo-alveolar glands, embedded in a mass of muscular tissue, which 'stands before' the outlet of

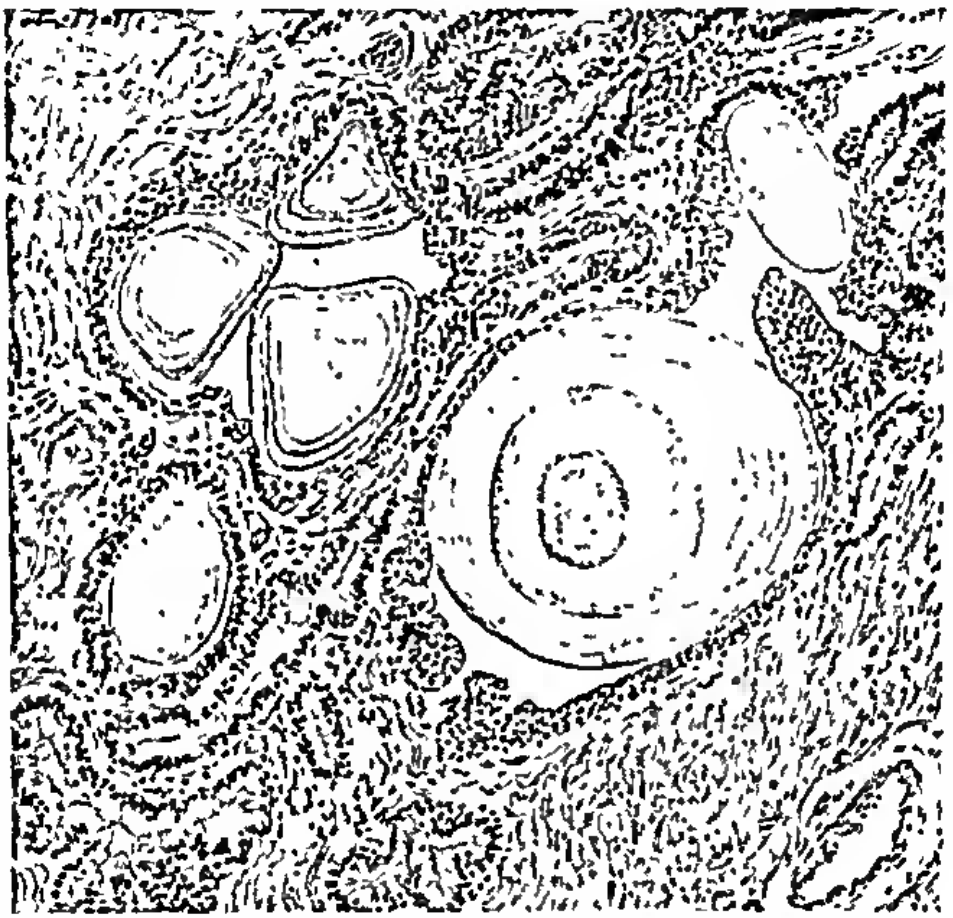


FIG. 453.—A SECTION OF THE HUMAN PROSTATE SHOWING CONCRETIONS. Zenker fixation, hæmatoxylin and eosin.

the bladder. The smooth muscle of the adult prostate forms a quarter or more of the bulk of the organ, and together with an elastic connective tissue, it unites the numerous glands in a compact mass. The development of these glands up to the time of birth has been studied by Lowsley.[1] He finds that the prostate includes from fifty-three to seventy-four separate glands (the average number being sixty-three) which are grouped in five lobes. The middle lobe consists of nine to ten large glands growing out from the dorsal side of the urethra, between the bladder and the openings of the ejaculatory ducts. The glands of the posterior lobe grow out from the dorsal wall of the urethra below the ejaculatory ducts; those

[1] LOWSLEY, 1912.

of the right and left lobes develop from the sides of the prostatic urethra; and those of the anterior lobe proceed from its ventral surface. The anterior lobe is well-developed in young embryos, but it 'shrinks into

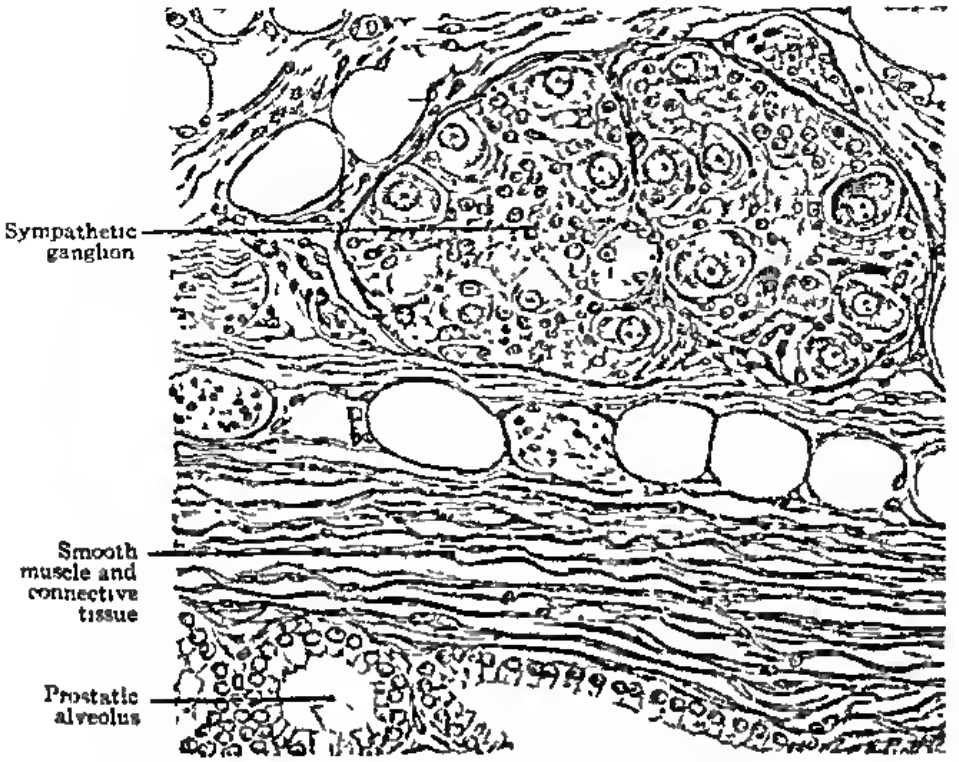


FIG 454.—SECTION THROUGH THE CAPSULE OF THE PROSTATE OF A MONKEY SHOWING A SYMPATHETIC GANGLION
Formaldehyde fixation, hæmatoxylin and eosin

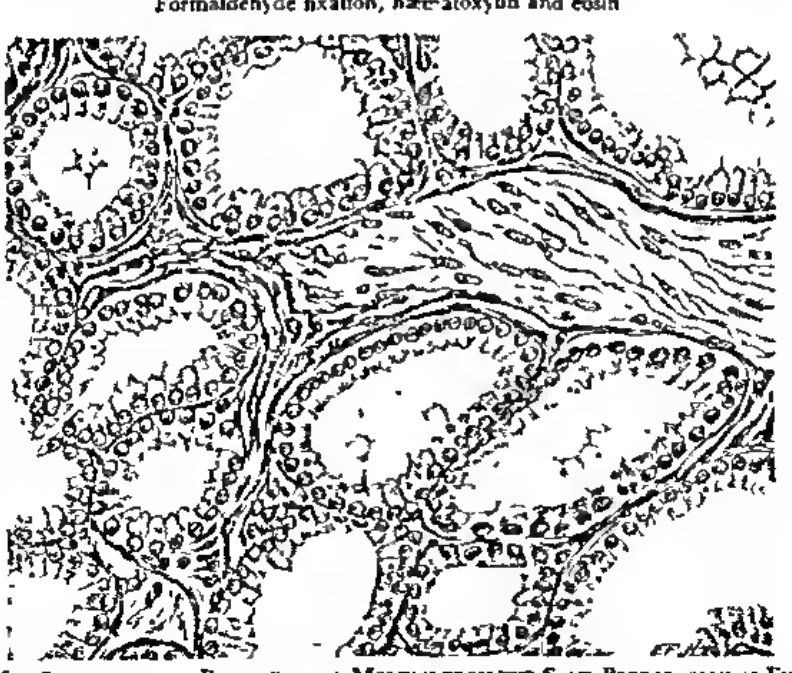


FIG 455.—SECTION OF THE PROSTATE OF A MONKEY FROM THE SAME PREPARATION AS FIG 454.
Formaldehyde fixation, hæmatoxylin and eosin

insignificance at the twenty-second week.' It may persist in the adult, but the great mass of the prostatic glands is at the sides and back of the prostatic urethra. The number of glands apparently becomes reduced. In the adult it is said to be from thirty to fifty.

The glandular epithelium is simple and either cuboidal or columnar. It may appear stratified as it passes over the folds in the walls of the tubules, and some of the lateral pouches of the main glandular spaces may show small, darkly-staining basal cells either scattered or forming a continuous basal layer.[1] Secretory granules are evident, and the secretion as seen in the lumens consists of similar granules and larger vesicular structures which may represent the detached tops of the cells (apocrine secretion) as well as desquamated cells. The maintenance of the prostate, as of the seminal vesicles (p. 505), seems to depend on the presence of a testicular hormone.[2] Near the outlet of the larger ducts the epithelium is like that of the bladder and prostatic urethra. In the prostatic alveoli,

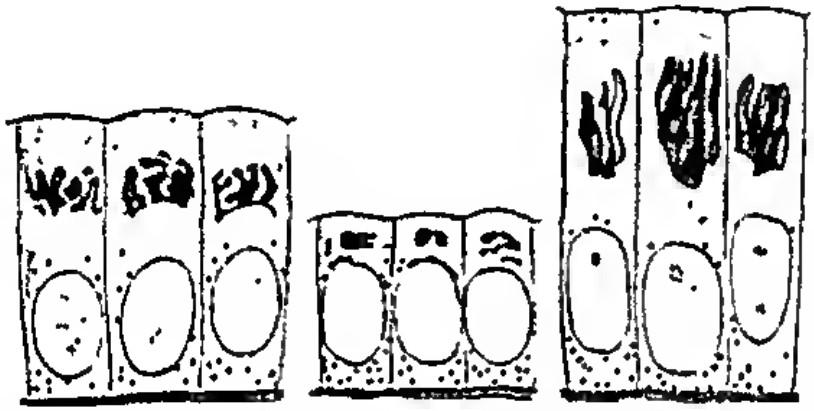


FIG. 456.—THE PROSTATE GLAND OF THE RAT AS AN INDICATOR FOR TESTIS HORMONE

A, normal, B, 20 days after castration, C, 20 days after castration and injected with testis extract. (Moore, Price and Gallagher. Courtesy of the Wistar Institute.) Note the different heights of the epithelial cells and the development of the Golgi apparatus.

of older persons especially, concretions of various forms and sizes (granules, round, oval or irregularly angular bodies from 0.2 to 2 mm. in diameter) occur; they are often pigmented a question of 'wear and tear' and as seen in sections they may exhibit concentric layers (Fig. 453). Striated concretions show a double refraction with polarized light. Their reactions on treatment with iodine solutions suggest amyloid. These concretions are probably deposited around fragments of cells. Octahedral crystals also occur in the prostatic secretion, which is a thin milky emulsion, faintly alkaline; it has a characteristic odor, whereas the other constituents of the seminal fluid are said to be odorless.

The smooth muscle fibers are found everywhere between the prostatic lobules; toward the urethra they thicken to form the internal sphincter of the bladder. The prostate is surrounded by a capsule of three layers, which Stieve[3] designates as (1) the *stratum musculare*, (2) the

[1] GRYNFELTT, 1934. [2] MOORE, PRICE AND GALLAGHER, 1930.
[3] STIEVE, 1930.

stratum fibrosum and (3) the *stratum vasculosum*. The muscular layer is the innermost and is comprised chiefly of smooth muscle fibers which lie close upon the surface of the organ where they connect with the muscle in the inter-alveolar septa. Between the muscle fibers there is found a fine network consisting principally of elastic fibers, a few fibrocytes and histiocytes. The middle layer is made up of loose collagenous tissue, and a feltwork of finer and coarser elastic fibers. In the interstices lie fibrocytes and histiocytes and isolated smooth muscle cells which form a very open network binding together the fibrous and muscular layers. Toward

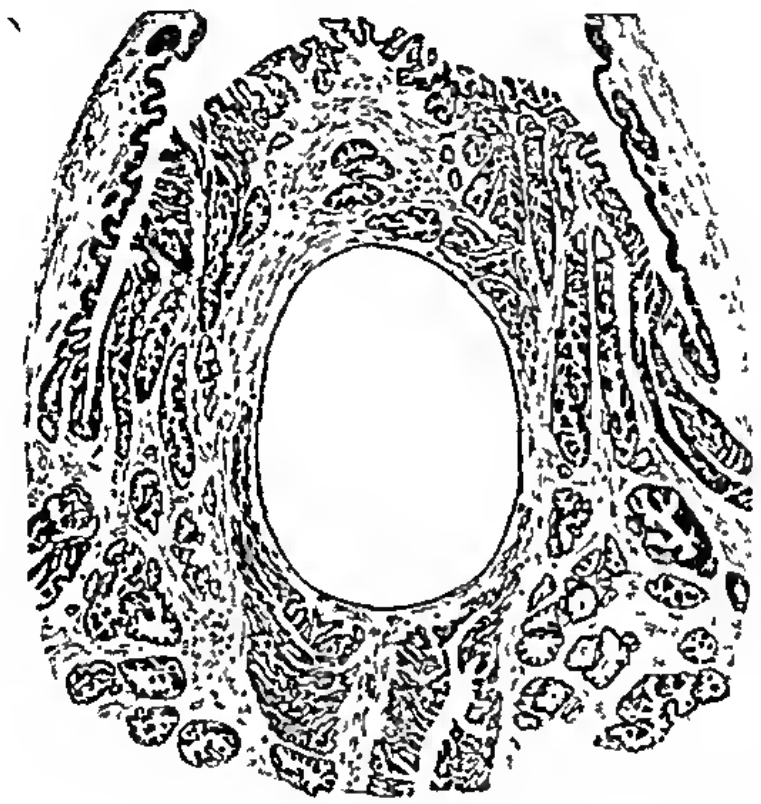


FIG. 457.—SECTION THROUGH THE COLLICULUS SEMINALIS SHOWING IN THE CENTER THE UTRICULUS PROSTATICUS. THIRTY-FOUR YEAR OLD MAN

Formaldehyde fixation; Delafield's hæmatoxylin and erythrosin. × 16 (Stieve)

the rectum the connective tissue is thicker and embedded in it are rather thick nerve fibers, ganglion cells and numerous fine blood vessels. Some of the nerve cells contain two or more nuclei (Fig. 454). In the outer layer the blood vessels are mostly wider and the walls of the veins are poor in muscle.

The ducts of the prostate are of smaller caliber than the irregular glandular portions, and concretions may block the outlets of individual glands. Continued secretion may cause distention, accompanied by a flattening of the epithelium, and by an increase of the muscular tissue in an endeavor to relieve the pressure. The enlarged gland may ultimately constrict the urethra, which it nearly encircles. In rodents one pair of the lobes of the prostate is differentiated to supply the material

which causes the coagulation of the seminal fluid in the vagina, and the formation of the vaginal plug.[1]

The *utriculus prostaticus* (uterus masculinus, vagina masculina) is a small pocket lined with stratified epithelium, opening into the dorsal wall of the urethra midway between the orifices of the ejaculatory ducts, or a little above them. It is sometimes absent, and is occasionally quite deep. Lowsley failed to find any small prostatic tubules opening into it, such as have been reported as occasionally present. The utriculus prostaticus is the lower end of the Müllerian ducts, which have fused, and it corresponds with the vagina in the female.

URETHRA AND PENIS

The urethra in the male is divided into three parts—the *pars prostatica*, the *pars membranacea* and the *pars cavernosa*. The true urethra is the part from the trigone of the bladder to the openings of the right and left ejaculatory ducts and it corresponds to the urethra in the female. The rest of the prostatic part, the whole of membranous and cavernous parts is the uro-genital sinus—a long narrow, common tube for the passage or urine and semen. The form and thickness of the epithelium varies in the same and in the different parts. Near the bladder it is transitional, but the outer cells gradually become elongated and the type of epithelium changes to pseudostratified. Except for short spaces where simple or two-layered columnar cells are found the pseudostratified epithelium continues into the cavernous portion. In the dilatation of the urethra near its distal end, the *fossa navicularis*, the epithelium becomes stratified with its outer cells squamous; the underlying papillæ of the lamina propria become prominent, and the whole is the beginning of the gradual transition from mucous membrane to skin.

Glands. Small groups of mucous cells are scattered along the urethra; and in the cavernous part, especially on the upper wall, they form pockets called *urethral glands* (of Littré). Often these pockets are on the sides of epithelial pits so that the glands are branched. Non-glandular pits also occur, known as *urethral lacunæ*, and the 'paraurethral ducts' near the external orifice are large lacunæ of various sorts. The development of the urethral glands has been studied by Johnson.[2]

Two glands of considerable importance empty by irregularly dilated ducts, 1½ in. long, into the beginning of the cavernous urethra. The bodies of these *bulbo-urethral glands* (Cowper's glands) are found one on each side of the membranous urethra, in close relation with striated and smooth muscle fibers. The end pieces are partly alveolar and partly

[1] Engle, 1926 [2] Johnson, 1920.

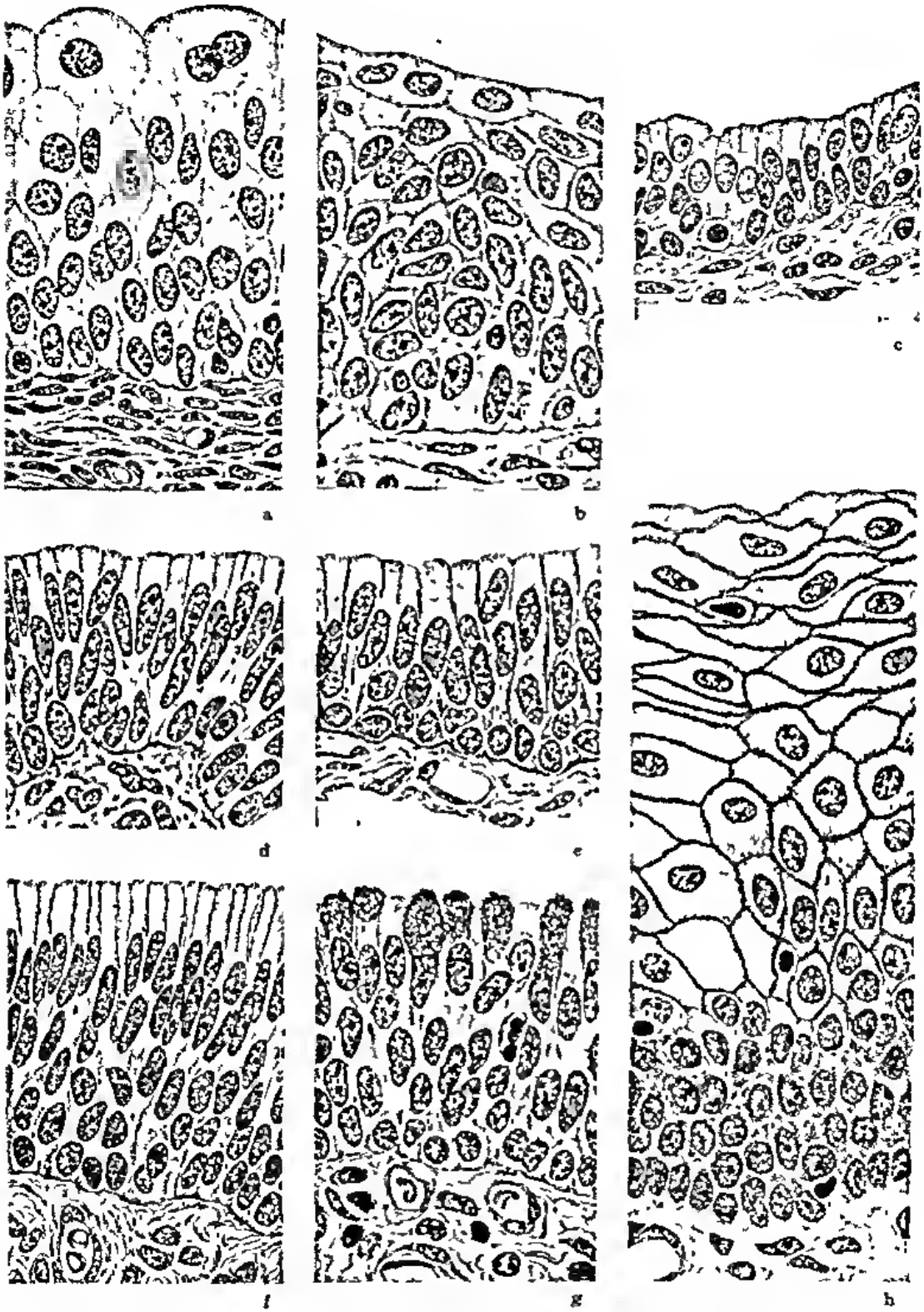


FIG 458.—SECTIONS SHOWING THE CHANGING CHARACTER OF THE EPITHELIUM IN THE DIFFERENT PARTS OF THE MALE URETHRA (Sheve)

a, near the bladder; b, inner portion of prostatic part, c, outer portion of prostatic part; d, diaphragmatic part; e, ampulla; f, middle of cavernous part, g, inner part of fossa navicularis, showing goblet cells, h, outer part of fossa naviculatis.

tubular. They consist of mucous cells and produce a clear, glairy mucus, discharged during sexual excitement. The cells show very different appearances corresponding to functional states. At rest they are simple columnar, with a granular cytoplasm and a clear spherical nucleus; after coitus they are cuboidal and appear empty. Transitional phases are seen between these two stages. The epithelium possesses prominent terminal bars, the cells rest upon a clear basement membrane and basket cells have been observed. The interstitial tissue is made up principally of collagenous fibers, with few elastic fibers intermingled. Between the connective tissue fibers there are seen fibrocytes and histiocytes, and numerous fine capillary vessels. The excretory ducts are surrounded by a distinct basement membrane, connective tissue fibers and thin rings of smooth muscles—the epithelium is composed of a single layer of low cells. The ducts may connect directly with the end pieces, or a secretory duct may intervene.

Muscle. The muscularis of the prostatic part of the urethra consists of an inner longitudinal and an outer circular layer of smooth muscle. Both layers continue throughout the membranous part; the circular layer ends in the beginning of the cavernous urethra leaving only oblique and longitudinal bundles in its distal part.

Corpus Cavernosum Urethræ. In the submucosa of the cavernous urethra there are many veins which become larger and more numerous in and beyond the muscularis. This vascular tissue which surrounds the urethra is limited by a dense elastic connective tissue layer, the *tunica albuginea*, and the structure which is thus bounded is the *corpus cavernosum urethræ*. Toward the perineum it ends in a round enlargement, the *bulbus urethræ*, and distally it terminates in the *glans penis*. The urethra enters the upper surface of this corpus cavernosum near the bulbus. Branches of the internal pudendal artery, namely, the arteriæ bulbi and the urethral arteries, penetrate the albuginea, and the former pass the length of the cavernous body and end in the glans. These arteries have particularly thick walls of circular muscle, and in cross sections the intima may be seen to form coarse, rounded projections into the lumen. These projections contain longitudinal muscles and subdivisions of the inner circular elastic membrane. The arteries in the corpus cavernosum produce capillaries found chiefly toward the albuginea. The capillaries empty into thin-walled venous spaces which appear as endothelium-lined clefts in a connective tissue containing many smooth muscle fibers. The cavernous body is permeated with these spaces which, at times of sexual excitement, become distended with blood, reducing the tissue between them to thin trabeculæ. Such distensible vascular tissue is known as *erectile tissue*. Some arteries connect directly with the venous spaces,

and such as appear coiled or C-shaped in a collapsed condition are called *arteriæ helicinæ*. The *venæ cavernosæ* have such very thick walls that they resemble arteries. They contain an abundance of inner longitudinal muscle fibers, and since these are not evenly distributed but occur in columns, the lumen of the veins is usually crescentic or stellate in cross section. Emissary veins pass out through the albuginea and empty into the median dorsal vein of the penis.

The *corpora cavernosa penis* are a pair of structures similar to the cavernous body of the urethra, and are found side by side above it. The

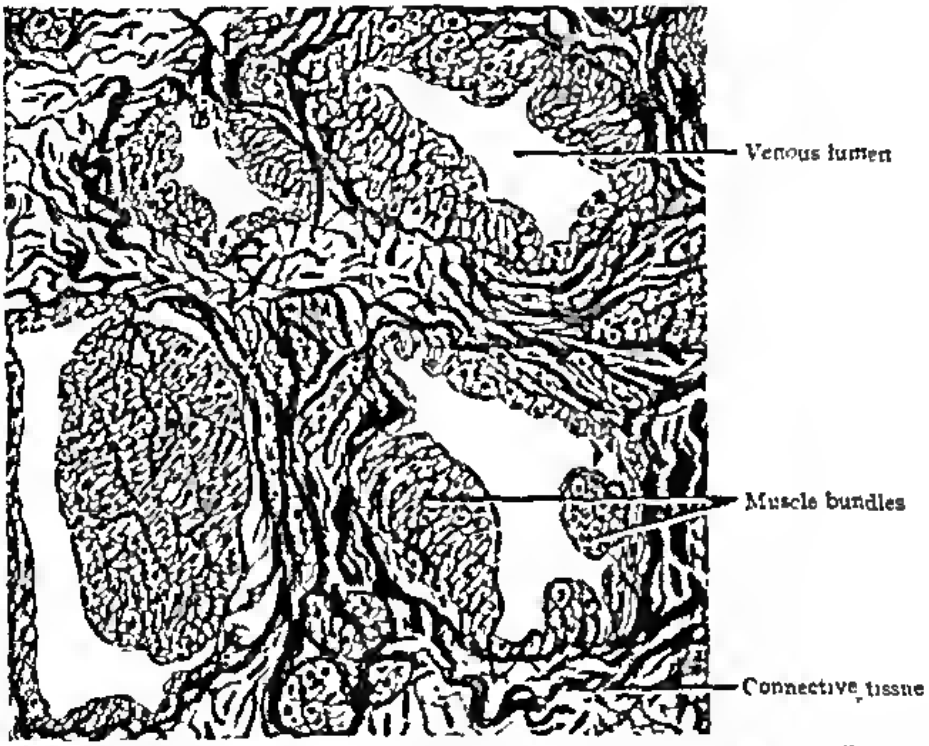


FIG 459.—PORTION OF A CROSS SECTION THROUGH THE CORPUS CAVERNOSUM SINUS UROGENITALIS (URETHRA) × 200. Zenker fixation, eosin, phosphomolybdic acid and methyl blue (von Möllendorff).

septum between them is perforated distally so that they communicate with one another. Each is surrounded by a very dense albuginea, *1 mm.* thick, divisible into an outer longitudinal and an inner circular layer of fibrous tissue. The septum is formed by the median fusion of these layers. The cavernous or erectile tissue of which these corpora are composed is essentially like that around the urethra.

All three cavernous bodies are surrounded by fascia and subcutaneous tissue containing blood vessels, lymphatics and nerves. The lymphatic vessels form a superficial and a deep set, the latter receiving branches from the urethra. The principal sensory nerves are the medullated *dorsal nerves of the penis*. They terminate in many tactile corpuscles in the papillæ beneath the skin, in bulbous and genital corpuscles in the deeper connective tissue, and in lamellar corpuscles found near or in the cavernous bodies. Free endings also occur. The sympathetic nerves are from a

continuation of the prostatic plexus and supply the numerous smooth muscles of the trabeculæ and cavernous blood vessels, and also the urethral glands. They are said to be joined by fibers from the lower spinal nerves, the *nervi erigentes.*

The skin of the penis is thin, elastic, fat-free and darker in color than the skin generally. The stratified squamous epithelium becomes thinner on the inner surface of the *prepuce* and a stratum lucidum is said to be absent on the *glans penis.* Sebaceous and sweat glands are found on the outer surface, but they are absent or few on the inner surface of the prepuce. There seems to be marked individual variation in both the number and distribution of glands. Epithelial crypts frequently have been taken for glands. In some animals, definite fatty-secreting glands, *glandulæ odoriferæ,* are present on the corona glandis or on the inner surface of the prepuce. These glands first described in the orang-utan are often referred to as the *glands* of *Tyson.* They are not found in man. Beneath the skin on the body of the penis there is a layer of smooth muscle fibers continuous with the *tunica dartos* of the scrotum forming a closed net bound together by elastic tendons. Arteries are embedded in this 'elastic-muscular system,' which according to Nagel[1] controls local blood pressure and therefore, serves as a heat regulator for the testes and probably prevents a hyperemia of the prepuce.

FEMALE GENITAL ORGANS

Development and General Features. Although it is probable that sex is determined at the time of the fertilization of the ovum, and that it cannot be modified by subsequent conditions of any sort, the sex of young embryos cannot be recognized. All human embryos of 13 mm. possess a prominent genital papilla; they have both Wolffian and Müllerian ducts, in so far as the latter have developed, and they contain genital ridges which are still in an 'indifferent stage'—it cannot be said whether they will become ovaries or testes—(*cf.* Fig. 425, p. 480). Slight sex differences *in the shape of the genital papilla and in the position* and extent of the cloacal membrane have been described, and may serve to shorten the indifferent period.[2] In the female the Müllerian ducts become highly developed, the Wolffian ducts degenerate, and the genital ridges produce ovaries.

The Mullerian Ducts. Before reaching the urogenital sinus, the lower ends of the Müllerian ducts are in contact, being situated between the Wolffian ducts (Fig. 460).[3] A fusion of the Müllerian ducts begins just above their lower termination and extends downward to the urogenital sinus.[4] Thus the entire ducts form a Y-shaped structure. The sinus sends

[1] Nagel, 1939. [2] Spaulding, 1921. [3] Keibel, 1896. [4] Hunter, 1930.

out paired sino-vaginal bulbs embracing the ends of the ducts. At first all these structures are solid cords, but the cells in the center break down and a lumen appears in embryos of about 150–200 mm. Thus the lower end of the vagina is from the sinus, therefore entodermal,[1] while the upper end is from the fused mesodermal Müllerian ducts. The lower end of the vagina is at first closed by a fold, the *hymen*, between the sinus and the bulbs, but this also becomes perforate later, usually forming a crescentic fold on the dorsal side of the entrance to the vagina.

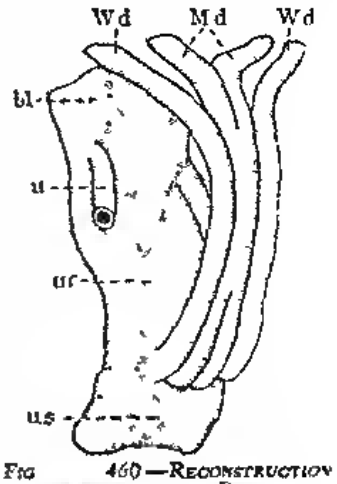


FIG 460.—RECONSTRUCTION SHOWING THE FUSION OF THE MÜLLERIAN DUCTS (Keibel)

bl., Bladder, M. d., Müllerian duct, u., ureter, ur., urethra, u.s., urogenital sinus, W.d., Wolffian duct

Above the vagina the Müllerian ducts form the lining of the uterus, which develops from the upper part of the stem of the Y, and from the inner ends of its arms. This region of junction becomes surrounded by a very thick layer of smooth muscle. The occasional occurrence of a median septum in the uterus or vagina,

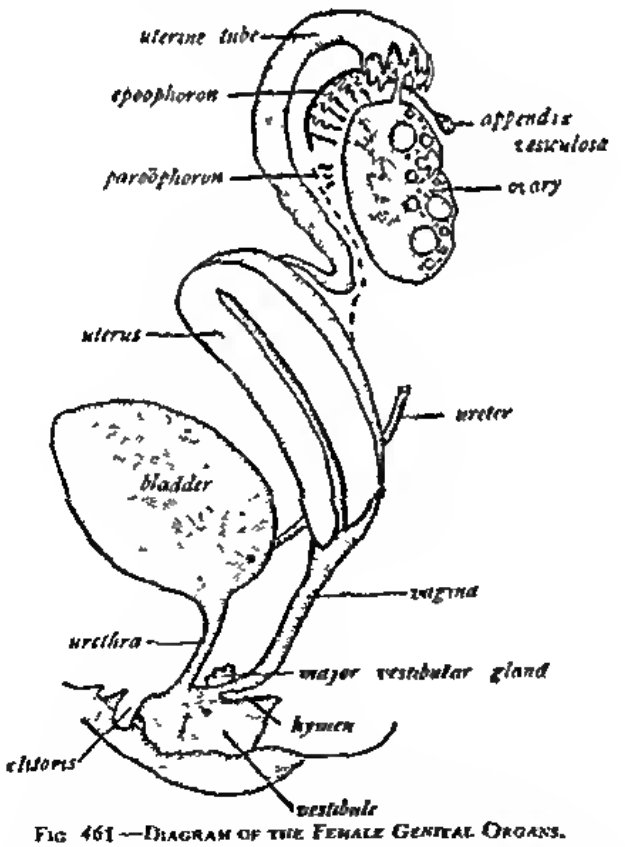


FIG 461.—DIAGRAM OF THE FEMALE GENITAL ORGANS.

dividing them into right and left halves, is due to imperfect fusion of the Müllerian ducts.

[1] KOFF, 1933.

The outer portions of the Müllerian ducts retain relatively thin walls and become the *uterine* (or Fallopian) *tubes*. Each opens freely through its fimbriated extremity into the abdominal cavity.

The Wolffian Bodies and Wolffian Ducts. In the female these structures become functionless and degenerate. Their principal derivative is a group of blind tubules, which may readily be seen in the translucent mesentery-like membrane extending between the ovary and tube. These tubules were named the 'organ of Rosenmüller' after their discoverer, who described them in 1802, and were called the 'parovarium' (later corrected to paroöphoron) because of their position beside the ovary; but when it was shown that these tubules were homologous with the epididymis, they were given a corresponding name, and are now known as the *epoophoron* (ἐπί, upon; ᾠοφόρος, ovary). The epoöphoron consists of '8 to 20' *transverse ducts*, which begin with blind ends in or near the upper end of the ovary and follow a more or less convoluted course to the *longitudinal duct*, into which they empty (Fig. 461). They are lined with simple cuboidal or columnar epithelium, sometimes ciliated, and are surrounded with muscle fibers. Occasionally there are detached solid cords in their vicinity, and sometimes the tubes become cystic. Obviously they correspond with the efferent ducts of the testis, and the longitudinal duct, into which they empty, represents the duct of the epididymis. Some of the transverse tubules, or the main duct itself, may extend into soft round nodules of tissue projecting from the mesentery, to which they may be attached by slender pedicles. These *appendices vesiculosæ* correspond with the appendix of the epididymis. Frequently there is a vesicular appendix entirely separate from the epoöphoron, situated near the fimbriated orifice of the uterine tube, and said by Felix to develop around an accessory Müllerian duct. Although accessory ducts have not been found in the male, the relations of this structure to the Müllerian duct suggest a comparison with the appendix testis. Both in the female and the male the appendages have been described as of two sorts, connected with the Müllerian and Wolffian ducts respectively.

The occasional presence of uterine tissue within the cortex of the ovary is thought to represent the lower end of a short accessory Müllerian duct enclosed by the developing gland. The organ at present called the paroöphoron is a remnant of the Wolffian tubules corresponding with the paradidymis, occasionally found near the hilum of the ovary, disappearing by the fifth year

The lower end of the Wolffian duct, which corresponds with the ductus deferens, may remain as the *canal of Gartner* This canal terminates near the hymen. It may extend upward beside the vagina, and be enclosed in the musculature of the lower part of the uterus; usually it is entirely obliterated

Development of the Ovary. Like the testis, the ovary is formed from the middle portion of the genital ridge. The peritoneum which covers it gives rise to the mass of cells in its interior, and deep within, the cells become arranged in medullary cords and a *rete ovarii*, the latter developing probably from the anterior third of the genital ridge. The rete cords do not connect with the Wolffian tubules. They acquire lumens about birth, and are bounded by simple epithelium; they remain in the adult and may become cystic.[1]

[1] Wilkerson, 1923

The medullary cords contain sex cells and supporting cells, and become segmented by connective tissue into rounded or irregular groups, in which the ova are surrounded by the indifferent cells. These groups, or primary follicles, are embedded in a connective tissue stroma, thought by some to be derived from the peritoneal proliferation, by others to have grown in from the surrounding mesenchyma. The original cell groups thus formed during fetal life develop into large hollow structures, as will be explained later, but all degenerate without coming to maturity. Meanwhile the ovary increases greatly in size, new material being added peripherally from the peritoneal layer or by the multiplication of the cells directly beneath it. The degenerated old cords and follicles form a medulla (hence the term 'medullary cords') covered except at the mesovarium by the newer material or cortex. In late fetal life new cords are being formed by invagination of the peritoneal epithelium, containing the two types of cells (Pflüger's egg-tubes). New follicles are made from the inner end of these cords. After birth the peritoneal layer becomes separated from the cords by a tunica albuginea. The activity of the follicles ceases somewhat, so that a definite cortex appears, marked by the presence of immature follicles in a connective tissue stroma. A good description of the development in the cat is given by Kingsbury.[1]

In the rete ovarii and the cords there is an obvious homology with the rete and tubules of the testis. Some writers would restrict this homology to the fetal cords and follicles, all of which degenerate early. The later growth of follicles would then be considered as a female characteristic superposed on the male structure.

Felix considers that the follicles develop, for the most part at least, directly from the tissue of the genital ridge, and states that tubes or cords growing in from the peritoneal epithelium, as described by Pflüger, do not exist in the human ovary.

Ligaments. As the Müllerian ducts come together below, they occupy ridges covered with peritoneum. These ridges coalesce so as to form a partition which crosses the pelvis from side to side and rises upward from its floor. Ventral to the partition is the bladder, separated from it by the vesico-uterine pouch; dorsal to it is the rectum, separated by the deeper recto-uterine pouch; and within it are the uterus and tubes. In the adult these folds of peritoneum extending laterally from the uterus constitute its *broad ligaments.* The Wolffian bodies and ovaries, which at first occupy vertical ridges on each side of the root of the mesentery, appear to slip down or descend into the interior of the broad ligaments, from the dorsal surfaces of which the ovaries later project.

Above each ovary there is a band of fibrous tissue which extends to the orifice of the tube, and running along this band there is a fimbria

[1] Kingsbury, 1913.

known as the *fimbria ovarica;* this arrangement apparently serves to keep the orifice of the tube in close relation with the ovary. Below the ovary, between the laminæ of the broad ligament, a cord of fibrous tissue passes from it to the musculature of the uterus, lying just below the uterine tube; this is the *ovarian ligament.* The *round ligaments* start from the uterine musculature not far from the ends of the ovarian ligaments. They pass downward, one on each side within the broad ligament, and terminate in the folds which correspond with those of the scrotum. The ovarian and round ligaments are believed to be subdivisions of a single structure equivalent to the *gubernaculum testis.*

The External Genital Organs. The urogenital sinus, which receives the urethra and vagina, becomes a shallow space called the *vestibule* (Fig. 461). The genital papilla, with the *glans* at its apex, becomes relatively shorter as the female embryo develops. It forms the *clitoris,* analogous with the penis, and is covered by the lesser genital folds, the *labia minora* (see Figs. 428 and 429). The labia form a prepuce for the clitoris but do not unite beneath it to make a raphé; they remain separate, as parts of the lateral boundaries of the vestibule. The larger genital folds, *labia majora,* likewise remain separate. They receive the ends of the round ligaments of the uterus which pass into them over the pubic bones, sometimes accompanied by a prolongation of the peritoneal cavity forming a processus vaginalis. In late stages of development the labia majora become large enough to conceal the clitoris and labia minora, which previously project between them.[1]

OVARY

The ovary is an oval body about an inch and a half long, covered by a modified portion of the peritoneum, the epithelial cells of which are cuboidal or low columnar. It consists of a cortex, composed of a very cellular connective tissue stroma, embedded in which are ovarian follicles in various stages of growth, and a less cellular connective tissue medulla, containing numerous blood vessels.

The relation of the cortical stroma to the looser tissue of the medulla is so characteristic that sections of the human ovary containing few ova and no active follicles may be readily identified. Usually a section of the ovary may be recognized as such without magnification, owing to the presence of the large cysts or follicles in which the maturing ova are contained. These grow so large within the cortex that they may invade the territory of the medulla, and are numerous even in childhood. Along its *hilum* it is attached to a mesentery, the *mesovarium,* which is a subdivision of the broad ligament of the uterus. The epithelium of the mesovarium

[1] Spaulding, 1921.

is continuous with that of the ovary, and its connective tissue joins the mass which forms the ovarian medulla. This tissue, rich in elastic fibers and containing strands of smooth muscle, surrounds the vessels and nerves. The blood vessels are abundant, and they pursue a very tortuous course both in the mesovarium and within the ovary. This is strikingly shown in Clark's injections.[1] They are derived in part from branches of the uterine vessels, but are chiefly the terminations of the ovarian artery and vein. Large stems traverse the medulla and form capillary

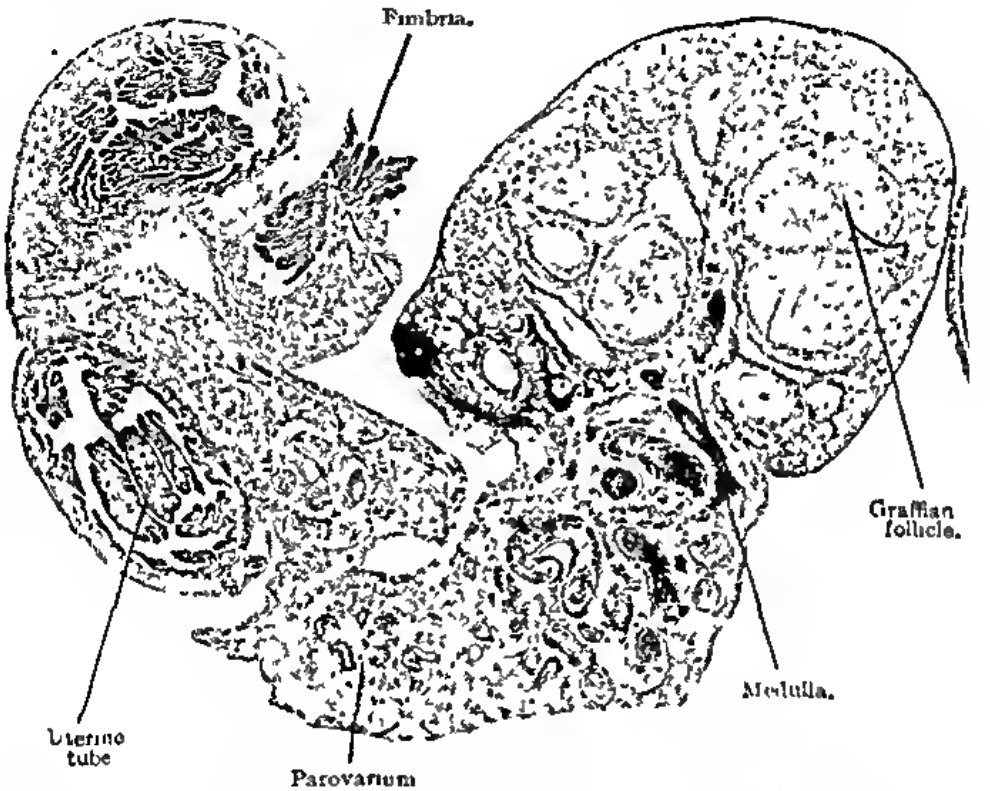


FIG. 462.—OVARY, MESOVARIUM AND UTERINE TUBE, MONKEY (PHOTOGRAPH OF SECTION) × 16.

plexuses around the follicles in the cortex. Thin-walled lymphatic vessels arise in the cortex below the rather dense sub-peritoneal layer (*or tunica albuginea*) and pass out at the hilum. The nerves are chiefly non-medullated sympathetic fibers, derived from the plexus which accompanies the ovarian artery, and distributed to the blood vessels. Ganglion cells have been found near the hilum, and a few medullated fibers occur. It is said that certain fibers end in contact with the cells of the follicles.

Growth of the Follicles. It is probable that all the sexual cells which are to be produced in a life-time are present in the ovaries at birth. At that stage, at least, many of those previously formed have already degenerated; and the ovaries contain a great excess of ova, all but a few hundred of which are destined to atrophy within the limits of the genital glands.

[1] CLARK, J. G., 1900.

In so far as the sexual cells have ceased to multiply and have entered upon the growth period, they represent the last generation of oögonia, and are being transformed into primary oöcytes. During this transforma-

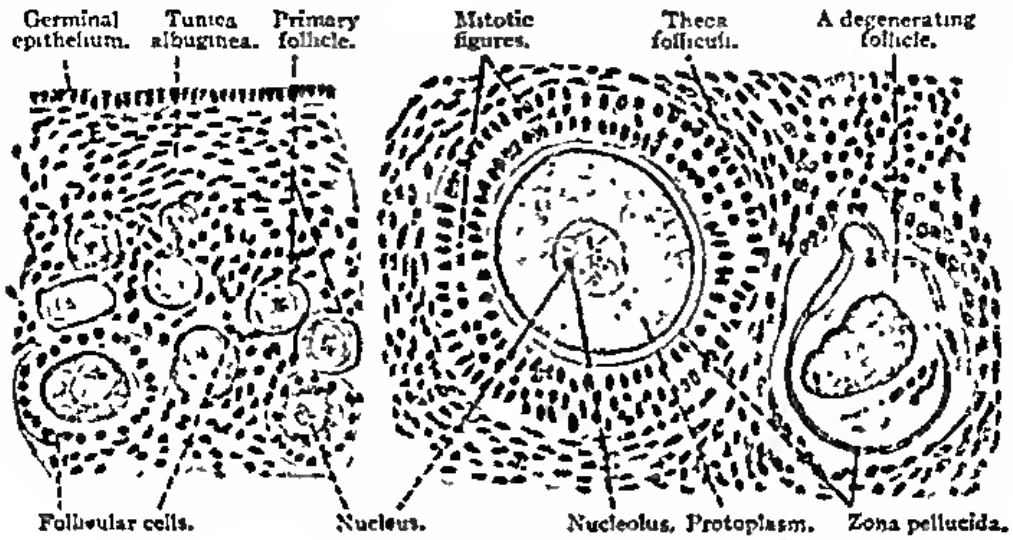


FIG 463.—FROM A SECTION OF A RABBIT'S OVARY × 240

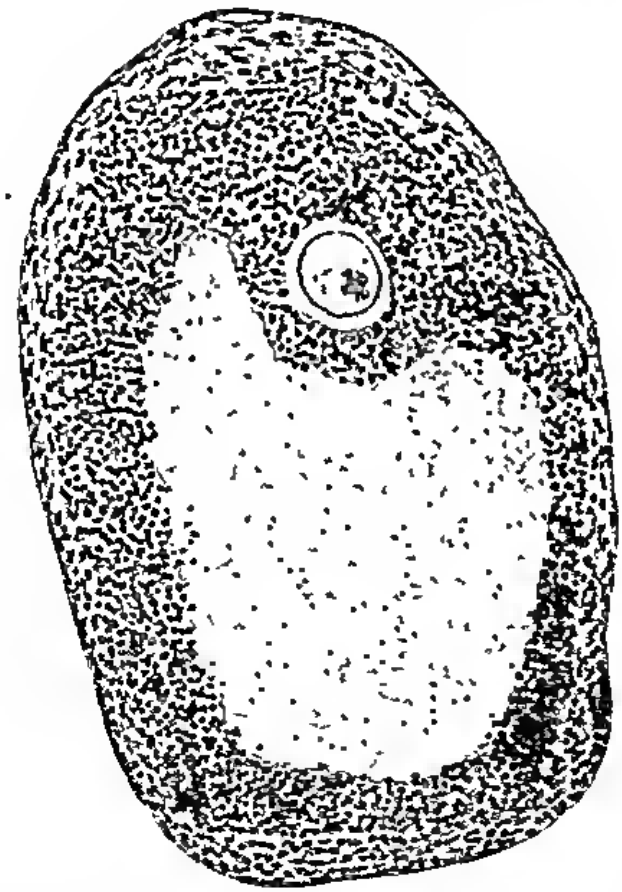


FIG 454.—GROWTH PHASE OF HUMAN PRIMARY OOCYTE, SHOWING STRUCTURE OF FOLLICLE, DISCUS PROLIGERUS AND ZONA PELLUCIDA × 125 (From Hoadley and Simons, 1928)

tion they increase greatly in size, finally becoming about 0.3 mm. in diameter. These egg cells have already been described in detail (p. 36). They are conspicuous in sections as large, round, deeply staining cells,

with round or oval vesicular nuclei, each containing a prominent nucleolus. The cells become so large that frequently they are cut into several sections, and portions of protoplasm without nuclei are to be expected. The larger oöcytes are surrounded by the clear, radially striated *zona pellucida* (Figs. 463 and 464); their cytoplasm may contain the vitelline bodies previously described. The final stages of maturation, accompanied by the formation of polar bodies, doubtlessly takes place within the zona pellucida in man as in other mammals. Mitotic division has been reported in a few instances.[1]

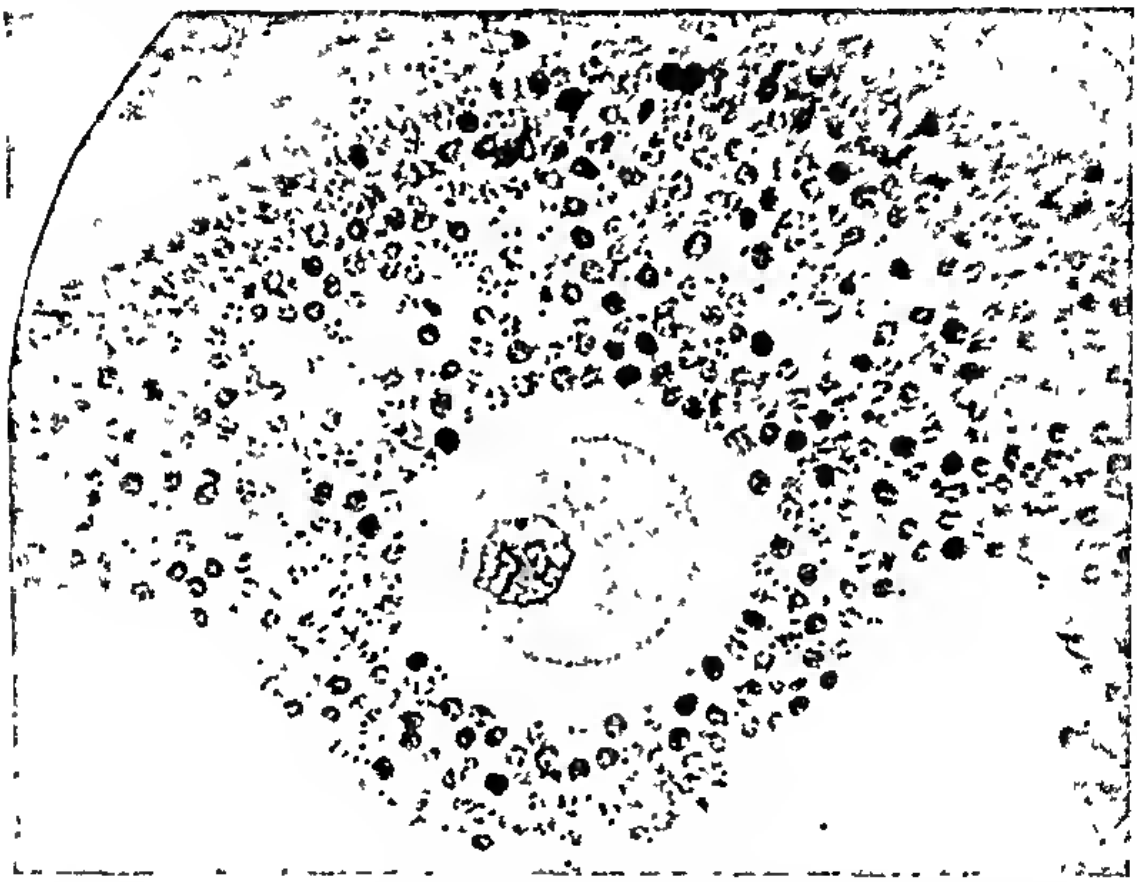


Fig. 465.—Human Oöcyte in Growth Phase with Enveloping Discus Proligerus and Zona Pellucida. (From Hoadley and Simons, 1928.)

The follicles are composed of oöcytes and their surrounding cells. After the groups of egg cells and indifferent cells become subdivided, each oöcyte is typically surrounded by a single layer of flat follicular cells, and this *primary follicle* lies isolated in the stroma of the cortex, beneath the tunica albuginea (Fig. 463). As the follicle enlarges, the follicular cells become more numerous, and thus crowd each other into cuboidal and columnar shapes, and later form a stratified layer. A crescentic cleft filled with fluid appears in the midst of the stratified epithelium on one side of the follicle, and by the accumulation of fluid, or *liquor folliculi*, this cleft becomes a spherical cavity. The fluid is regarded by some as a

[1] Hoadley and Sibions, 1928.

transudate from the blood vessels, which are abundant in the stroma outside of the follicle. Others consider that it is actively secreted by the cells of the follicle, certain of which undergo liquefaction. Occasionally other tiny spaces, surrounded by a row of radially disposed epithelial cells (Call-Exner bodies), occur in the stratified layer; they contain a stainable fluid differing from that in the main cavity. As the liquor in the main cavity increases, the follicle enlarges, and the stratified epithelium becomes a progressively thinner layer, the *stratum granulosum*. The oöcyte is on one side of the follicle, and is contained in a heap of cells known as the *cumulus oophorus* (formerly called the discus proligerus). This is connected with the wall of the follicle, but in certain sections it may appear completely detached.

As the follicle increases in size, it moulds the surrounding connective tissue stroma into a sheath, the *theca folliculi*. This later becomes differentiated into a vascular *tunica interna*, and a fibrous *tunica externa*. The tunica interna contains many cells with abundant protoplasm. It is separated from the epithelium of the follicle by a delicate membrana propria.

In distinction from the solid *primary follicles*, those with cavities are known as *vesicular follicles* (Graafian follicles). They increase in diameter from 0.5 to 12.0 mm., and are then ready to discharge the contained oöcyte. Occasionally a single follicle has two oöcytes, and rarely more. Kennedy[1] reports several cases of polyovular follicles in man, and thinks they are not extremely rare. As many as eleven oöcytes have been counted in one follicle. It cannot be stated whether the additional oöcytes develop by division of the oögonium within a primary follicle, or by the failure of a group of primitive sexual cells to become separated from one another.

Ovulation and the Corpus Luteum. Around the mature vesicular follicle, the tunica interna becomes very thick and cellular, forming elevations toward the stratum granulosum. At this stage the follicle is large, being about half an inch in diameter, and one surface of it is so close to the peritoneal epithelium as to cause it to bulge and then to rupture. Through the opening thus made the liquor folliculi escapes, together with the oöcyte. The latter is said to become detached by the formation of fluid-filled spaces between the cells of the cumulus; it generally carries with it more or less of the innermost layer of the cumulus, and these cells, because of their radial arrangement, are termed the *corona radiata*. As the oöcyte leaves the follicle, there is apparently a chance for it to become lost in the abdominal cavity, but the fimbriated orifice of the tube is near at hand, and the stroke of its cilia produces a current towards

[1] KENNEDY, 1924.

its entrance. In a Guinea-pig, Hensen observed that the fimbriæ were in very active motion, sweeping here and there over the surface of the ovary so powerfully that the effect of ciliary action must have been trivial. The ova, surrounded by the mucoid cells of the follicles, adhered more closely to the fimbriæ than to the smooth surface of the ovary. Except toward the time of ovulation, Hensen found that the fimbriæ

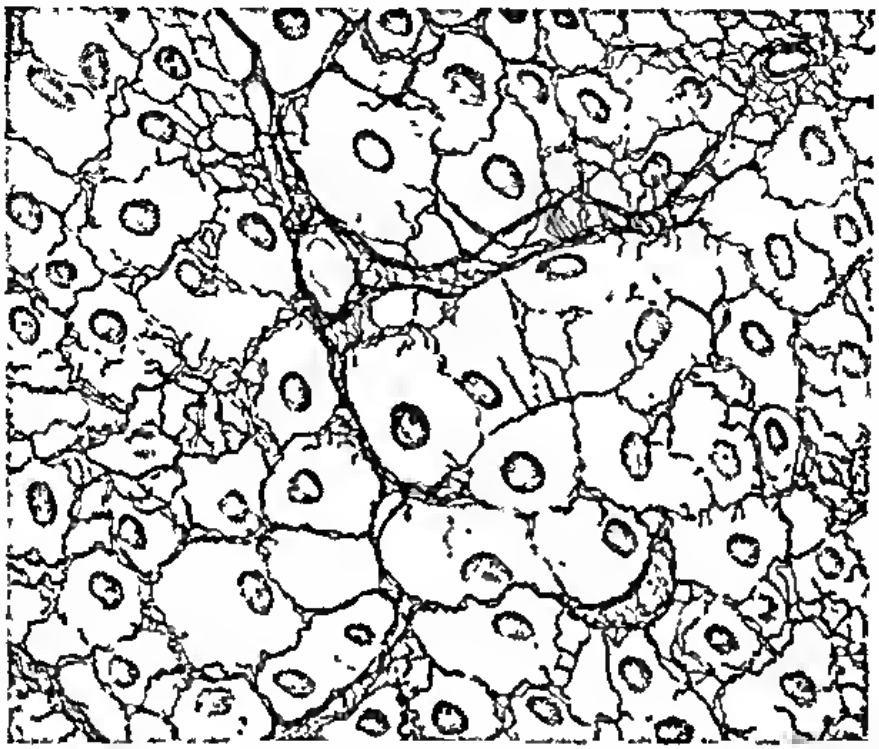


FIG. 466.—ARGYROPHIL FIBERS SURROUNDING THE INDIVIDUAL CELLS OF THE CORPUS LUTEUM. PAP'S METHOD (Bachmann).

were relatively inactive.[1] The discharge of the ovum from the follicle is known as *ovulation*.

Thomson[2] finds that ovulation is not the result of the increased amount of the liquor folliculi secreted by the stratum granulosum. The cells of this layer, after secreting a small amount of this fluid, may remain inactive for long periods. The Call-Exner bodies are areas of their degenerative liquefaction. The impulse causing distention of the follicle comes from without as an increased blood supply in the theca interna. Tissue fluids are forced into the follicle, often lifting the passive follicle cells from the theca and thus loosening the ovum. Muscle fibers, which he recognizes in the theca externa, also play a part. He suggests that the action may result either from the general turgescence of the genital organs in menstruation, or from a more sudden increase of blood during sexual excitement.

It may be noted that in approaching the peritoneal epithelium, through which the rupture occurs, the follicle must push aside or distend the connective tissue of the tunica albuginea. This is ordinarily a rather weak layer, but it has been suggested that in some cases it is more highly developed and acts as an obstruction to ovulation.

[1] HENSEN, 1875. [2] THOMSON, 1919.

After ovulation, blood escapes from the capillaries of the tunica interna and forms a clot within the empty follicle. This may be preceded by a flow of clear serous fluid. The clot is sometimes called the *corpus hæmorrhagicum*. On all sides it is surrounded by the cells of the stratum granulosum, which enlarge and become filled with droplets of a yellow fatty pigment and are hence known as *lutein cells*. They are large cells, often without definite cell walls, arranged in irregular masses. They form a yellow convoluted zone which may easily be seen without magnification; the entire structure is then known as the *corpus luteum*, *i.e.*, yellow body. Vascular strands of connective tissue from the theca extend between the lutein cells and enter the central clot, and some of these cells of the theca interna may change to lutein cells, and soon become indistinguishable from the others.[1] The extravasated blood breaks down into granules and hæmatoidin crystals, and is gradually absorbed. It is replaced by gelatinous connective tissue which finally contracts into a dense white fibrous nodule, and this scar is known as the *corpus albicans*, *i.e.*, white body. Meanwhile the lutein cells undergo hyaline degeneration and become resorbed. The surface of the ovary, which is smooth in childhood, becomes pitted and irregular with the increasing formation of these corpora albicantia.

Provided that pregnancy does not take place, the corpus luteum reaches its maximum development in about two weeks after ovulation, and it becomes reduced to a scar in about two months. If pregnancy occurs, it enlarges further and persists at the height of its development until the fifth or sixth month. Its diameter is then 1.5–3.0 cm., and at the end of pregnancy it is still quite large and yellow. If the corpus luteum is removed, the ovum fails to become attached to the wall of the uterus. There is both experimental and histological evidence that it produces an internal secretion which is probably received by the blood vessels invading it from the theca. In order to distinguish between the corpus luteum of pregnancy and that of unproductive ovulation, the former is called the *true* corpus luteum; the latter is the *corpus luteum spurium*.

Many follicles degenerate at various stages in their evolution without discharging their ova. This *atresia* which may happen at any period of the follicle's growth has been studied by Allen and his associates.[2] In the primary follicles it is accompanied by the invasion of phagocytes through the layer of follicle cells, while in the vesicular follicles the death of the ovum may cause an increase in the liquor folliculi with the formation of a cyst and the disintegration of the cells of the stratum granulosum. The zona pellucida, which surrounds the oöcyte, may become conspicuously folded and persist for some time. The basement membrane

[1] Corner, *1919*. [2] Allen, Pratt, Newell and Bland, *1930*.

of the stratum granulosum may also thicken and become convoluted. These degenerating or *atretic follicles* are finally reduced to inconspicuous scars. After the menopause the degeneration of the oöcytes becomes general.

Within the stroma of the cortex, *interstitial cells* are found, which resemble lutein cells but are smaller. They have been compared with the interstitial cells of the testis, and are said to contain secretory granules. Kingsbury[1] states that they are modified stroma or theca cells, and hence of connective tissue origin. The granules are of a lipoid nature, and repre-

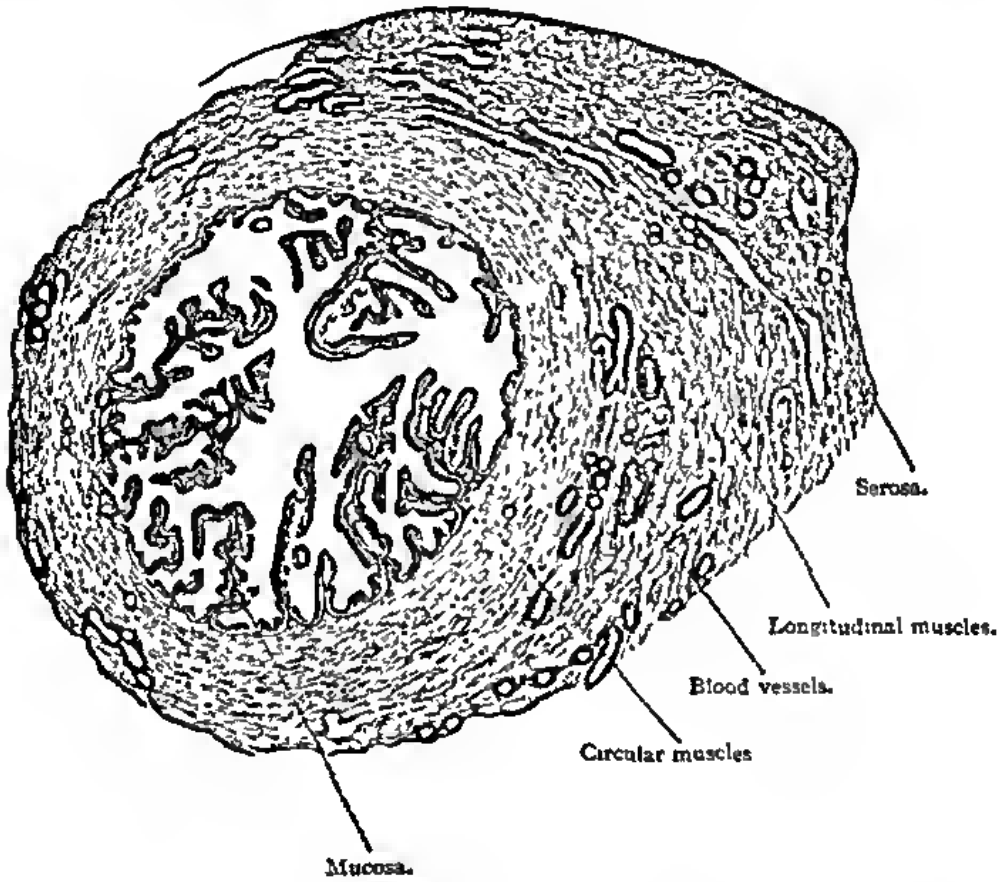


FIG. 467.—CROSS SECTION, NEAR THE AMPULLA, OF A UTERINE TUBE FROM AN ADULT WOMAN.

sent degenerative conditions, as in atretic follicles. He finds insufficient evidence for regarding them as a gland of internal secretion, and questions whether the interstitial cells of the testis have that function. Corner[2] thinks they do not exist in the adult human ovary, though the theca cells of degenerating follicles may be mistaken for them. Others have located hormones in the liquor folliculi which have profound effects on the uterus, vagina, and mammary glands, and induce all the characteristics of the œstrous cycle.[3] (See p. 535.)

UTERINE TUBES

Each uterine tube is about 5 inches long and extends from its orifice in the abdominal cavity to its outlet in the uterus. It is divided into the

[1] KINGSBURY, 1914. [2] CORNER, 1932. [3] ALLEN AND OTHERS, 1925.

fimbriated funnel or *infundibulum;* the *ampulla* or distensible outer two-thirds, the lumen of which is about a quarter of an inch in diameter; the *isthmus* or narrow inner third, not sharply separated from the ampulla; and the *uterine portion* which extends through the musculature of the uterus to the uterine orifice. The wall of the tube is composed of three layers, a mucosa, muscularis, and serosa (in addition to which a tela

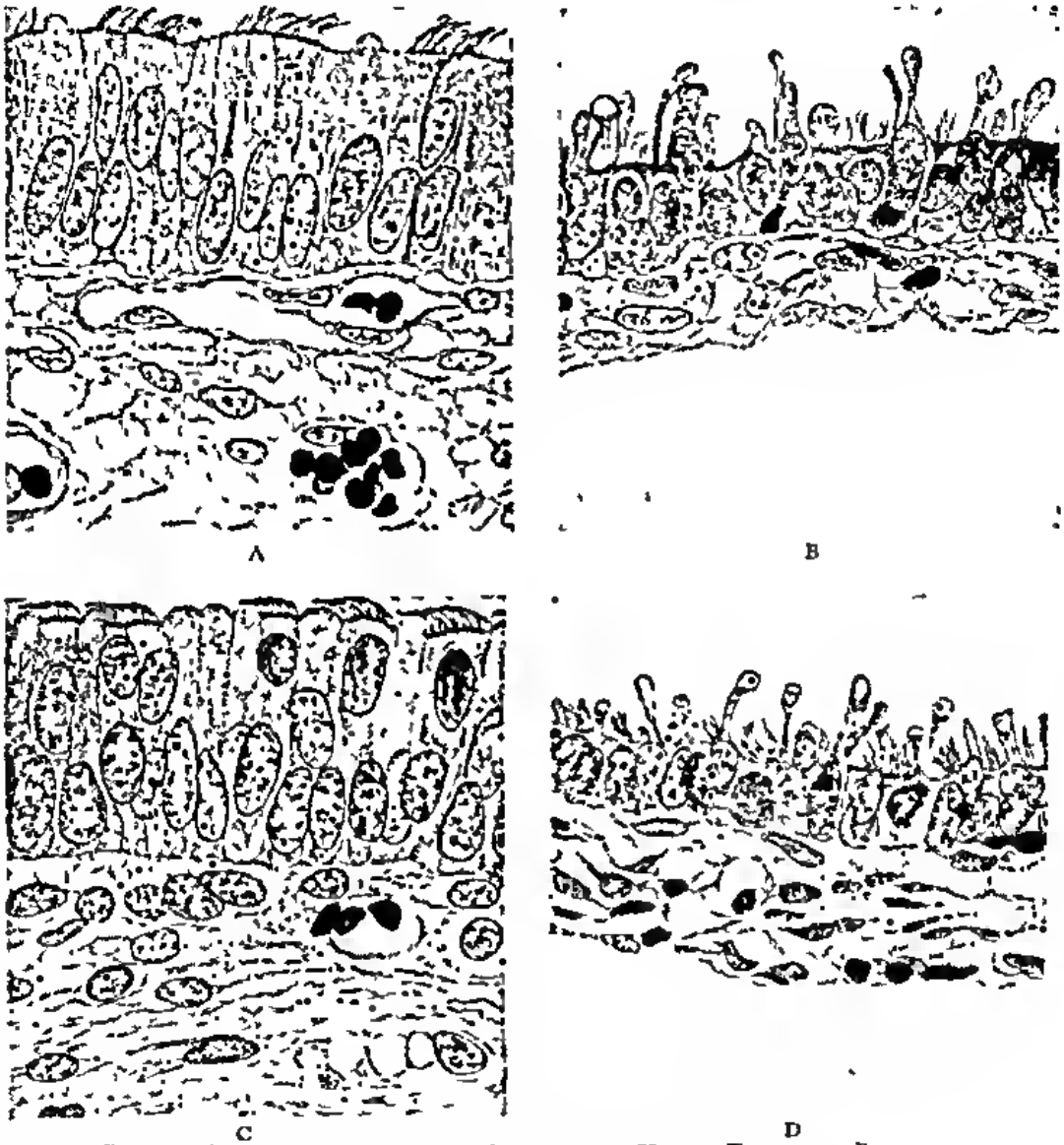


FIG. 468.—SECTIONS OF EPITHELIUM AND STROMA OF THE UTERINE TUBE OF THE PIG.

A, during œstrus (ova found in tubes), B, 12th day after ovulation, C, during the latter part of the third week following ovulation, D, during the third week of pregnancy. Embryos of 23 somites (17th–18th day found in the uterus) × 700 (Snyder)

submucosa is enumerated in the Basle nomenclature). The mucosa is thrown into thin longitudinal folds, which are low in the isthmus, but tall and branched in the ampulla. Occasionally the branches anastomose, enclosing a pocket, but glands are absent. The epithelium is chiefly simple columnar and ciliated, the stroke of the cilia being toward the uterus; but there are areas of non-ciliated cells which are said to produce a mucoid fluid. The two types of cells are connected by intermediate

forms. During the menstrual cycle and in pregnancy the cells undergo definite regular changes, becoming lower in the premenstrual period and taller or even pseudostratified at mid interval.[1] Areas of flat cells may occur at the periphery of the lumen. Mucous cells are absent.

The folds of the mucous membrane are occasionally indented or overhanging, so that in transverse sections detached fragments may appear, suggestive of villi (Fig. 467); but the fact that almost all of the many projections connect with the submucous layers indicates that they are elongated folds. Each of them contains a thin layer of cellular connective tissue, in which there are small arteries and veins running chiefly lengthwise of the tube. Lymphocytes occur in the meshes of the tissue and lymphatic vessels have been reported. Occasionally strands of smooth

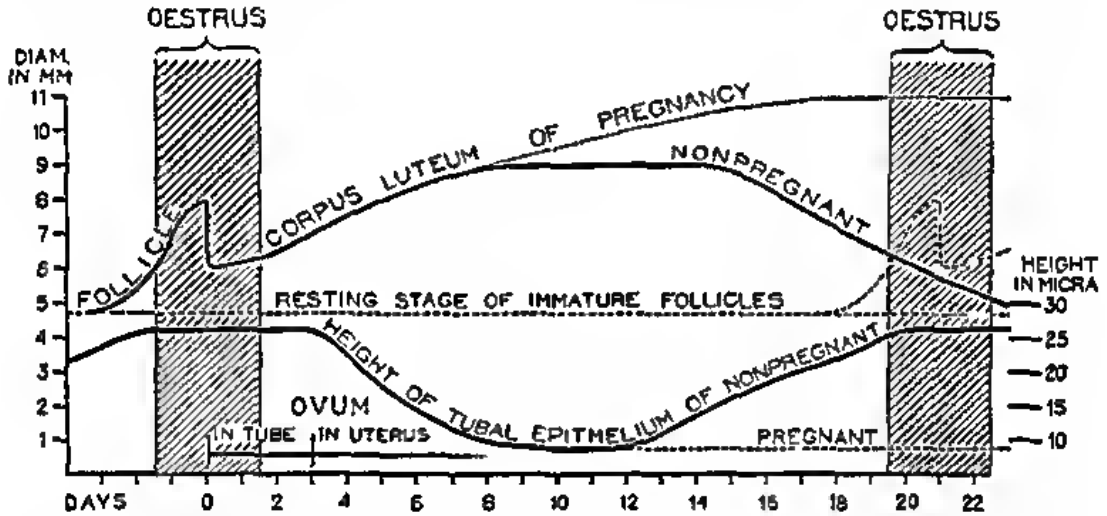


Fig. 469.—Curve Showing Height of Epithelium of the Uterine Tube of the Pig at Successive Stages of the Ovulation Cycle and Early Pregnancy, in Comparison with Corner's Diagram of the Ovarian Cycle. (Snyder.)

muscle fibers are found within the folds. The mucous membrane rests directly upon the tunica muscularis which consists of a thick inner circular layer and a thin outer longitudinal layer of smooth muscle fibers, but both layers are resolved into coarse bundles by the abundance of intermuscular connective tissue. The muscle fibers (in the sow) are said to vary in size during different periods of the œstrous cycle.

Since the uterine tubes are embedded in the broad ligaments, they are not closely invested by the peritoneum. There is a considerable layer (serosa) of loose connective tissue outside of the muscularis, and toward the ovary this tissue may include sections of the tubules of the epoöphoron. It contains the branches of the ovarian and uterine blood vessels which supply the tube. These are accompanied by lymphatic vessels and nerves. The latter innervate the tubal musculature and the mucous membrane.

Although the tubes are probably normally closed, the uninterrupted passage from vagina to abdominal cavity should be remembered as an opportunity for infection.

[1] Snyder, 1923 and 1924.

The cilia are presumably active only after ovulation, and sweep the ovum toward the uterus, creating at the same time a current against which the spermatozoa tend to force their way. Active peristalsis of the tube at this time has been reported in animals.[1] *Grynfeltt*[2] *finds true erectile tissue in the fimbria which could aid the muscles in their* activity. Fertilization normally takes place in the tube in other animals and doubtless in man also.

Cases of extrauterine pregnancy occur in which the ovum is retained in the tube, caught perhaps in the pockets between folds. This is called tubal pregnancy, and progresses until the enlarging embryo ruptures the tube wall (usually in about six weeks), calling for operative interference. Abdominal pregnancy may be the result of the failure of an ovum to enter the tube, or of the extrusion of the developing embryo from the tube, either by the fimbriated extremity or through an early rupture of the wall which subsequently heals. Placental attachment in such cases has been found on the peritoneum of the abdominal wall or on most of the abdominal organs. Growth may continue, or lime salts may be deposited; but usually everything is absorbed. Ovarian pregnancy, the attempt of the fertilized ovum to develop within the follicle, has been reported by Hunter,[3] and others, but doubted by Kampmeier.[4]

UTERUS

The uterus is a pyriform, muscular organ, flattened dorso-ventrally. It is about two and a half inches long, receiving the uterine tubes at its upper end or fundus, and ending below in the vagina. It is divided into *corpus* (and *fundus*) and *cervix*. The corpus and fundus together have a triangular cavity, which opens into the *canal of the cervix* through the *internal orifice;* the canal communicates with the vagina through the *external orifice* of the uterus. The lining of the cervix presents a feather-like arrangement of folds on its dorsal and ventral surfaces; these are the *plicæ palmatæ*. The walls of the uterus consist of a mucosa, muscularis, and serosa (constituting the endometrium, myometrium, and perimetrium, respectively).

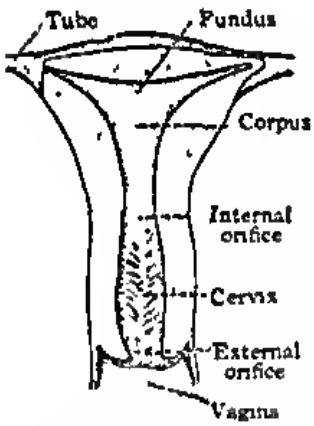


FIG. 470.—THE DORSAL HALF OF A VIRGIN UTERUS (two-thirds natural size) (Rieffel.)

The corpus and fundus are lined with simple columnar epithelium, some areas of which are ciliated. The cilia have been described as difficult to preserve, and their absence from certain cells has been attributed to faulty fixation. According to Gage the uterine cilia are as readily preserved as those which occur elsewhere, and he finds that only one cell among fifteen or twenty is actually ciliated, but this ratio is said to change periodically during the menstrual cycle.[5] Mucous cells are absent. The epithelium forms slender tubular pits, the *uterine glands*, but in the

[1] WISLOCKI AND GUTTMACHER, 1924 [2] GRYNFELTT, 1924. [3] HUNTER, 1921.
[4] KAMPMEIER, 1929. [5] LUCAS, 1932.

resting human uterus these produce no definite secretion. They are branched tortuous tubes extending through the broad mucosa (which is 1 mm. thick), and invading to a slight extent the muscular tissue beneath. They have been carefully modelled by Baumgartner.[1] Occasionally they anastomose with one another, and in their deeper portion they have long horizontal branches, at right angles with the main tube. Sometimes a small group of glands opens into a single depression of the surface epithelium. In older persons the glands degenerate, losing their connections with the surface and becoming cystic. Each gland is surrounded

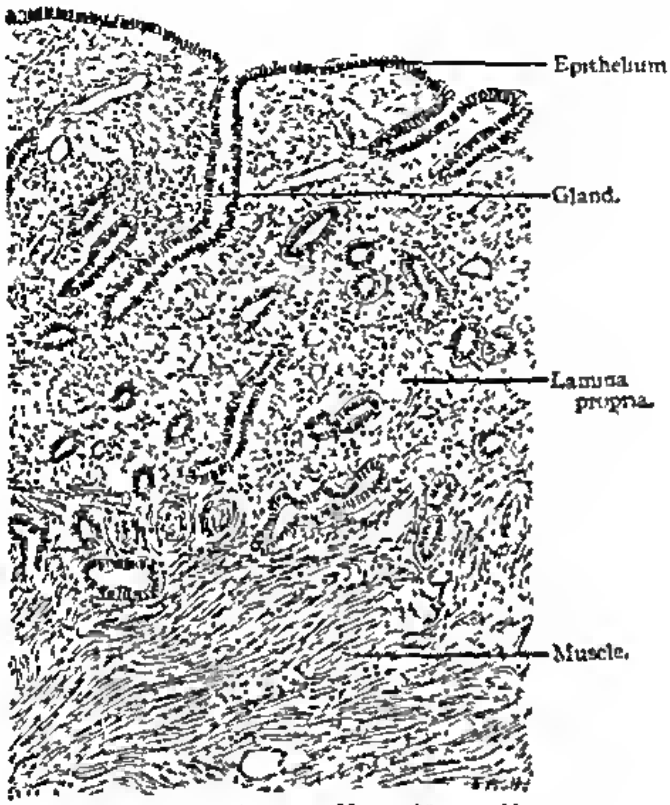


FIG. 471.—MUCOSA OF HUMAN UTERUS, VIRGIN

by a delicate basement membrane, and between them there is an abundant lamina propria of reticular tissue, containing many nuclei, and with numerous lymphocytes. In the reticular tissue are blood vessels forming a rich capillary network around the glands and especially beneath the free surface. Lymphatic vessels form a wide-meshed plexus with blind extensions. The whole mucosa forms the *endometrium*.

The upper and larger part of the cervix of the uterus is likewise lined with simple columnar, occasionally ciliated, epithelium, which forms tubular *cervical glands* more richly branched than those of the corpus. The cells are taller than those of the body of the uterus (60 μ as compared with 20 μ). Slender mucous goblet cells are occasionally found, and

[1] BAUMGARTNER AND OTHERS, 1920.

their number increases during pregnancy, when the glands discharge a secretion which occludes the canal of the cervix. Often the glands produce macroscopic retention cysts, named 'ovules of Naboth.' Toward the external orifice of the uterus the epithelium becomes stratified and squamous, and rests on connective tissue papillæ. Thus it resembles the

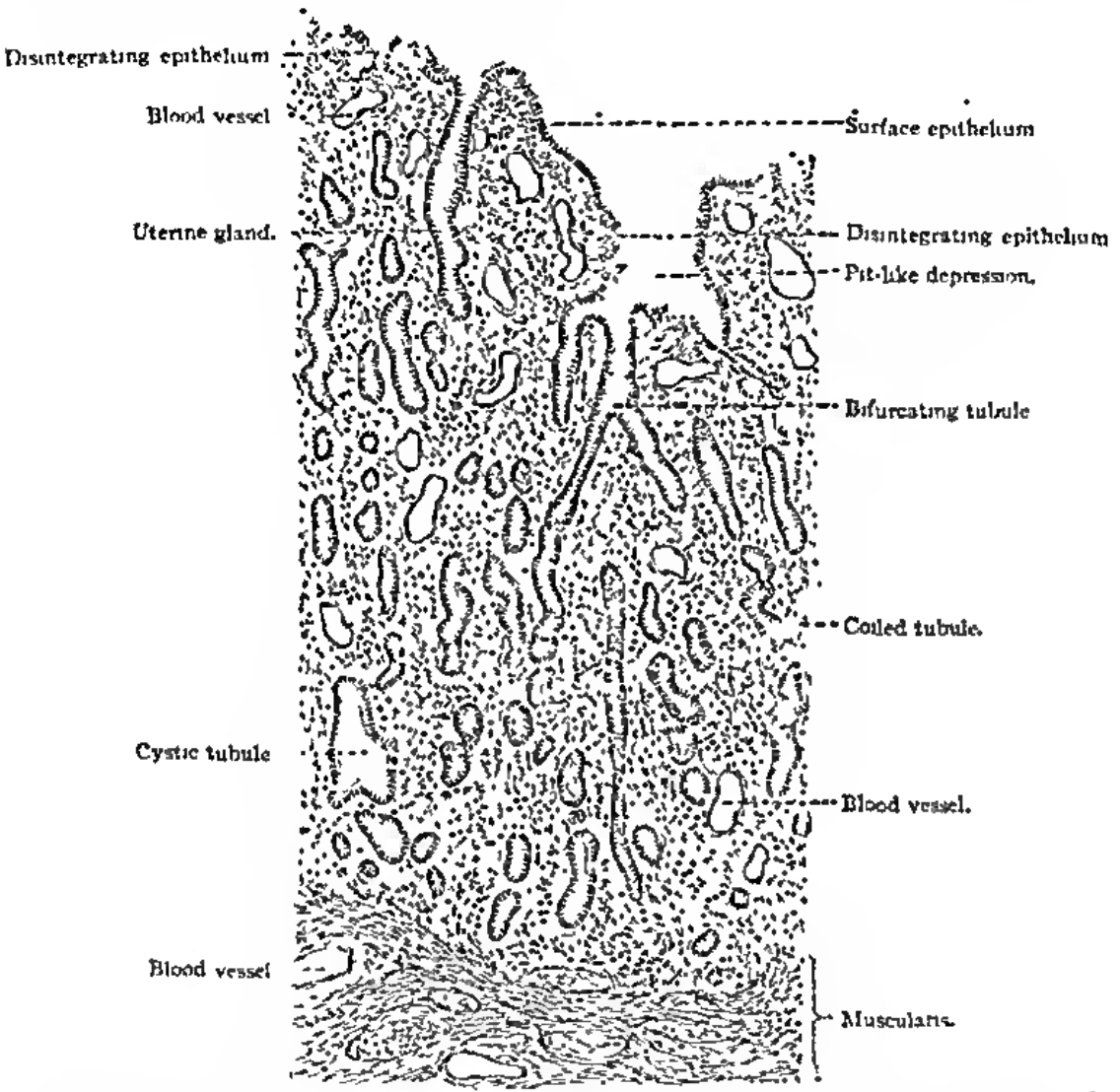


FIG. 472.—MUCOUS MEMBRANE OF A VIRGIN UTERUS DURING THE FIRST DAY OF DESQUAMATION. × 30. (*Schaper.*)

lining of the vagina of which it is a continuation, and after the first childbirth the stratified epithelium extends further up into the cervix than before.

The musculature of the corpus is a thick investment of interwoven bundles of smooth muscle fibers, which cannot be subdivided into well-defined layers. Attempts have been made to divide the myometrium into several layers—up to four—but such divisions seem artificial and confusing. It begins immediately outside the lamina propria, but the border between the two is very irregular, so that the bottoms of some of the

glands lie deep in the clefts between the muscle bundles. Branched and interconnecting muscle cells are also said to be present, so that the musculature somewhat resembles that of the heart, except that cross striations and intercalated discs are absent. Between the muscle bundles lies considerable connective tissue. In the middle region numerous blood vessels are present, and this region is known as the 'stratum vasculare'; here the fibers are chiefly circularly disposed. The muscles external to this form the 'stratum supravasculare,' and the fibers are chiefly longitudinal; both cells and nuclei are larger in this layer. They continue the longitudinal layer of the uterine tube; some of them are said to enter the round ligaments, which contain also some striated fibers; and others spread into the broad ligaments.

In the cervix the three strata of muscle fibers are found to be very distinct—inner and outer longitudinal, and middle circular. Although the uterus generally contains few elastic fibers, found only in its peripheral layers and running perpendicular to the plane of contraction of the muscles, elastic fibers are abundant in this position in the lower segment of the corpus and vaginal portion of the uterus.

During the first half of pregnancy both elastic and muscular fibers increase in size and number, new muscle cells being added by a transformation of connective tissue elements, according to Stieve.[1] Many of these new cells remain after childbirth, so that the uterus never regains its virgin size In the second half of pregnancy, the elastic fibers decrease in the musculature, but increase in the perimetrium (Stöhr) The way in which the thick layer of muscles in the resting uterus becomes arranged in the thin layer of late pregnancy is an unsolved problem, similar to that presented by the musculature of the bladder and intestine during distention.

The serosa covering the dorsal and ventral surfaces of the uterus is in part a well-defined layer, the perimetrium, but it blends with the connective tissue of the broad ligaments laterally, and here, from its position beside the uterus, is known as the 'parametrium.' Embedded in the parametrium and main trunks of the uterine vessels run along the lateral margins of cervix and corpus, both artery and vein showing many kinks and convolutions. The vessels are thus apparently adapted to the future expansion of the uterus, but when it retracts after pregnancy they are said to show more pronounced bendings, as if they had been permanently elongated. The parametrium contains also numerous lymphatic vessels, together with the ganglionated sympathetic *utero-vaginal plexus.* Nerves from this plexus and from the third and fourth sacral nerves supply the uterus.

[1] STIEVE, 1929.

COMMON ORIGIN AND DIVERSE PRODUCTS OF THE MALE AND FEMALE PARTS*

Origin	Male product	Female product
Genital ridge	Testis	Ovary
(its lower part)	Gubernaculum testis	(Chorda uteroinguinalis)
Wolffian body	Epididymis	Epoöphoron
Certain Wolffian tubules	Ductuli efferentes	Ductuli epoöphori transversi
Other Wolffian tubules	Ductuli aberrantes	
Other Wolffian tubules	Paradidymis	Paroöphoron
Wolffian duct	Ductus epididymidis Ductus deferens Ductus ejaculatorius	Ductus epoöphori longitudinalis Ductus paraurethrales of Gartner
Cystic end of duct (stalked hydatids)	Appendix epididymidis	Appendix vesiculosa
Müllerian ducts	Appendix testis Utriculus prostaticus	Uterine tubes Uterus Vagina
Urogenital sinus	Urethra distal to the ejaculatory ducts	Vestibulum vaginæ
	Gl prostaticæ	Gl. urethrales
		Gl. paraurethrales of Skene
	Gl. bulbourethralis of Cowper	Gl. vestibularis major of Bartholin
	Gl. urethrales of Littré	Gl. vestibulares minores
	Lacunæ urethrales	
Genital papilla	Penis	Clitoris
Phallic folds	Scrotum	Labia majora

* Arranged by Dr F T Lewis

Menstruation. Menstruation is the periodic degeneration and removal of the superficial part of the mucosa of the corpus and fundus, accompanied by hæmorrhage from the vessels of the lamina propria, and its subsequent growth and regeneration, and occurs in sympathetic rhythm with the ripening and discharge of the ova and the growth of the corpora lutea. The process has been very carefully described by Bartelmez.[1] Three successive stages may be distinguished, namely (1) the stage of *congestion*, lasting four to five days; (2) the stage of *desquamation* and *hæmorrhage*, four days; and (3) the stage of *regeneration* and *repair*, seven days. Thus the entire process requires about sixteen days, and after an interval of twelve days the cycle begins anew. There is con-

[1] Bartelmez, 1933.

siderably variation in these periods, however, both among different women and in subsequent cycles in individuals.[1]

For four or five days before the discharge occurs, the thickness of the mucosa increases greatly, partly because of the congestion of the vessels, partly from an increase in the intercellular fluid (œdema). The reticular cells are thus separated from each other, especially in the upper layers. The glands become wider, longer, and more tortuous, opening between irregular swellings of the superficial epithelium. Their changes during both menstruation and early pregnancy have been studied by O'Leary[2] who describes the formation of new branches called by him 'basal buds,'

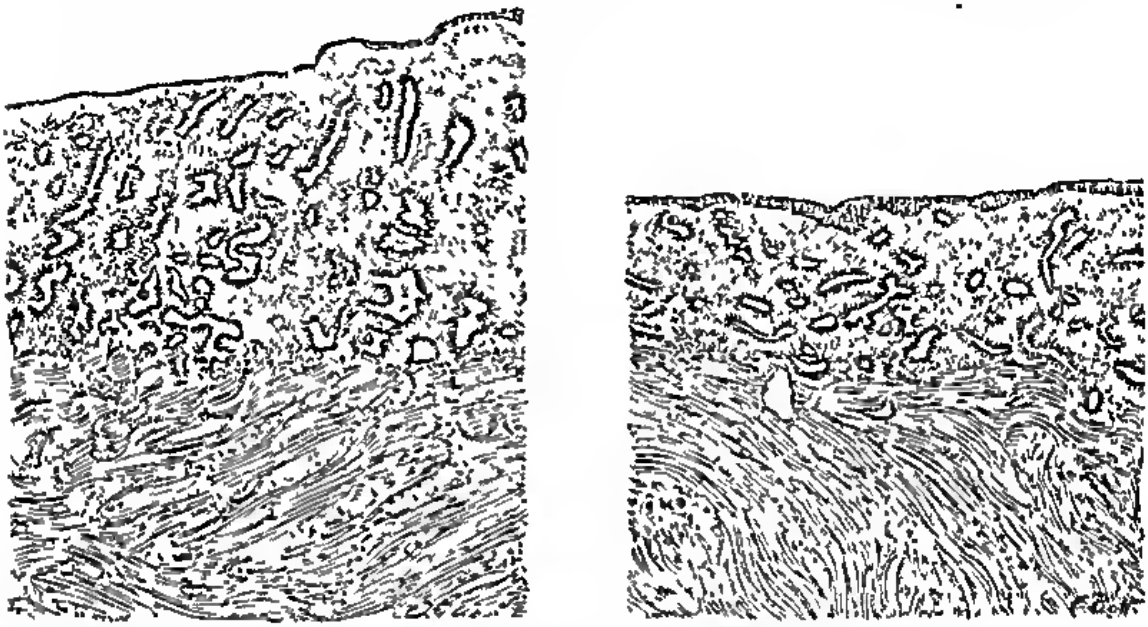


FIG 473.—HUMAN ENDOMETRIUM.
A, third day, B, seventh day of the menstrual cycle Formaldehyde fixation, hæmatoxylin and eosin

which extend from the lower ends of the glands toward the surface. The gland cells apparently secrete, and mitotic figures are to be seen in some of them, as well as in the stroma cells. Red corpuscles pass out between the endothelial cells of the distended veins and capillaries, and form subepithelial masses, which however do not form clots, because of the presence of reticular tissue. This stage of congestion and tumefaction is followed by one of hæmorrhage and desquamation. The epithelium of the surface and outermost parts of the glands becomes reduced to granular débris, or it may be detached in shreds. The underlying vessels rupture and add to the blood which had escaped by diapedesis. A denuded surface remains, on to which open the necks of the glands. In the stage of regeneration the glands supply the necessary epithelium. By mitotic division within, the glands cells are forced out of the necks

[1] KING, J. L., 1926. [2] O'LEARY, 1929.

and spread over the surface, at first as a layer of thin squamous cells, later becoming cuboidal and then columnar as the normal number is attained. The lamina propria is renewed, and the whole mucosa returns to its resting condition. Occasionally a gland neck may be blocked in some manner and be covered in by reticular tissue, thus leading to

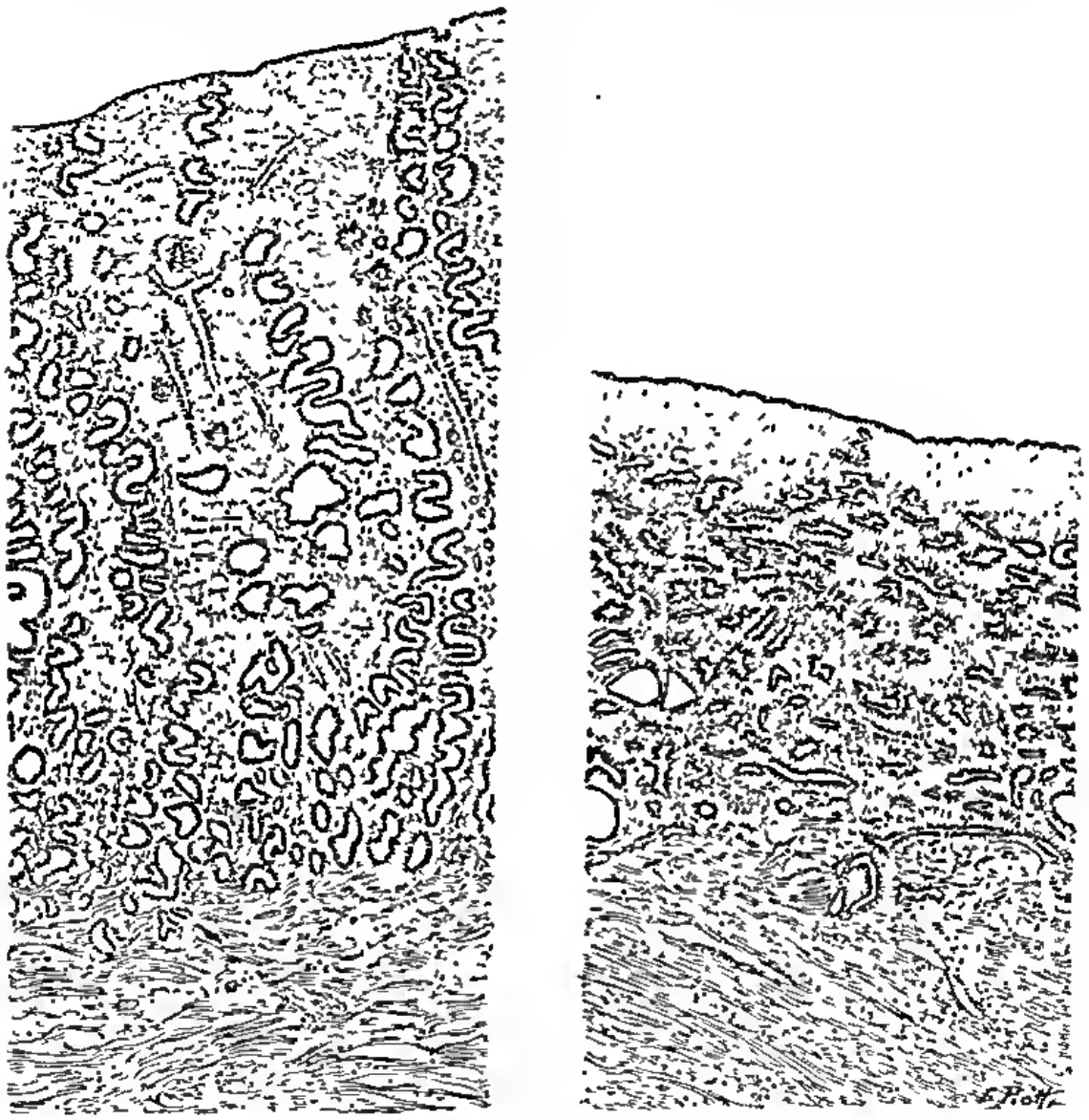


FIG. 474.—HUMAN ENDOMETRIUM

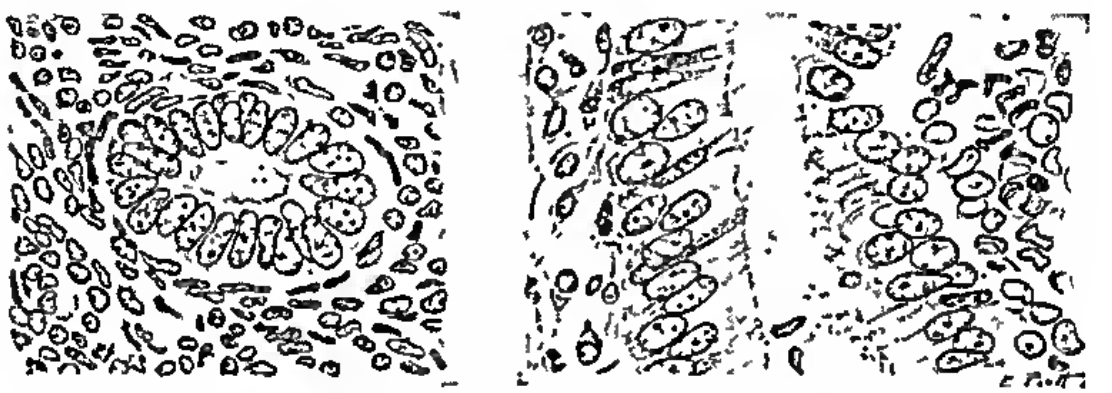
A, eighteenth day, B, twenty-fifth day of the menstrual cycle. Formaldehyde fixation, hæmatoxylin and eosin.

possible cysts. The cervix takes no part in menstruation except that the secretion of its glands may increase during the stage of congestion. The different periods of the menstrual cycle are reflected in definite changes in the vaginal epithelium and vaginal content. (See numerous papers by Stockard[1] and others.)

In a section, the presence of degenerating or desquamating surface epithelium is not in itself sufficient to denote menstruation. Such appear-

ances may be due to post-mortem changes, as is so frequently seen in sections of the intestinal tract, or to faulty preservation. The enlarged blood vessels in the upper portion of the lamina propria, the masses of escaped blood corpuscles, the loose texture of the reticular tissue, and the swollen epithelial cells in the necks of the glands are much surer guides to the condition.

Beginning at puberty (13–15 years) menstruation takes place normally once in 28 days for 33 years, more or less During pregnancy it is interrupted, although the time when it should occur may be indicated by slight uterine contractions and finally by those which cause the delivery of the child. Thus the duration of pregnancy is described as ten menstrual cycles. The immediate cause of menstruation seems to be the production of hormones in the ovary.



A B

FIG 475.—UTERINE GLANDS, RESTING (TRANSVERSE SECTION), AND MENSTRUATING (LONGITUDINAL SECTION), SHOWING SECRETORY ACTIVITY

The hormones of the ovary have been the object of intense study within the last few years Apparently two main hormones have been recognized: the first, called theelin (from θῆλυς, thelus = female) or folliculin or estrin (because it produces œstrus in animals), is derived from the follicles, either liquor or granulosa cells; the second, called corporin or lutein or progestin, comes from the corpora lutea At present it is believed that they are under the control of the anterior lobe of the pituitary gland. Briefly the pubertal growth of the female sex organs and the cyclic changes occurring with menstruation may be outlined as follows.

The sex hormone A of the pituitary gland activates the ovary to produce follicles which secrete theelin, and the theelin causes the changes in the accessory sex organs and stimulates rapid growth of the endometrium. The luteinizing hormone B of the anterior lobe causes rupture of the follicles and the formation of the corpora lutea. These latter in turn secrete more theelin and also corporin, which cause progestational changes in the endometrium. The excess of theelin in the blood is thought to inhibit the production of the pituitary hormones, the system being thus self-curbing. The loss of new theelin results in the retrogression of the corpora lutea and the hæmorrhagic disintegration of the endometrium (its growth stimulus having been removed) which is called menstruation. With the elimination of the excess theelin the cycle is repeated.

If pregnancy intervenes, the fetal chorion is thought to develop hormones, similar to those of the anterior lobe of the hypophysis, called prolan and separable also into A

[1] STOCKARD, 1923.

and B with comparable functions. Prolan is apparently not inhibited by the excess of theelin, so that the ovary continues to be stimulated to form theelin and progestin during pregnancy, and the changes in the breast leading to lactation are due to their presence. Prolan is abundantly found also in the blood and urine of pregnant women, and its action on the ovaries of test animals is now one of the most reliable tests of pregnancy.

The ovarian hormone A, theelin, can apparently be recovered in small quantities from *some of the male tissues, from certain plants, and from some plant products. Chemically* it shows relationship with the common vitamin D, and also to certain of the tar derivatives which produce experimental cancer.[1] Growth-stimulation seems to be the common attribute, but definite understanding of the process is still lacking.

Corner's[2] observations on monkeys indicate that 'menstruation frequently occurs without ovulation, and is therefore not dependent on the presence of a corpus luteum. However, when ovulation occurs, it seems to take place at a definite time, about twelve or fourteen days before the onset of menstruation. Menstruation without ovulation is not preceded by the so-called premenstrual changes of the endometrium, which occur only after the formation of the corpus luteum. The cause and meaning of menstruation, in this species, are not at present known.' It is generally accepted that human menstruation may take place without ovulation, and that ovulation may occur between menstrual periods, and also during pregnancy. It may even occur in children before menstruation has begun. Nevertheless, ovulation probably occurs usually and normally at the close of menstruation. Coitus is not considered to be a factor in inducing ovulation, but it is said that in the rabbit and ferret, and in pigeons, ovulation may fail to occur in the absence of the male. It should be remembered, however, that the œstrous cycle of most mammals is not strictly comparable[3] with menstruation, to which only man and the higher monkeys are subject.

The following considerations are also important in establishing the age of young embryos. The time required for spermatozoa to travel to the upper end of the tube, where fertilization takes place, is probably about twenty-four hours. There they may fertilize the ovum at once if ovulation has just occurred. They retain their vitality and are capable of fertilizing the ovum during a period of ten days in the rabbit, but it is doubtful if human spermatozoa can live more than two or three days in the uterus.

The Discovery of Mammalian Ova. During the seventeenth century the ovary was called the *testis muliebris*, or *testis femineus*. It was believed to produce the mucoid secretion which escapes from the genital orifice, and this was regarded as seminal fluid. The uterine tubes were accordingly the *vasa deferentia mulierum*, serving to convey this fluid to the uterus, where, through a mixture and interaction of the male and female semina, an embryo was produced. Aristotle had argued to the contrary, but his opinion was summarily disposed of by Bartholin, who discussed the ovaries as follows:[4]

'Their function is to produce semen in their own way, which Aristotle, against all reason and observation, has dared to deny to women, contrary to the express teaching of Hippocrates.'

The ancient doctrine of Aristotle, expounded in his treatise on the generation of animals, was based upon the familiar facts that menstruation marks the beginning, and ceases at the end, of the child-bearing period; and moreover menstruation is interrupted while the embryo is being formed. Therefore he concluded that the menstruum supplies the substance and material for the new body, which arises, like the curd in

[1] Overholser and Allen, 1933. [2] Corner, 1927. [3] Hartman, 1929.
[4] Bartholin, 1666

milk, through the agency of the semen. The semen engenders; the menstruum nourishes. The theory had already been advanced that the semen comes from all parts of the body, and that its particles reproduce the structures from which they are derived. This enticing speculation, revived by Darwin in his theory of pangenesis, had been discussed at length and rejected by Aristotle.

Generation, therefore, was considered to result from the mixing of two fluids, and would have remained a barren physico-chemical problem until recent times, if further morphological observations had not been made. The view of Bartholin had at least the merit of definitely associating the ovary with the reproductive function Vesalius and Fallopius had seen the follicles and corpora lutea; Fallopius described them as 'vesicles filled with water or aqueous humor, some limpid and other yellow.'[1] Many others had observed them, and from their resemblance to the ova of birds they had even been called 'ova,' when in 1672 a young Dutch physician, Regnerus de Graaf, made his thorough study of the female genital tract.

De Graaf concluded that the 'semen mulicbre' is not produced by the 'testes mulicbres,' but that the general function of the latter is 'to produce and nourish ova, and bring them to maturity.' Consequently he proposed to substitute the name *ovary*, and to call the *tubes oviducts*. He declared that the ova escaped from the follicles through minute apertures (in the rabbit admitting a bristle) and made their way through the tubes to the uterus, in which they developed. The abnormal formation of a human embryo within the tube was figured and, to a certain extent, explained. De Graaf studied many mammals, and especially rabbits. He found minute ova in the oviducts and observed the follicles from which they had escaped. In older stages he recorded a general agreement between the number of corpora lutea and embryos. Since, however, he frequently referred to the entire follicles as ova, his results were not promptly accepted; the diameter of the isthmus of the tube is so small that the entrance of the follicles into the uterus was considered impossible. It was a matter of easy observation to determine more precisely the relation of the ova to the follicles After many years this was done by von Baer, an eminent embryologist, whose studies of the chick are regarded as 'the most profound, exhaustive and original contribution to embryology which has ever been made' (Minot). This work bears the famous subtitle '*Beobachtung und Reflexion*'—the German expression of Haller's '*Observations suivies de Reflexion*' and de Graaf's '*Cogitationes atque observationes*.' After describing the condition of the ova in the tubes of the bitch, von Baer writes:

'It remained for me to ascertain the condition of ova in the ovary, for it seemed clearer than day that ova so small as those found in the tubes did not represent Graafian follicles expelled from the ovary; and I did not consider it probable that such solid bodies had been coagulated from the fluid of the vesicles. Now, contemplating the ovaries before making an incision, I clearly distinguished in almost every vesicle, a yellowish-white point unattached to the walls, which swam about freely in the fluid when the vesicle was pressed upon with a probe. Led on by a certain curiosity, rather than moved by hope that with the naked eye I had seen ovules in the ovaries through all the coats of the Graafian follicle, I opened a vesicle, and taking out a point in question on the blade of a knife, I placed it under the microscope. I was overcome with amazement when I saw the ovule, now recognized outside of the tubes, so clearly that a blind man could hardly doubt it. Surely it is strange and unexpected that an object so persistently sought for, and endlessly described as inextricable, in every physiological compendium, could so easily be placed before the eyes.'[2]

[1] FALLOPIUS, 1588. [2] VON BAER, 1828.

Thus the ova in mammalian ovaries, which had long been believed to exist, were first definitely seen within the follicles one hundred and fifty years after the discovery of the microscopic spermatozoa, the existence of which had never been suspected.

PLACENTATION*

The early developmental history of the primate egg is a matter of recent record.[1] In the rhesus monkey development of the ovum has been followed in great detail from the maturation of the egg in the ovary, through stages of fertilization and segmentation in the tubes, to the final

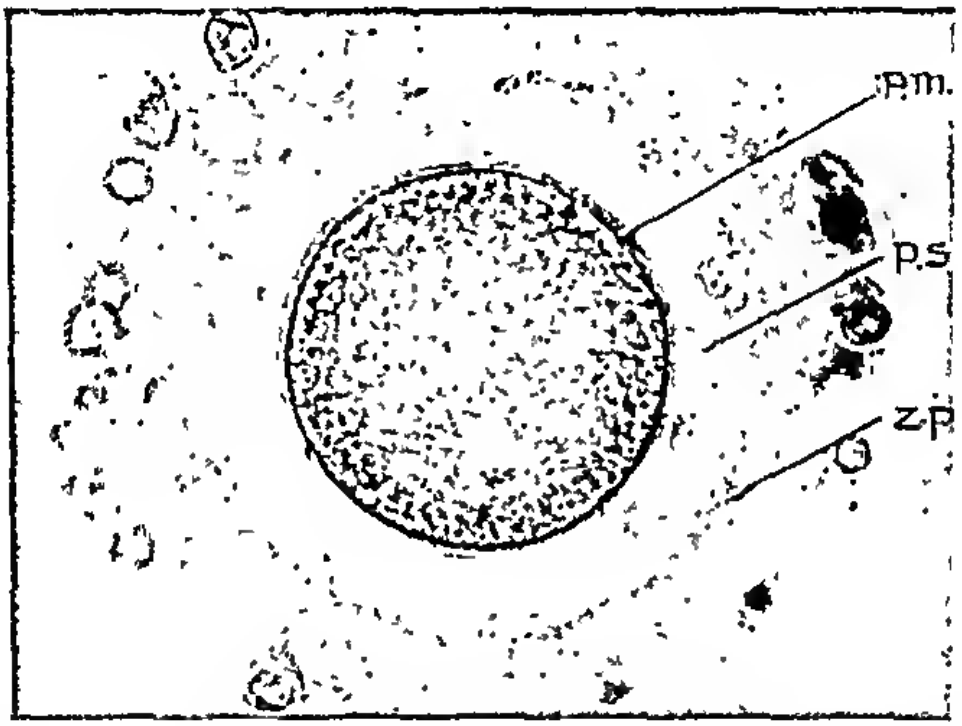


FIG. 476.—UNFERTILIZED HUMAN TUBAL EGG

The dark vitellus is surrounded by the plasma membrane (p m), a wide perivitelline space (p s) and the zona pellucida (z.p.) × 400 (Hertig)

implantation of the blastocyst in the wall of the uterus and the formation of the placenta.

In the human, also, very important advances have been made recently by the discovery of a number of very early implanting ova.[2,3] The history of the human egg remains, however, incomplete in that no one thus far has secured fertilized, segmenting tubal eggs, free blastocysts or eggs in the very first hours of implantation. An excellent example of an unfertilized human tubal egg is illustrated in figure 476.

The free blastocyst of the rhesus monkey just before implantation is a nearly spherical hollow object, some 0.2 to 0.3 mm. in diameter, consisting of a thin shell of extraembryonic or trophoblastic cells and a

* This section on placentation was written by Dr. George B. Wislocki.

[1] Carnegie Institution of Washington, Publ. 538, 1941.

[2] HERTIG AND ROCK, 1941. [3] ROCK AND HERTIG, 1942.

mass of embryo-forming cells attached at one pole to the inner surface of the trophoblast. Primitive entodermal cells form a membrane beneath the primordial embryonic mass. The trophoblastic or auxiliary cells constituting the outer surface of the blastocyst are relatively precocious, and it becomes their function to attach the ovum to the endometrium and to provide a suitable chamber and culture medium in which the embryonic rudiment can survive and undergo development. The zona pellucida disappears; as long as it is present the trophoblast cannot

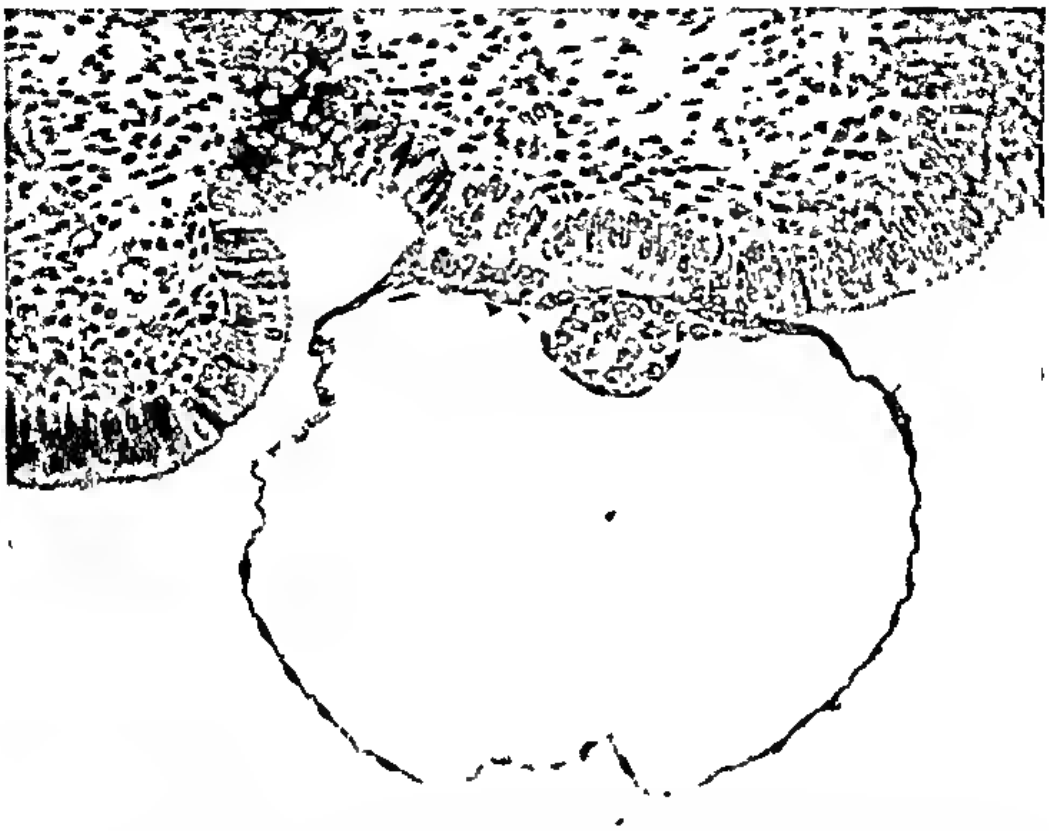


FIG. 477.—EIGHT- TO NINE-DAY OLD BLASTOCYST OF A RHESUS MONKEY BECOMING ATTACHED TO THE ENDOMETRIUM. SYNCYTIAL TROPHOBLAST AT THE EMBRYONIC POLE IS BEGINNING TO INVADE THE ENDOMETRIUM WITH CYTOLYSIS OF THE EPITHELIUM. THE EMBRYO-FORMING CELLS FORM A DISCRETE CLUMP SEPARATED FROM THE CAVITY OF THE BLASTOCYST BY A MEMBRANE OF PRIMITIVE ENTODERM. × 200. (Heuser and Streeter.)

come in contact with the uterine epithelium. The term 'trophoblast,' that is, 'nutritive layer,' was introduced by the Dutch embryologist Hubrecht.

On the 9th day after fertilization, the blastocyst of the rhesus monkey begins to implant on the endometrial wall (Fig. 477). The trophoblastic cells proliferate rapidly in a coronal area at the embryonic pole of the the ovum and a number of points of attachment to the uterine epithelium are formed. In the ensuing hours the trophoblast begins to cause a dissolution of the epithelium and to come in contact with the endometrial stroma; the latter, in the neighborhood of the ovum, shows an intense edema and enlargement of capillaries.

The youngest fertilized human ovum was discovered recently by Hertig and Rock in a uterus removed surgically on the 24th day of a 27- to 31-day menstrual cycle. A single coitus was recorded seven and a half days prior to operation. The blastocyst is superficially implanted on the posterior wall of the uterus, but is well attached at its embryonic pole, appearing as a shallow, saucer-like mass 0.46 × 0.42 mm. in diameters (Fig. 478). The endometrium, which is in the twenty-second day of its cycle, is edematous and the uterine glands are secreting actively. The trophoblast at its site of attachment to the endometrium

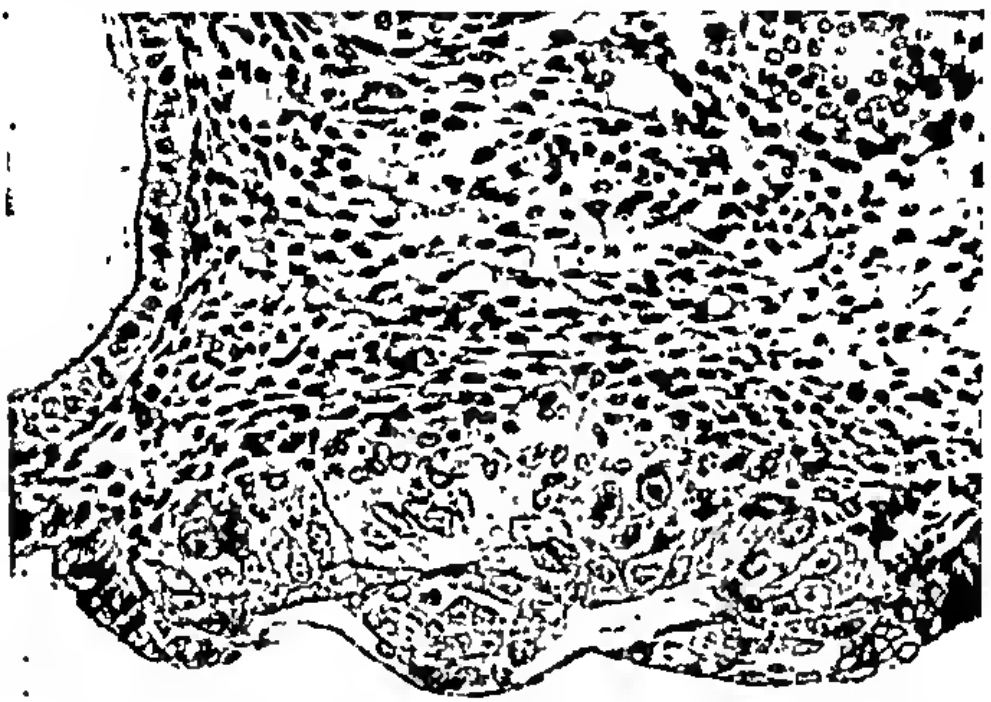


FIG. 478.—A 7½-DAY OLD HUMAN OVUM IMPLANTED ON PHYSIOLOGICALLY EDEMATOUS 22-DAY SECRETORY ENDOMETRIUM. AT ITS SITE OF ATTACHMENT THE OVUM SHOWS A THICK PROLIFERATION OF THE TROPHOBLAST WHICH IS MOSTLY OF THE SYNCYTIAL TYPE. THE EMBRYO-FORMING CELLS CONSTITUTE A CENTRAL MASS LYING JUST BELOW THE PLATE OF THICKENED TROPHOBLAST. × 300. (Hertig and Rock.)

has proliferated to form a thickened plate composed mainly of darkly staining masses of syncytium.

In the human the developing egg undergoes *interstitial implantation* or *nidation*, as a result of which the ovum sinks into the endometrium to become encapsulated by it, unlike that of the rhesus monkey which undergoes superficial nidation. The trophoblast occurs in two forms as large clear cells referred to as the *cytotrophoblast*, and as darkly staining masses with no cell boundaries termed the *syncytial trophoblast*. In the earliest stages the latter preponderates and the entire interstitially implanted egg is becoming surrounded by proliferating syncytial trophoblast. The syncytium becomes loosened up by liquefaction of part of its cytoplasm with the formation of holes or lacunæ which

receive blood extravasating into them from dilated maternal capillaries. Their walls become eroded by the action of the proliferating trophoblast.

A surface view of the endometrium with the implantation site of a normal eleven day human ovum is shown in figure 479 at × 8 magnification. At the site of nidation there is a slightly raised, pale gray, glistening translucent area a little less than 1 mm. in diameter, surrounded by a bright red area attributable to congestion of blood vessels in the surrounding endometrium. Fissures are visible in the endometrium which are associated with the openings of uterine glands.

Fig. 479.—Surface View of the Endometrium and Implantation Site of an 11-day Old Human Egg. × 8 (*Hertig and Rock*)

Microscopic examination of sections of the eleven day egg reveals further differentiation of the trophoblast and of the blood lacunæ (Fig. 480). The trophoblast which is increasing rapidly in volume is now composed of a well-defined inner cytotrophoblastic layer and of a much broader outer layer of syncytial trophoblast which contains an intercommunicating network of lacunæ. Stagnant maternal blood which partly fills the trophoblastic lacunæ serves as a source of nourishment for the growing embryo and its auxiliary tissues or adnexa. The endometrial epithelium has regenerated and has closed the defect at the site where the ovum originally penetrated. The amnion is in process of forming as a dome-like membrane over the ectodermal plate; it arises by the flattening out of lining cells which are derived from the adjacent cytotrophoblast. This mode of origin of the amnion, encountered in primates, is unlike the formation by folding of the extraembryonic somatopleure found in many other animals (*e.g.*, rabbit, *pig*). According to Hertig,[1] the cytotrophoblast gives rise at this period by differentiation and delamination *in situ* to the earliest mesenchyma as well as to primitive angioblasts which are the forerunners of fetal vascular plexuses. The bilaminar embryonal germ disc consists of a thick plate of ectoderm and of a thin ventral layer of primitive entoderm. The central cavity within the blastocyst according to Hertig and Rock is lined dorsally by the primitive entoderm, but elsewhere is surrounded by a membrane of flattened

[1] Hertig, 1935.

mesenchymal cells (exocœlomic or Heuser's membrane). This cavity should be interpreted according to them as the extraembryonic cœlom or chorionic cavity. Others regard the cavity in question as representing the yolk sac,[1] and identify the confluent spaces in the loose extraembryonic mesenchyma surrounding it as the exocœlom. The latter interpretation seems more reasonable and has been adopted in figure 480.

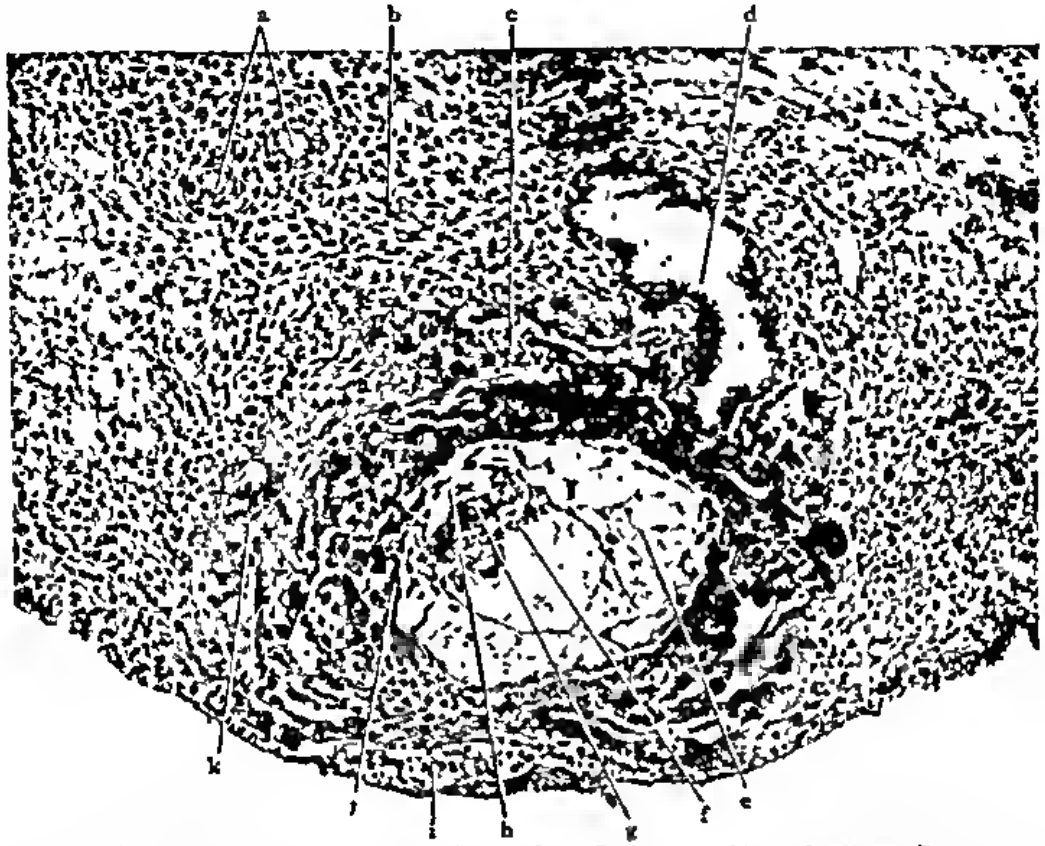


FIG. 480.—A SECTION THROUGH AN 11-DAY OLD HUMAN OVUM EMBEDDED IN 25-DAY SECRETORY ENDOMETRIUM

Within the syncytial trophoblast (k) is an intercommunicating network of lacunar spaces which contain some extravasated maternal blood (c). The bilaminar embryonic germ disk is seen toward the upper left, with the amniotic cavity above and the yolk sac cavity below. There is a suggestion of early decidua around the ovum. Above the ovum to the right is an enlarged, secretory, endometrial gland (d), whereas above and to the left the edematous stroma contains a coiled artery (a, b). e, exocœlom, f, the ventrally situated entoderm bounding the yolk sac, g, ectodermal embryonic shield, h, amniotic cavity enclosed by amnion, which is delaminating in area from the adjacent cytotrophoblast, i, repairing endometrial epithelium, j, cytotrophoblast, giving rise to syncytium and extraembryonic mesoderm. × 100. (Hertig and Rock.)

Implantation represents the initial phase in the process of placentation. By the fifteenth day the lacunæ have become very large and confluent and communicate freely with maternal veins or venous sinuses. The trophoblast differentiates around the enlarging lacunæ in the form of elongated cords or strands composed individually of a core of cytotrophoblast, with a covering of syncytium which everywhere lines the blood lacunæ. The cytotrophoblast has increased in relative amount and many mitoses are found within it. It constitutes a germinal bed from which the trophoblastic syncytium is differentiated, while at the same

[1] LEWIS, F. T., 1939.

time it gives origin, according to a recent investigation by Hertig,[1] to the extraembryonic mesoderm.

The cords of trophoblast which extend outward from the surface of the extraembryonic somatopleure (chorion) are called the *primary chorionic villi*. After about the 15th day, mesoderm begins to appear in the proximal attached portions of the cords and differentiates progressively toward their growing distal ends. The differentiation of mesoderm in this manner gradually converts the primary chorionic villi into

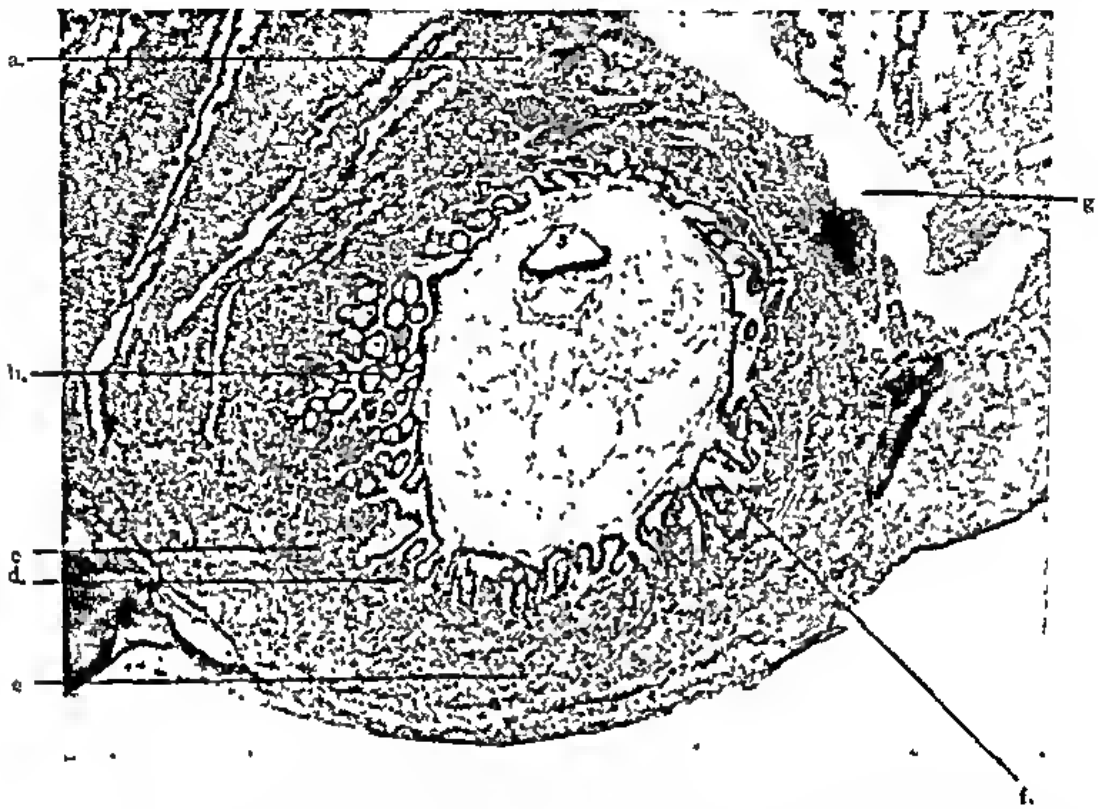


FIG. 481.—A SECTION THROUGH A 17-DAY OLD HUMAN OVUM

Observe the embryonic shield with the amniotic cavity above it and the entodermal yolk sac below it. The dark chorion encloses the large exocoelom and is connected above to the embryo by the mesodermal body stalk. Secondary villi containing cores of mesoderm and developing angioblast are in process of development (b). Peripheral to these is a lamina composed largely of cytotrophoblast constituting cell columns (d) and the developing trophoblastic shell (c). Surrounding the latter with an indefinite boundary between them is the decidua. Below, separating the egg from the uterine cavity, is a broad zone of decidua capsularis (e). a, decidua basalis, f, intervillous space, g, dilated maternal venous sinus. × 30. (*Heuser, Hertig and Rock.*)

secondary or *definitive placental villi*. Each secondary chorionic villus contains a core of mesoderm in which a plexus of embryonic blood vessels will soon develop and is surrounded by a sheath of cytotrophoblast which is in turn covered by a mantle of syncytial trophoblast. The distal ends of the villi continue to grow for a considerable period in the form of columns of cytotrophoblast into which mesoderm extends very gradually. The distal tips of these cytotrophoblastic cell columns unite on the periphery of the growing ovum to constitute the trophoblastic shell which is composed in the main of cellular trophoblast, but also

[1] HERTIG, 1935.

contains irregular strands of syncytial trophoblast some of which penetrate the adjacent endometrium. The beginning of many of these features can be seen in a photograph (Fig. 481) of a human egg of the 17th day.

The extraembryonal mesoderm, the cœlom and the mesodermal primordium of the *connecting* or *body stalk* are formed precociously in human and monkey ova. The body stalk constitutes a mass of mesoderm uniting the chorion to the caudal part of the embryonic shield. In it the major blood vessels connecting the chorion with the embryo will develop.

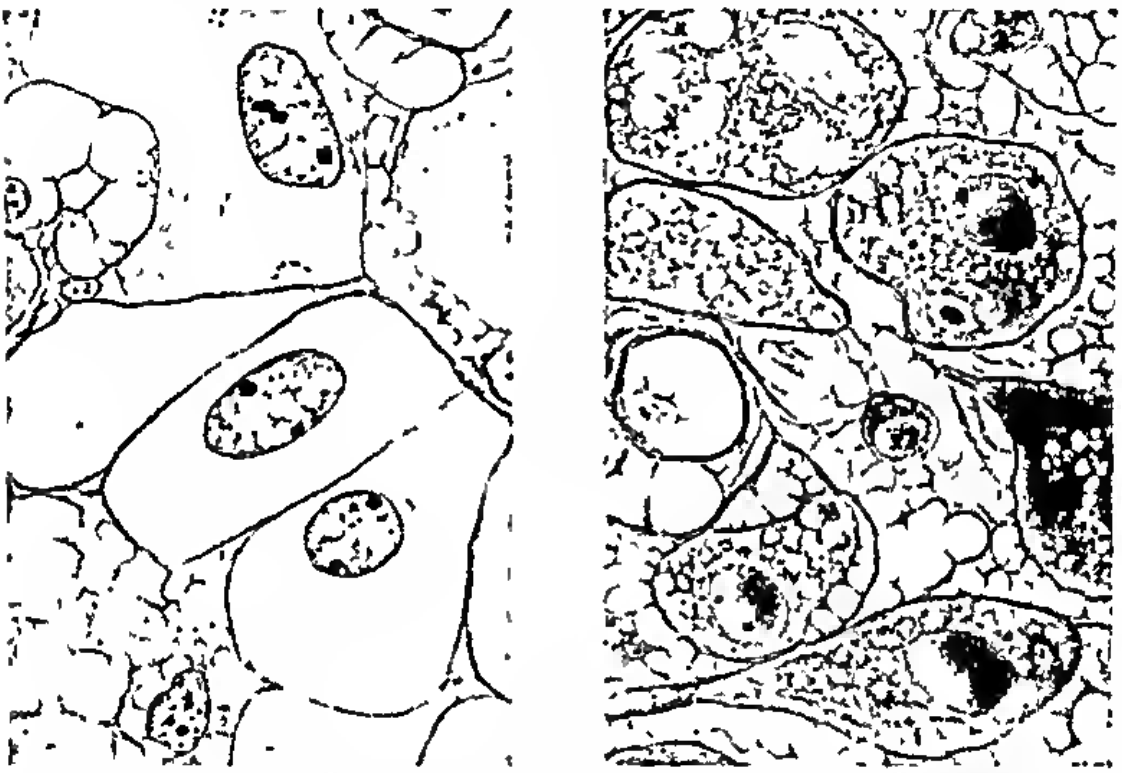


FIG. 482.—TYPICAL DECIDUAL CELLS FROM THE ENDOMETRIUM OF A HUMAN EMBRYO OF ONE MONTH

The cells on the left, stained with iron haematoxylin, have a uniformly stippled cytoplasm. In the picture on the right the cells have been stained to show the mitochondria, in addition, their cytoplasm is vacuolated. × 1600 (Wislocki and Bennett.)

By subsequent elongation and modification it forms the umbilical cord. The body stalk is the homologue of the allantoic stalk which in many mammals contains a sizable allantoic diverticulum. In man and monkey the allantoic diverticulum arising from the entoderm of the hind-gut remains rudimentary and of microscopic dimension. In figure 481 a small portion of the body stalk is visible seemingly connecting the wall of the amnion to the chorion.

As the ovum grows, the endometrium surrounding it undergoes significant changes. The zone in which the border of the trophoblastic shell intermingles with the endometrial tissue has been variously called the 'junctional,' 'composite,' or 'penetration' zone. In it the maternal tissue undergoes degeneration and necrosis. The endometrium subjacent

to the junctional zone is divided into an outer cellular part, the *stratum compactum*, and a deeper part, the *stratum spongiosum*, characterized by the presence of very much dilated, secreting glands. The spongy layer extends down to the myometrium, although a narrow intervening basal zone containing the fundic ends of the uterine glands is often distinguish-

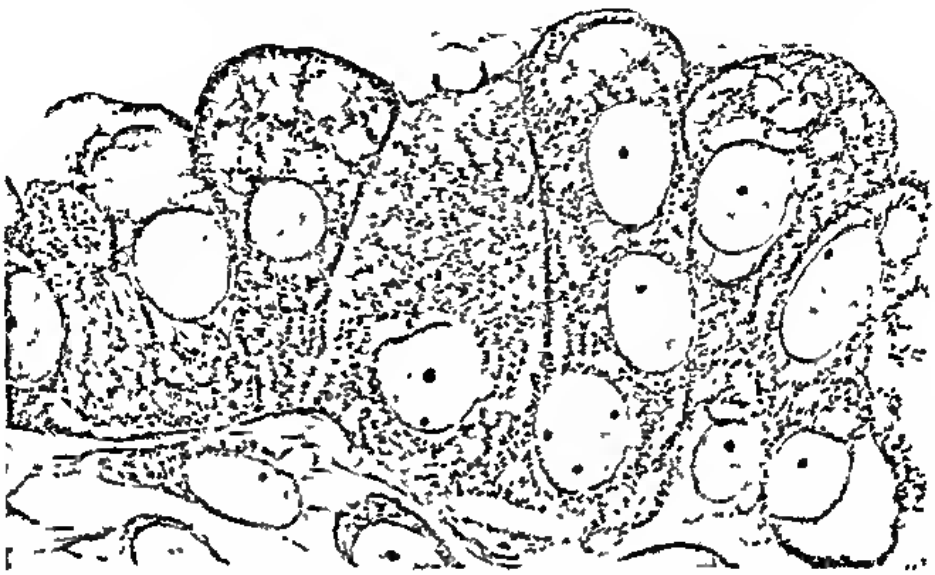


FIG. 483.—A PORTION OF THE EPITHELIUM LINING A SECRETORY UTERINE GLAND IN THE DECIDUA BASALIS BENEATH THE PLACENTA OF A 30 DAY OLD HUMAN EMBRYO. THE SECTION HAS BEEN STAINED FOR MITOCHONDRIA. × 1600 (Wislocki and Bennett.)

FIG. 484.—EPITHELIAL CELLS LINING A UTERINE GLAND FROM THE *DECIDUA BASALIS*, STAINED WITH OSMIC ACID BY CHAMPY'S FIXATION, TO DEMONSTRATE THE PRESENCE OF LIPOIDS. × 1600. (Wislocki and Bennett.)

able. The cells of the compact zone become transformed into decidual cells, the main variety of which is a very characteristic large polygonal cell. The majority of these contain cytoplasmic glycogen, while some also exhibit lipoidal droplets as well as vacuoles (Fig. 482). The tall, frequently bulbous, epithelial cells lining the endometrial glands are also rich in mitochondria, glycogen and lipoid granules (Figs. 483 and 484).

The compact layer of the endometrium becomes transformed into decidua throughout the entire extent of the uterine cavity. Hence, the decidua is divided for convenience into three regions, in reference to its relationship to the ovum. That directly beneath the implanted ovum forms the maternal part of the placenta and is called the *decidua basalis* (or *serotina*); that separating the embedded egg from the uterine cavity is designated as the *decidua capsularis* (or *reflexa*); whereas the part which lines the remainder of the uterus is referred to as the *decidua vera* (Fig. 481). The decidua is cast off at the time of birth, with the fetal mem-

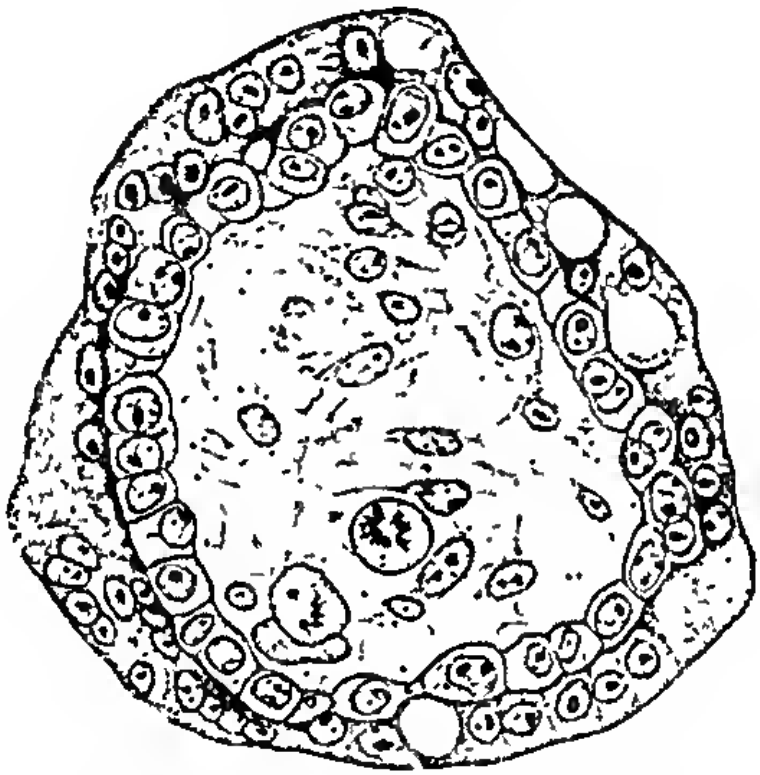


FIG. 485.—CROSS SECTION OF A YOUNG HUMAN CHORIONIC VILLUS SHOWING THE AXIAL MESODERMAL CORE SURROUNDED BY THE TWO-LAYERED CHORIONIC EPITHELIUM, COMPOSED OF AN INNER LAYER OF LANGHANS CELLS (CYTOTROPHOBLAST) AND OF AN OUTER LAYER OF SYNCYTIUM (SYNCYTIAL TROPHOBLAST). VACUOLES, VARIABLE IN SIZE AND DISTRIBUTION OCCUR IN THE SYNCYTIUM. × 550 (Hamilton and Gladstone.)

branes, and the endometrium renews itself from the deep, residual portion of the stratum spongiosum and the basal zone.

Chorionic villi grow at first everywhere over the circumference of the chorion. Basally where the thick, well-vascularized endometrium favors their growth the villi become long, branched and profuse; they constitute the *chorion frondosum* and give rise to the definitive saucer-shaped placenta. Over the outer portion of the chorionic wall which bulges toward the uterine cavity the villi are much shorter. By the third month of gestation the villi, together with the decidua capsularis dwindle, leaving the chorion as a relatively smooth membrane termed the *chorion læve*. As the fetus enlarges and its membranes expand, the chorion læve

eventually fuses with the decidua vera of the opposite uterine wall, thereby filling and obliterating the uterine cavity.

The chorionic villus represents the most important structure of the placenta (Figs. 485 and 486). It is by means of the villi that the placenta exercises its essential functions as a site of transfer between the maternal and fetal circulations. Through the trophoblastic parenchyma covering the villi those materials must pass which are necessary for the nutrition and growth of the embryo, as well as, in the reverse direction, those substances which the growing organism finds it necessary to excrete.

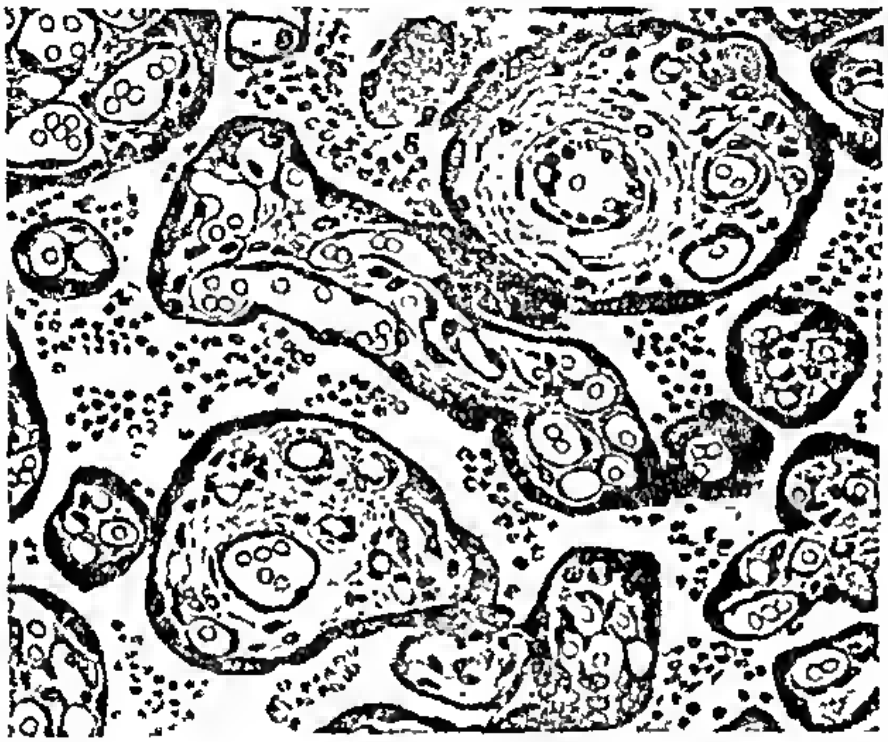


FIG. 486.—THE STRUCTURE OF THE PLACENTAL VILLI AS SEEN IN SECTION IN AN ADVANCED PLACENTA OF A GORILLA. THE CHORIONIC VILLI IN MAN AND THE ANTHROPOID APES ARE IDENTICAL IN STRUCTURE. SURROUNDING THE VILLI, THE INTERVILLOUS SPACE IS SEEN CONTAINING GROUPS OF MATERNAL ERYTHROCYTES.

The villi are composed of a mesodermal core containing wide fetal capillaries and of a surface layer of deeply staining syncytium. Note the occasional syncytial anastomoses between adjacent villi. × 275. (J. P. Hill.)

As the primary villi are being converted into the secondary villi, the maternal blood lacunæ associated with them become increasingly confluent until a more or less continuous *intervillous space* is created around them, which acquires definite afferent and efferent connections with the arteries and veins of the uterine wall (Fig. 492). In consequence of this, maternal blood begins to circulate in the intervillous space and to bathe the surfaces of the villi. In the mesodermal stroma of the villi the embryonic circulation also becomes established. Angioblastic plexuses arise at an early stage in the chorion and wall of the yolk sac and become connected with the blood vessels and developing heart within the growing embryo, so that toward the end of the first month the fetal circulation is formed and blood begins to circulate in the

capillaries of the chorionic villi. The secondary or definitive villi continue to grow and branch. A number of large villous stems extend across the intervillous space to serve as anchoring villi which attach the placenta to the decidua, whereas a multitude of lesser, more delicate villi project freely into the intervillous space where their surfaces are bathed by circulating maternal blood. Many of the relationships are shown diagrammatically in figure 492.

A typical cross section of a young villus in which embryonic blood vessels are not yet present is illustrated in figure 485. One observes a central core of loose mesenchymal tissue in which cell divisions are taking place surrounded by two distinct layers of chorionic epithelium

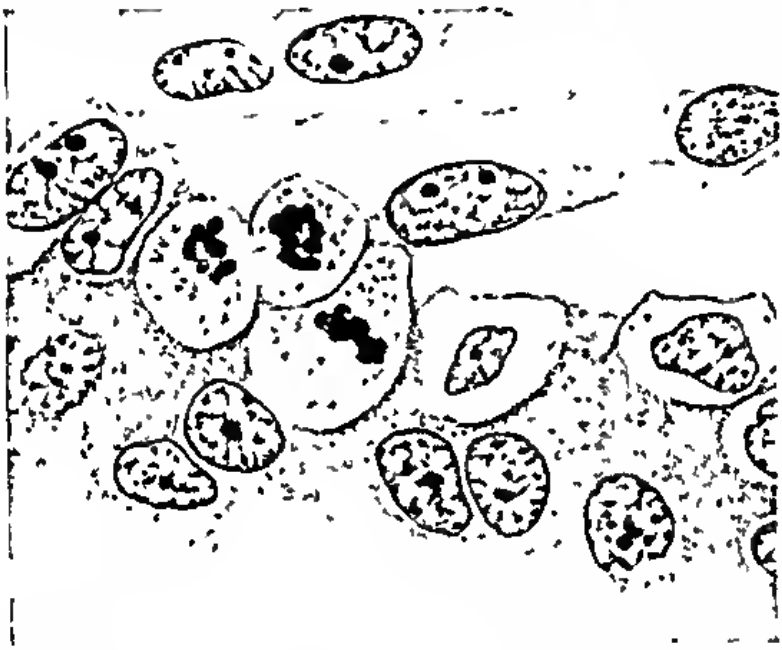


FIG. 487.—A PORTION OF A CHORIONIC VILLUS OF THE PLACENTA OF A 13-DAY HUMAN EMBRYO. OBSERVE SEVERAL OF THE LANGHANS CELLS WHICH ARE DIVIDING MITOTICALLY. × 1600. (Wislocki and Bennett.)

(trophoblast). The cytotrophoblastic layer is composed of variously large, discrete, pale cells with relatively large nuclei. The cytotrophoblastic cells which are present upon the secondary villi are usually referred to specifically as *Langhans cells* after their discoverer. Overlying the Langhans epithelium is a darker, variably thick layer of syncytial trophoblast in which small nuclei are irregularly dispersed. Figure 486 illustrates a group of villi at a much later stage and shows to advantage the thin-walled, sinusoidal capillaries of the stroma. The intervillous space containing maternal erythrocytes surrounds the villi.

The Langhans epithelium is shown in detail in figures 487, 488, and 489. These cells constitute a germinal bed in which frequent mitoses are seen and from which the syncytium is derived (Fig. 487). The much smaller, more irregular and darkly staining nuclei of the syncytium have never been observed to undergo direct mitosis, so that amitosis has been

postulated as the manner by which they divide in so far as their number cannot be accounted for by direct derivation from the Langhans cells.

The Langhans cells contain a goodly number of mitochondria, a Golgi apparatus, a variable number of faint vacuoles and small amounts of glycogen, but no lipoidal droplets. They also possess certain cytoplasmic granules which give an oxydase reaction with indophenol blue. The Langhans cells diminish steadily in number during the course of

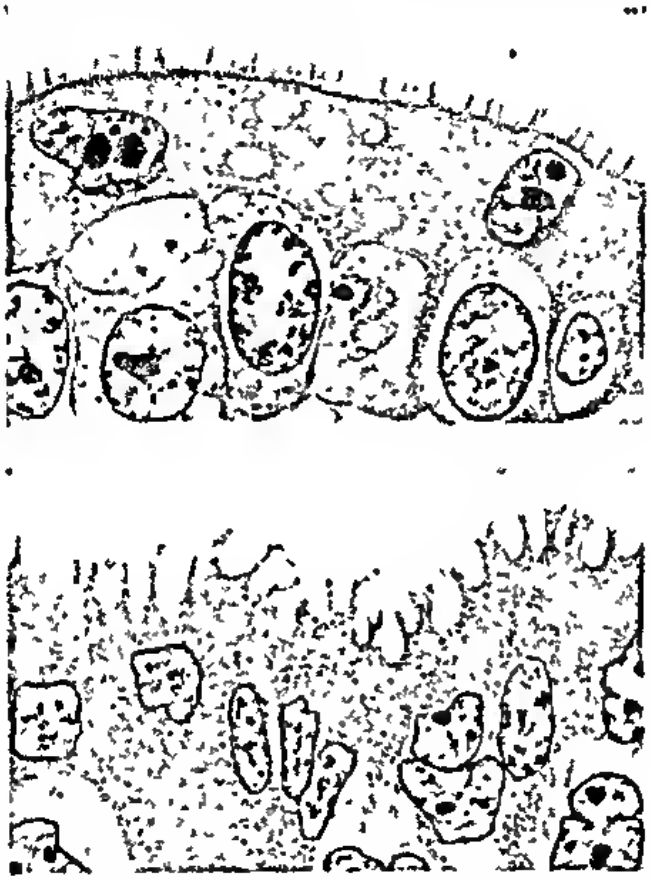


FIG. 488.—TWO VIEWS OF THE TROPHOBLAST OF A HUMAN CHORIONIC VILLUS TO ILLUSTRATE THE VARIABILITY OF THE SURFACE OF THE SYNCYTIUM

Above, the surface possesses a brush-like border, whereas, below, it forms cytoplasmic streamers. × 1600. (Wislocki and Bennett.)

pregnancy, so that from the fifth month on to the end of gestation very few of them are encountered. Some have claimed that there are none at all after the sixth month.

The syncytium is cytologically a very complex structure.[1] It is thicker in the early period and becomes progressively thinner as gestation advances, but it is present on the villi until term, although a variable fraction of it is said to undergo a form of hyaline degeneration before pregnancy terminates. The syncytium possesses an outer surface which is extremely variable in structure, ranging from a brush-like border composed of minute hairs through various intermediate appearances

[1] WISLOCKI AND BENNETT, 1943

to a condition where the surface bears delicate cytoplasmic streamers and fronds (Fig. 488). These variable surface appearances in fixed material suggest that the living cytoplasm of the syncytium may be unstable and possess considerable plasticity. The presence of irregular strands as well as knobs and tags of nucleated syncytium in various parts of the placenta, especially in the early phases of its development, has suggested to many that the cytoplasm of the syncytium may undergo flowing or streaming movements. In confirmation of this, recent studies

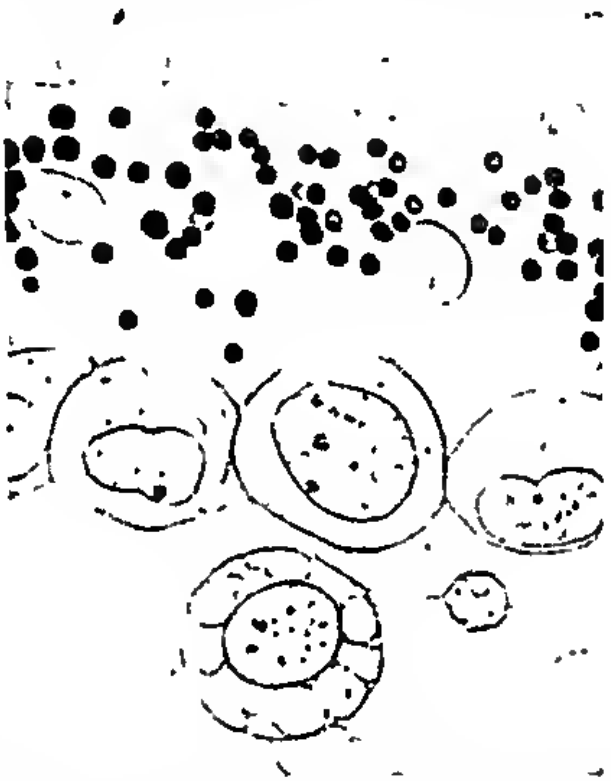


FIG. 489.—THE TROPHOBLAST OF A HUMAN CHORIONIC VILLUS OF 30 DAYS STAINED WITH OSMIC ACID BY CHAMPY'S FIXATION, TO ILLUSTRATE THE ABUNDANCE OF LIPOID DROPLETS IN THE SYNCYTIUM AND THEIR ABSENCE IN THE LANGHANS CELLS

In the lower half of the drawing, in the stroma beneath the trophoblast, a typical, vacuolated Hofbauer cell is visible. × 1600 (Wislocki and Bennett.)

on explanted bits of placenta grown in tissue cultures indicate that the cytoplasm of both the syncytial and the cellular trophoblast is capable of flowing and giving rise to a variety of streamers and thread-like processes.[1,2]

The cytoplasm of the syncytial trophoblast is delicately stippled and especially in the early phases of its development has a foamy or delicately vacuolated appearance. Larger vacuoles arise in irregular locations as a result apparently of fusion of smaller vacuoles. Mitochondria are abundantly demonstrable by appropriate methods, as well as dispersed Golgi material and lipoid droplets. Glycogen is usually absent, although traces of it have been recorded. Oxydase granules have been demon-

[1] FRIEDHEIM, 1929. [2] JONES, GEY AND GEY, 1943.

strated. Under the polarizing microscope the syncytium is observed to be rich in birefringent material both in the deeper portion of the cytoplasm and along its outer surface.

Lipoidal droplets, demonstrable by Sudan III as well as by osmic acid, are exceedingly abundant and relatively large in the early months of pregnancy (Fig. 489). The osmophilic droplets diminish in size as pregnancy advances, but even at term extremely minute lipoidal granules are still abundantly present. When fresh villi or frozen sections are treated with phenylhydrazine, yellow phenylhydrazones are formed in

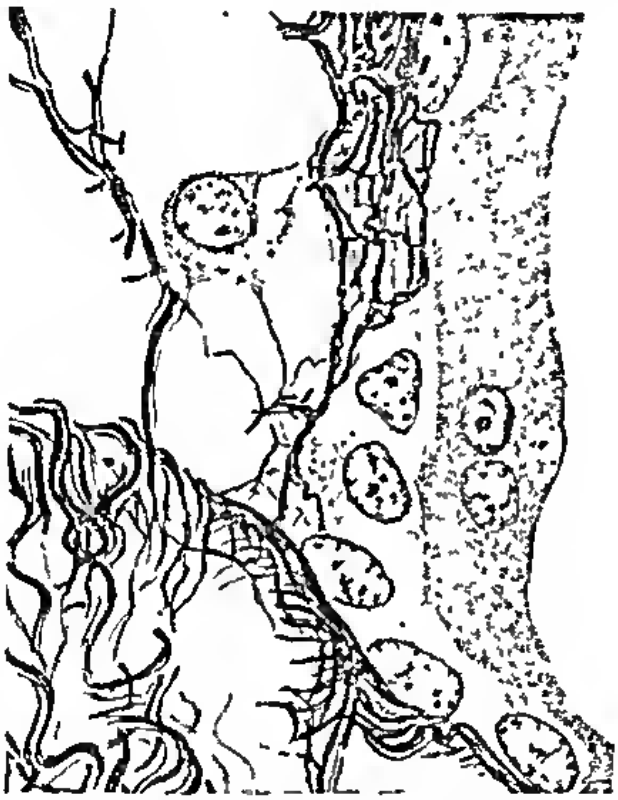


FIG. 490.—A PORTION OF A HUMAN CHORIONIC VILLUS WITH THE RETICULAR CONNECTIVE TISSUE FIBERS IMPREGNATED WITH SILVER BY BODIAN'S METHOD. × 1600. (Wislocki and Bennett.)

the syncytium, a reaction which indicates the presence of ketone bodies and aldehydes.[1] After treatment of the sections with acetone, the birefringence disappears and no phenylhydrazones are formed. The presence of lipoidal material which is birefringent in association with yellow phenylhydrazones suggests that the syncytium is the site of formation of the steroid hormones which the placenta is known to produce.[2] The stainable lipoid in the placenta is probably in good part 'intrinsic' or 'metabolic' lipoid, rather than 'extrinsic' fat in process of transmission from mother to fetus for purposes of nutrition.

The inner border of the syncytium, in contrast to the outer border, is relatively smooth and little differentiated. The Langhans cells, as

[1] BENNETT, 1940. [2] WISLOCKI AND BENNETT, 1943.

long as they persist, and the syncytium rest upon a condensed network of reticular connective tissue fibers (Fig. 490). The subjacent stroma is relatively acellular and contains scattered mesenchymal cells or fibroblasts. In addition it contains peculiar round cells of variable size which are extremely vacuolated and contain granules. These are called *Hofbauer cells* after the investigator who first gave a detailed description of them.[1] They are abundant in the first months of pregnancy, but diminish in number later on. Their nature is uncertain; some have regarded them as degenerating cells,[2] while others consider them to be macrophages because of the affinity of their cytoplasm for neutral red.[3] A typical example of a Hofbauer cell is shown in figure 489.

The chorionic villi at term call for separate mention. The syncytium covering them contains demonstrable particles of lipoid as well as mitochondria and its surface exhibits minute stubble-like irregularities but no typical brush-border. It is characteristic of the sinusoidal capillaries in the chorionic villi at term to press themselves closely against the syncytium in certain places. In such places the syncytium appears to become stretched over the capillaries in the form of a thin membrane. These thinned-out membranous areas have been termed 'epithelial plates,'[4] and it is possible that through them at this period the most active transfer of substances from mother to fetus and vice versa occurs. Thin as these areas, which separate the maternal and the fetal blood streams, may appear to be, they are always composed of three layers, namely, a tenuous lamina of syncytium, an exceedingly delicate network of argyrophil reticular fibers, and the cytoplasm of endothelial cells.

The term cytotrophoblast includes the Langhans cells of the chorionic villi as well as the masses of cells comprising the *trophoblastic cell columns* and the *trophoblastic shell*. In addition, cytotrophoblast proliferates in irregularly occurring localized areas on certain of the chorionic villi creating so-called *trophoblastic cell islands*, which are covered by a thin but often incomplete layer of syncytial trophoblast. Similar localized areas of cell proliferation are encountered in the chorionic membrane which bounds the intervillous space beneath the fetal surface (chorionic or closing plate) of the placenta (Fig. 492). These various masses of cytotrophoblast contain glycogen and between the cells a peculiar protein-containing fluid or interstitial substance accumulates. Little is known regarding this ground substance beyond the facts that it does not contain argyrophil or collagenous fibers and that in it with increasing age variable amounts of fibrin make their appearance. It seems likely that this ground substance is derived partly from the decidua, serving to convey nutriment to the growing cytotrophoblastic cell

[1] Hofbauer, 1905. [2] Meyer, 1921. [3] Lewis, W. H., 1924. [4] Bremer, 1916.

columns and shell. It may also be produced in part by the cytotrophoblastic cells themselves. Cellular trophoblast growing in tissue cultures possesses motility and exhibits streaming of its cytoplasm, and from such cultures chorionic gonadotropic hormone has been recovered.[1] Furthermore, the necrosis of the decidua as the trophoblast invades it has suggested to many that proteolytic enzymes must be liberated by both the

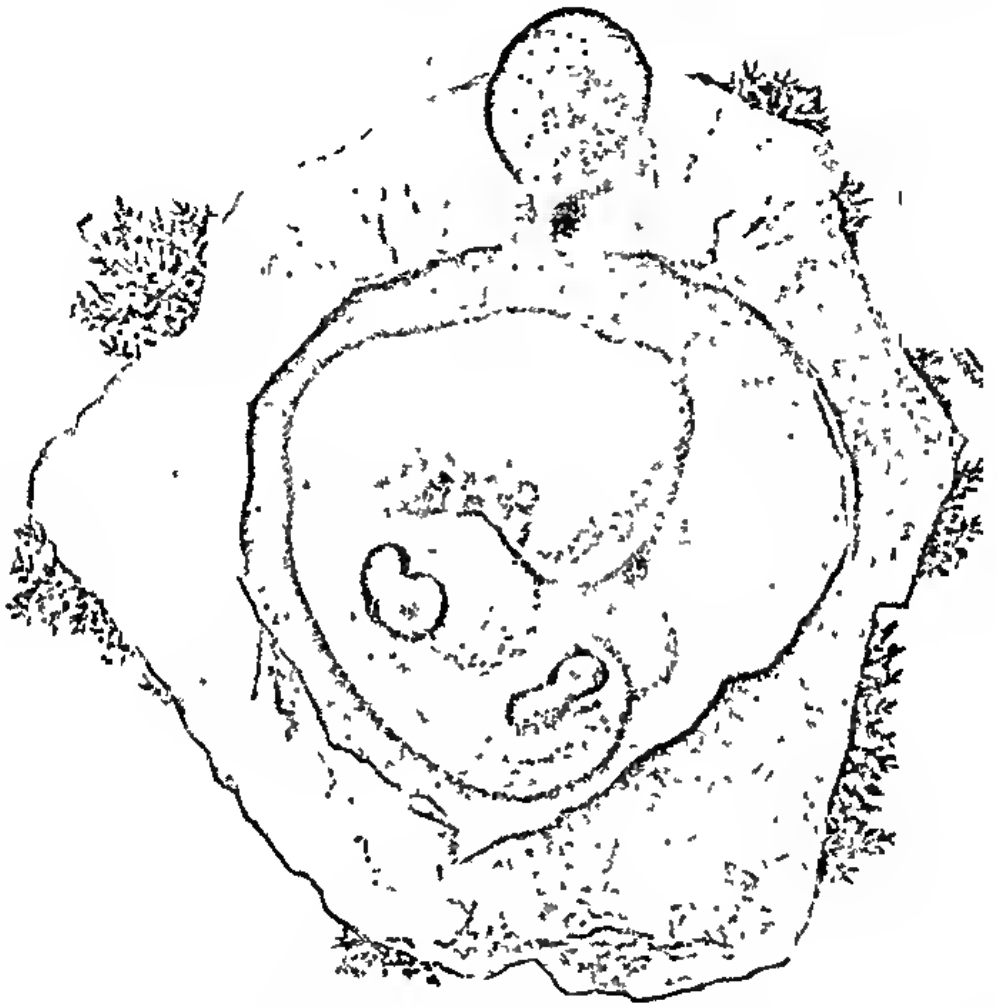


FIG. 491.—A NORMAL HUMAN EMBRYO OF 10 MM., APPROXIMATELY ONE MONTH OLD REMOVED SURGICALLY FROM THE UTERUS

The chorion laeve has been removed and a window has been cut in the amnion thus exposing the embryo. The vitelline duct can be seen extending to the yolk sac which rests in the exocoelom between the chorion and the amnion. × 5. (Minot)

syncytial and cellular trophoblast. The cytoplasm of the trophoblast of the cell columns and shell is frequently markedly vacuolated. These various observations suggest that the cellular trophoblast is an actively metabolizing tissue which probably produces and releases various substances into the interstitial matrix. It should be pointed out that the curve of excretion of chorionic gonadotropin coincides approximately in the human and in the chimpanzee[2] with the period of the expansion and decline of the cytotrophoblast. The ground substance of the cyto-

[1] JONES, GEY AND GEY, 1943. [2] ELDER AND BRUHN, 1939.

trophoblast was called 'canalized fibrin' by Langhans, but Grosser[1] renamed it 'fibrinoid,' meaning fibrin-like. Neither of these terms seems to characterize it aptly. Amongst its few properties which are known, the fact that fibrin is variably deposited in it does not warrant applying the designation 'fibrinoid' to this protein-containing fluid which by virtue of its heterogeneous origins must contain many other substances besides the precursors of fibrin. The fact that blood plasma,

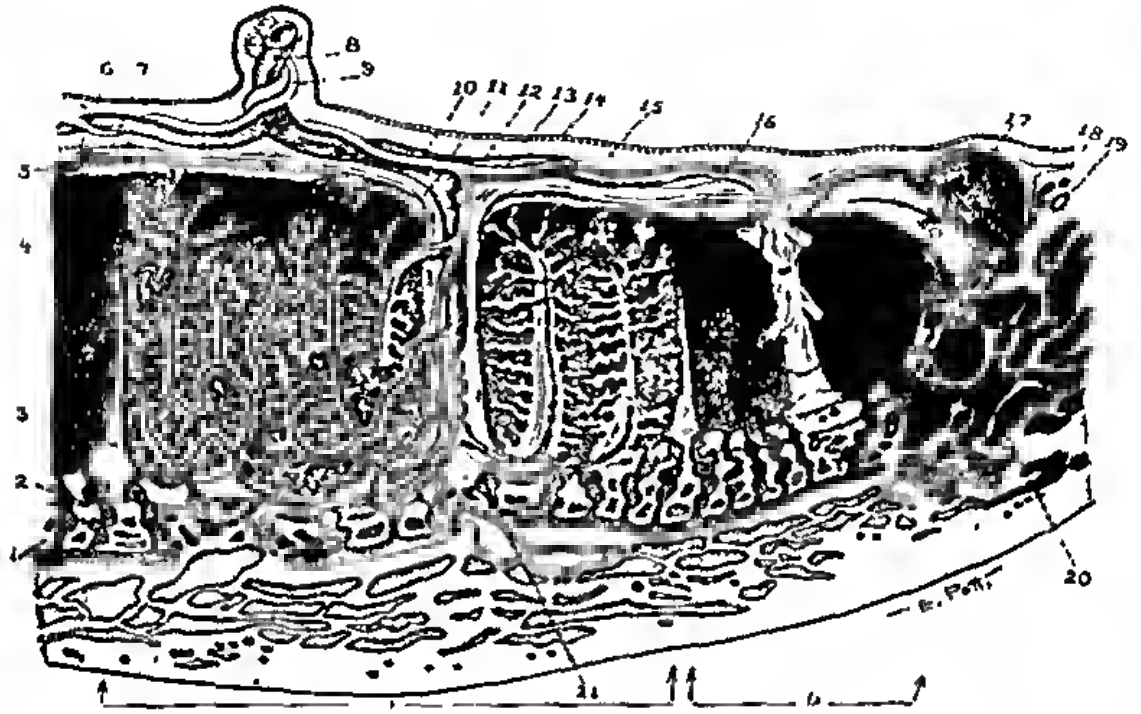


FIG. 492.—A DIAGRAM OF THE CIRCULATION IN THE HUMAN PLACENTA REDRAWN AND MODIFIED FROM SPANNER. Maternal arteries, red; maternal veins, blue; fetal arteries, white; fetal veins, gray. 1, Coiled arteries, 2, Nitabuch's fibrin layer, 3, septum composed of decidua and trophoblast, marking the boundary of a placental cotyledon (the limits of an individual cotyledon are indicated below by two arrows and the intervening line marked 'a'), 4, the intervillous space, 5, subchorial portion of intervillous space, 6, chorial trophoblast, 7, smooth muscle cells in wall of chorion, 8, umbilical vein, 9, an umbilical artery, 10, a placental artery, 11, smooth muscle in a stem villus, 12, a placental vein, 13, artery and vein of a stem villus, 14, the amniotic epithelium, 15, the chorionic plate, 16, subchorial fibrin, 17, border or marginal venous sinus, 18, chorion laeve; 19, parietal decidua; 20, collecting veins of border or marginal zone, 21, recurrent artery and vein. The arrow indicates the direction of flow of maternal blood from the coiled arteries, through the intervillous space, into the marginal sinus and veins. Two arrows below and the intervening line marked 'b' indicate the location of a border or marginal cotyledon. The veins of the stem villi as well as their recurrent branches possess sphincters which are indicated but not labelled. Spanner calls these vessels 'throttle veins.'

for example, contains fibrinogen and clots upon standing would not justify calling it a 'fibrinoid' substance.

The circulation of the fetal and maternal blood in the human placenta has been the subject of recent study by Spanner[2] and a diagram illustrating this investigator's concept of the placental circulation is presented (Fig. 492). The two umbilical arteries give rise to numerous placental arteries which spread fan-wise in the chorionic plate. From these radial trunks, branches are given off which follow the stem villi

[1] GROSSER, 1925 AND 1927. [2] SPANNER, 1936.

from the surface to the base of the fetal placenta. Each of these arteries breaks up into a number of recurrent branches which leave the stem villi to penetrate subsidiary villi, and these in turn branch and re-branch to break up finally into the capillary bed of the smallest villi. Each stem artery with its corona of recurrent branches resembles a candelabra. The venous blood is returned from the multitude of terminal villi through a system of veins which accompanies the arteries. The veins are peculiar according to Spanner in that they possess series of muscular sphincters which act as checks upon the returning venous flow ('throttle veins'). In the stroma of the chorionic plate as well as in the stem villi thin sheets of smooth muscle cells are described having no relations whatsoever to the walls of the fetal vessels.

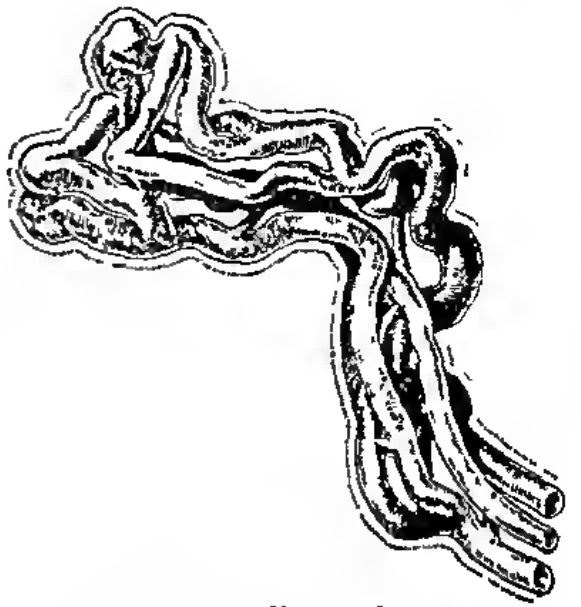


FIG 493.—THE INJECTED VASCULAR BED OF A TERMINAL CHORIONIC VILLUS FROM A HUMAN PLACENTA AT TERM (Spanner)

The maternal blood reaches the intervillous space surrounding the villi through a series of peculiar 'coiled arteries' (Fig. 494). These arise from branches of the uterine arteries which run mainly in the vascular layer of the myometrium. The coiled arteries are composed of three segments: (1) a short, slightly coiled connecting piece, which after dividing into two, produces (2) a wide, coiled segment of variable diameter, which ends (3) in several short, terminal pieces which are funnel-shaped and open into the intervillous space by narrow orifices. From the coiled segments, in addition, minute branches to the number of three or four are given off which supply the basal decidua breaking up into capillaries without communicating with the intervillous space.

Thus the maternal arterial blood reaches the placental labyrinth by a series of coiled arteries with constricted mouths which pierce the floor of the intervillous space. The blood then bathes the surfaces of the chorionic villi and passes towards the chorionic plate beneath which the venous blood collects. It then flows toward the periphery of the placental disc, where the venous blood finally enters a large circumferential venous sinus and a number of related marginal veins, to be returned to the deep uterine veins, so completing the placental circulation. According to Spanner no veins comparable to the arteries pierce the floor of the intervillous space in the human placenta.

Burwell[1] and collaborators have recently demonstrated that the principal alterations in the circulation in the pregnant woman include an increase in cardiac output, pulse rate, pulse pressure and total blood volume, a decrease in the arteriovenous difference, a loud bruit over the site of the placenta, and a rise in pressure in the veins of the lower extremities. They ascribe these changes to (1) an arterio-venous shunt through the placenta and (2) an obstruction to venous return from the lower extremities by the enlarged uterus. According to them, the

Fig. 494.—A Corrosion Preparation of an Isolated 'Coiled Artery' from the Human Decidua Basalis (Spanner).

maternal vascular system in the placenta as described by Spanner offers a connection between arteries and veins which has marked similarities to a large arterio-venous shunt. As the latter has demonstrated, about 500 arteries with a terminal diameter averaging 0.15 mm. empty directly (like the nozzles of garden hose) into the intervillous space. Hence relatively large arteries connect with relatively large veins through the capacious intervillous space without the interposition of arterioles or capillaries.

Daron[2] studied the arterial supply of the uterus of the rhesus monkey during the various phases of the menstrual cycle. He recorded two distinct types of arteries in the endometrium: (1) large, coiled arteries which are closely wound to form radial columns and which divide peripherally into numerous precapillary arterioles; and (2) small arteries which extend only into the basal zone of the mucous membrane where

[1] Burwell and others, 1938. [2] Daron, 1936.

they soon break up into capillaries. During gestation the large coiled arteries and the proximal portions of the precapillary arterioles become apparently the coiled segments and short, terminal end-pieces described by Spanner. The blood flow through the coiled arteries, according to Daron, is controlled by the pronounced capacity of certain radial myometrial arteries, which give rise to the coiled arteries, to undergo constriction, as well as by the tortuosity of the coiled arteries themselves.

The comparative anatomy and histology of the placenta is complex and the reader seeking information on this subject should consult the works of Grosser,[1] Mossman,[2] and Hill.[3] The yolk sac and allantois fuse variously in different mammals with the chorion to afford a bridge by means of which fetal blood vessels (either vitelline or allantoic) connect the chorion with the embryo Thus yolk-sac placentæ and chorio-allantoic placentæ are recognized, the former occurring in many orders of mammals (but not in man or monkeys) and usually preceding the latter in time of appearance and disappearance. Yet in certain mammals the two types continue to function concurrently. The placentæ of mammals have been classified according to external shape. Shape appears to be related to the size and rate of growth of the blastocyst, as well as to the form of the uterus, but study of shape alone has led so far to no significant understanding of comparative placental relationships. The placenta of the horse and pig is diffuse, that of ruminants multiplex or cotyledonary, that of carnivores zonary, while that of insectivores, bats, rodents and primates, is generally discoidal.

Grosser proposed classifying placentæ on the basis of their histological structure, taking into consideration the intimacy of contact between the chorion (trophoblast) and maternal tissues, thereby indicating the thickness and cellular constitution of the several cellular barriers separating the maternal and fetal blood streams.

In the *epitheliochorial placenta* (pig, horse) the chorion is merely applied to the epithelium of the intact uterine mucosa. In this type much of the nutriment is supplied by the secretion of the enlarged uterine glands ('uterine milk').

In the *syndesmochorial placenta* (ruminants) the uterine epithelium is destroyed, leaving the connective tissue stroma exposed to the trophoblastic covering of the chorion. In this and the preceding type, the chorion separates readily from the mucosa at birth and there is no true decidua and little bleeding.

In the *endotheliochorial placenta* (carnivores) there is destruction of the connective tissue of the mucosa leaving the maternal capillaries surrounded essentially by endothelium.

[1] Grosser, 1927. [2] Mossman, 1937. [3] Hill, J. P., 1932.

In the *hemochorial placenta* (insectivores, bats, higher primates, *some* rodents) the maternal blood, through the loss of the maternal capillary endothelium, comes into direct contact with the chorionic villi. In these types the endometrium becomes transformed into decidua and partially destroyed. At birth there may be considerable bleeding (man and monkeys).

Mossman has described a further extension of this classification in certain rodents in which he states that in addition to all of the maternal elements, the trophoblast disappears to a considerable degree from the surface of the chorionic trabeculæ, leaving the endothelial walls of the fetal capillaries directly exposed to the circumambient maternal blood. In keeping with the preceding terminology he calls this a *hemoendothelial* type of placenta.

The placenta is a barrier through which nutritive substances pass from mother to fetus at the same time that fetal waste products are transferred in the opposite direction. In addition, certain hormones (chorionic gonadotropin and steroid hormones) are produced by and released from the fetal placenta.[1] The syncytial trophoblast covering the chorionic villus constitutes the essential structural element of the placental barrier.

Debate has centered upon the question as to whether the placenta is primarily a semipermeable membrane, the distribution of substances between mother and fetus being governed principally by hydrostatic and diffusion pressures, or whether, in addition, the trophoblastic membrane exercises a selective regulation of certain substances by virtue of special physical or chemical properties. This question is unsettled, but the formulation proposed by Cunningham[2] is a useful one. He divides the substances which traverse the placenta into three categories:

"(1) those substances which are diffusible and which meet with no mechanism in the placenta capable of acting on them; these pass by diffusion from mother to fetus, or in the reverse direction without any mediation on the part of the placenta. This group contains most of the excretory products of the fetus, and large numbers of foreign substances, many of which are highly toxic; (2) a group of substances to which the maternal or fetal surfaces of the placental barrier are impermeable. Here may be grouped the formed elements which are normally present in the circulation and such foreign substances as insoluble salts (*e.g.*, barium sulphate) and foreign particulate matter such as India ink, cinnabar and bacteria; and (3) certain substances which meet a definite preformed regulatory mechanism in the placenta. At present this group must include most of those substances which are designed for the fetal

[1] Newton, 1938 [2] Cunningham, 1920 and 1922.

metabolism and certain important inorganic salts, especially those containing iron." To this classification should now be added as a further category those substances, such as certain proteolytic enzymes and hormones, which there is reason to believe are actually produced in and secreted by the trophoblast.

The failure of India ink to be taken in by the trophoblast in experiments on animals illustrates that phagocytosis of gross microscopic particles does not play an important rôle in placental transfer. Yet, it should be remarked that in young human and monkey embryos, at that period when the trophoblast is actively invading the endometrium at the implantation site, a certain degree of phagocytosis of clumps of degenerating maternal tissues has been observed. On the other hand, particles of lesser size such as vital dyes (trypan blue) are slowly absorbed by the trophoblast in a variety of animals, and rapidly so by the epithelium of the yolk sac placenta in rodents (Everett[1]). Yet, although absorbed and stored in the trophoblast and vitelline epithelium, little or none of these dyes is finally transmitted to the fetuses. This failure of vital dyes to traverse the placental barrier illustrates one aspect of the regulatory functions of the trophoblast.

The cytological complexity of the trophoblast, as described in previous paragraphs, also emphasizes that the placental barrier is structurally far more complicated than an ordinary semipermeable membrane.

UMBILICAL CORD

The *umbilical cord* is a translucent, glistening, white or pearly rope of tissue about 2 feet in length, extending from the umbilicus to the placenta. It consists of mucous tissue (p. 94) covered with epithelium, and contains at birth three large blood vessels, two *umbilical arteries* and one *umbilical vein* (Fig. 495, B). The parallel arteries generally wind around the vein making sometimes forty revolutions. The twist is to the left in about three-fourths of the cords, most of the others to the right, since very few are found untwisted. The surface of the cord shows corresponding spiral markings and often irregular protuberances called false knots. (True knots, tied by the intrauterine movements of the embryo, are very rare.) There are no lymphatic vessels or capillaries in the cord, and the large blood vessels do not anastomose. The walls of the arteries contain many muscle fibers, but very little elastic tissue, and they are usually found collapsed in sections; their contraction is of interest since nerves have been traced into the cord for only a very short distance. The vein generally remains open.

[1] EVERETT, 1935.

The *umbilical arteries* arise in young embryos as the main terminal branches into which the dorsal aorta bifurcates. These vessels curve ventrally on each side of the pelvis and pass out through the cord to the chorion; they are equidistant from the allantois which they accompany. In the adult the parts of these vessels near the aorta

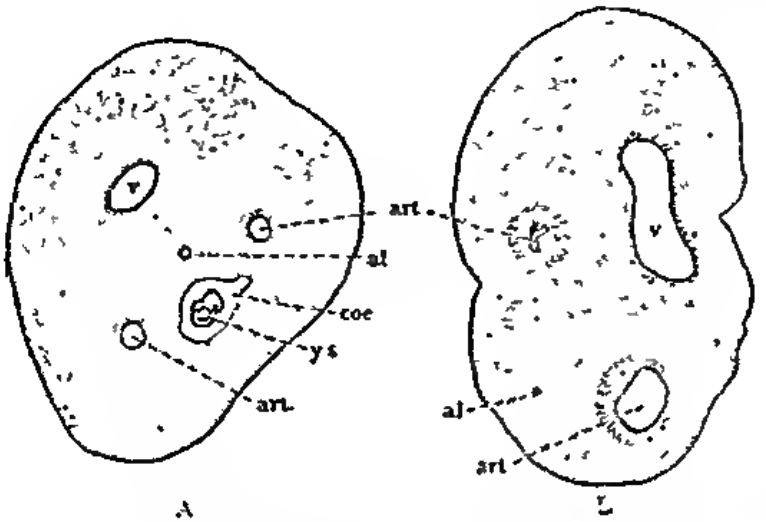


Fig. 495.—Cross Sections of Umbilical Cords.
A, from an embryo of two months. × 20. B, at birth. × 3. al., Allantois; art., artery; coe., coelom; v., vein; y. s., yolk stalk.

are known as the common iliac arteries, and the small offshoots from them, which have grown down the limbs, have become the external iliac arteries. The distal course of the original vessels may still be followed through the hypogastric arteries (internal iliacs) up on each side of the median line to the navel; toward the navel the vessels have become reduced to slender cords. The *umbilical vein*, within the cord, represents the fusion of a pair (p. 367). On entering the body it conveys the blood from the placenta, through the persistent left umbilical vein, directly to the liver, where it opens freely into the sinusoids, though its main channel is the ductus venosus running on the under side of the liver to empty into the vena cava inferior. In the adult its former course is marked by the round ligament of the liver and the ligament of the ductus venosus.

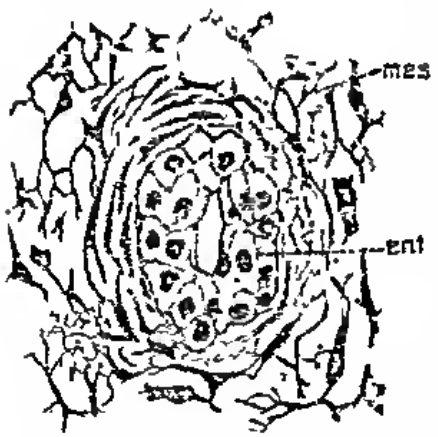


Fig. 496.—Cross Section of the Allantoic Duct, from the Umbilical Cord of a Human Embryo of Three Months. × 340 (Minot.)
Ent, Entodermal epithelium, *mes*, mesenchyma

The *allantois*, which the umbilical vessels accompany, at first extends the entire length of the cord as a slender epithelial tube. Its condition at three months is shown in Fig. 496. At birth it has become reduced to a very slender, and generally interrupted, solid strand of epithelial cells. That it may retain its continuity is stated by Ahlfeld.[1] This remnant may be sought for near the body of the embryo, and its tendency to retain its original position equidistant from the umbilical

[1] Ahlfeld, 1876.

arteries is the best guide for locating it. Within the body of the embryo the allantois is prolonged to the upper end of the bladder, with which it is continuous; this intra-abdominal part has long been called the *urachus* (*i.e.*, vas urinarium). If it remains pervious at birth, which is abnormal, urine may escape at the umbilicus.

The *yolk stalk*, surrounded by an extension of the body cavity, is found in young umbilical cords (Fig. 495, A). This stalk is a slender strand of mesoderm, containing the entodermal vitelline duct, and the vitelline vessels which accompany it to the yolk-sac. The loop of intestine from which the yolk stalk springs may also extend into the cavity of the cord, and, if it has not been drawn into the abdomen at birth, umbilical hernia results. If the cavity of the vitelline duct remains pervious at birth, the intestinal contents may escape at the umbilicus. (Such a condition is known as a fecal fistula, whereas the pervious urachus constitutes a urinary fistula.) Ordinarily the yolk stalk and its vitelline vessels, together with the cœlom of the cord, have been obliterated before birth, so that no trace of them remains in sections of the cord.

The *yolk-sac* may be found, with almost every placenta, as a very small cyst adherent to the amnion in the placental area. If the distal end of the cord is gently stretched, a wing-like fold appears, differing from all others by containing no large vessels; the fold indicates the direction of the yolk-sac, which may be exposed by stripping the amnion from the chorion. It may be beyond the limits of the placenta.

Amniotic villi are irregular, flat, opaque spots on the amnion near the distal end of the cord. They are often present and may suggest a diseased condition. They have been compared with the pointed epithelial elevations which cover the surface of the umbilical cord in ruminants, but the latter do not appear as areas of imperfect skin, and probably are entirely different structures They may appropriately be called villi, but the human 'villi' scarcely rise above the surface. Their significance is unknown.

VAGINA AND EXTERNAL GENITAL ORGANS

The *vagina* consists of a mucosa, submucosa, muscularis and fibrosa. Its epithelium is thick and stratified, its outer cells being squamous and easily detached. It rests upon low papillæ of the lamina propria, and is thrown into coarse folds or *rugæ*. Glands are absent. In the middle layers of the epithelium a dark zone can often be recognized in which the cells are undergoing cornification, but without the presence of eleidin granules, as in the skin. This zone differs in different regions of the vagina, and the picture changes at various periods of the menstrual cycle.[1] More elaborate studies of similar changes during œstrus in the

[1] Smith and Brunner, 1934.

vagina of animals have been made by others. The vaginal smears, first studied by Stockard and Papanicolaou,[1] reflect accurately the character of the surface epithelium and the amount of infiltration by leucocytes in

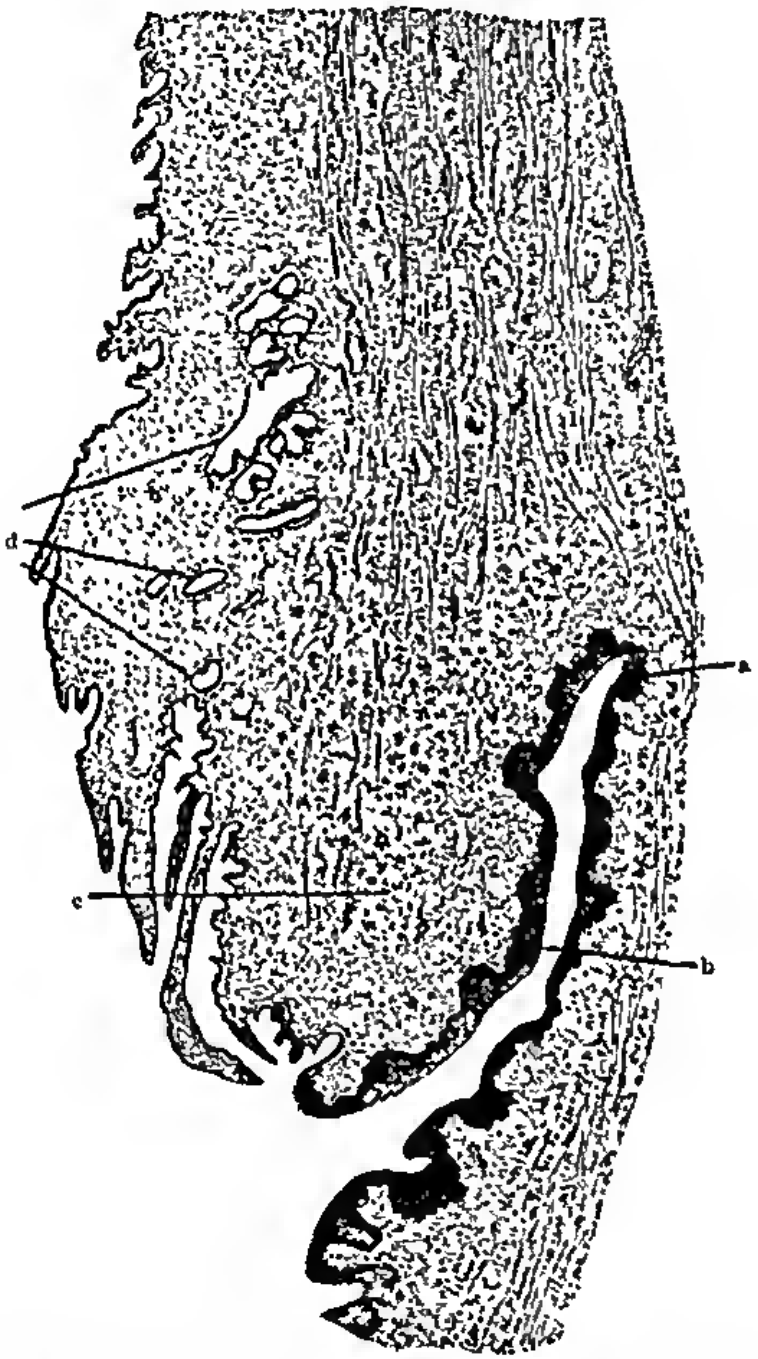


FIG. 497.—SAGITTAL SECTION OF THE CERVIX UTERI AND VAGINA OF A NEW-BORN CHILD. ONLY THE DORSAL HALF OF THE SECTION IS SHOWN. IRON-HÆMATOXYLIN AND PICRO-FUCHSIN. (SZYMONOWICZ.)
a, posterior fornix of vagina, b, epithelium of vagina, c, vaginal portion; d, cervical glands.

the different phases of the œstrous cycle, and in some animals have become the surest means of dating pregnancies. A few leucocytes are always present in the human epithelium. The lamina propria is a delicate connective tissue with few elastic fibers, containing a variable number of

[1] STOCKARD AND PAPANICOLAOU, 1917.

lymphocytes. Occasionally there are solitary nodules, above which numerous lymphocytes wander into the epithelium. The submucosa consists of loose connective tissue with coarse elastic fibers. The muscularis includes an inner circular and a small outer longitudinal layer of smooth muscle. The fibrosa is a firm connective tissue, well supplied with elastic elements. Blood and lymphatic vessels are found in the connective tissue layers, and wide veins form a close network between the muscle bundles. There is a ganglionated plexus of nerves in the fibrosa.

The mucous membrane of the vestibule differs from that of the vagina in possessing glands. The numerous *lesser vestibular glands*, 0.5–3 mm. in diameter, produce mucus; they occur chiefly near the clitoris and the outlet of the urethra. The pair of *large vestibular glands* (Bartholin's) also produce mucus; they correspond with the bulbo-urethral glands in the male and are of similar structure. The hymen consists of fine-fibered, vascular connective tissue covered with mucous membrane. The clitoris is an erectile body, resembling the penis. It includes two small *corpora cavernosa*. The glans clitoridis contains a thick net of veins. It is not, as in the male, at the tip of a corpus cavernosum urethræ which begins as a medium bulb in the perineal region; the bulbus in the female exists as a pair of highly vascular bodies, one on each side of the vestibule. Each is called a *bulbus vestibuli*. The labia minora contain sebaceous glands, 0.2–2.0 mm. in size, which are not connected with hair follicles; they first become distinct between the third and sixth years. The labia majora have the structure of skin.

SKIN

The skin (*cutis*) consists of an ectodermal epithelium, the *epidermis*, and a mesodermal connective tissue, the *corium* or dermis (Fig. 363). The epidermis is a specialized stratified squamous epithelium, and besides covering the entire body gives rise to hairs, nails, the enamel of the teeth, and several types of glands. Sweat glands and sebaceous glands, the latter usually associated with hairs, are the most widely distributed; locally the epidermis forms the *mammary glands*, *ceruminous glands* of the ear, *ciliary glands* of the eyelids, and other special types. The greater part of the surface of the skin presents many little furrows, the *sulci cutis*, which intersect so that they bound rectangular spaces. On the palms and soles the furrows are parallel for considerable distances, being separated from one another by slender ridges, the *cristæ cutis*, along the summits of which the sweat glands open. The ridges are most highly developed over the pads of tissue at the finger tips, where they present the familiar spiral and concentric patterns. These pads of connective tissue, the *toruli tactiles*, must not be confounded with elevations due to underlying mus-

cles. The patterns of these ridges are now used so widely in finger print identification that their classification has become a separate science.

In the embryo the surface layer of cells of the few-layered epithelium becomes specially differentiated. The cells become larger, dome-shaped, and stain more deeply (Fig. 498). Because of the fact that the growing hairs do not penetrate this layer, but cause it to be cast off, it has been called the *epitrichium* ('upon the hairs'). The layer is chiefly of interest because of its possible homology with the syncytial outer layer of the early chorion. The epitrichium has been found on the umbilical cord and in places on the amnion. The thickness of the skin on soles, palms, and finger tips is not due to pressure, like later calluses, as it is already present in the fetus.

In the adult epidermis two main layers may be distinguished, subdivisions of a single thick stratified epithelium. The outer layer is the

FIG. 498.—SKIN FROM THE OCCIPUT OF AN EMBRYO OF TWO AND ONE-HALF MONTHS (Bowen)
The outer layer of dark cells is the epitrichium.

stratum corneum, the inner the *stratum germinativum*. The latter rests by an irregular base on the papillæ of the corium. The basal cells constitute a single row of columnar cells with elongated nuclei and indistinct cell walls. The upper layers are the usual 14-sided cells of stratified epithelium, connected by intercellular bridges. In all the many layers of the stratum germinativum, and not only in the basal layer as might be supposed, mitoses occur[1] supplying new cells to replace those lost from the skin surface. Mitosis occurs in animals in a rhythmic variation; most of the growth occurring at night.[2]

The whole stratum germinativum was formerly called the stratum spinosum, from the appearance given by the bridges; and also the stratum mucosum, and rete Malpighi. It was first described by Malpighi, who recognized its soft or 'mucous' nature and referred to it as a rete because it forms a network between the rounded papillæ of the corium

The transition from the stratum germinativum to the stratum corneum is abrupt. It may be marked by an incomplete layer of coarsely granular cells, such as are highly developed in the skin of the palms and soles, where they form the *stratum granulosum*, which may be considered a subdivision of the stratum germinativum. As they move out into the stratum corneum the cells acquire a horny exoplasmic membrane; the bridges become short stiff spines; the cytoplasm and nucleus are dry and shrunken; and in the outermost cells the nucleus wholly disappears. This process, called 'keratinization,' is described by Ludford.[3] The cells

[1] THURINGER, 1928. [2] CARLETON, 1934. [3] LUDFORD, 1924*b*.

become flatter toward the surface, from which they are constantly being desquamated.

The stratum granulosum is an inconspicuous layer and the stratum corneum only a few cells thick in the skin over most of the body, but in sections of the palms and soles the process of cornification presents a

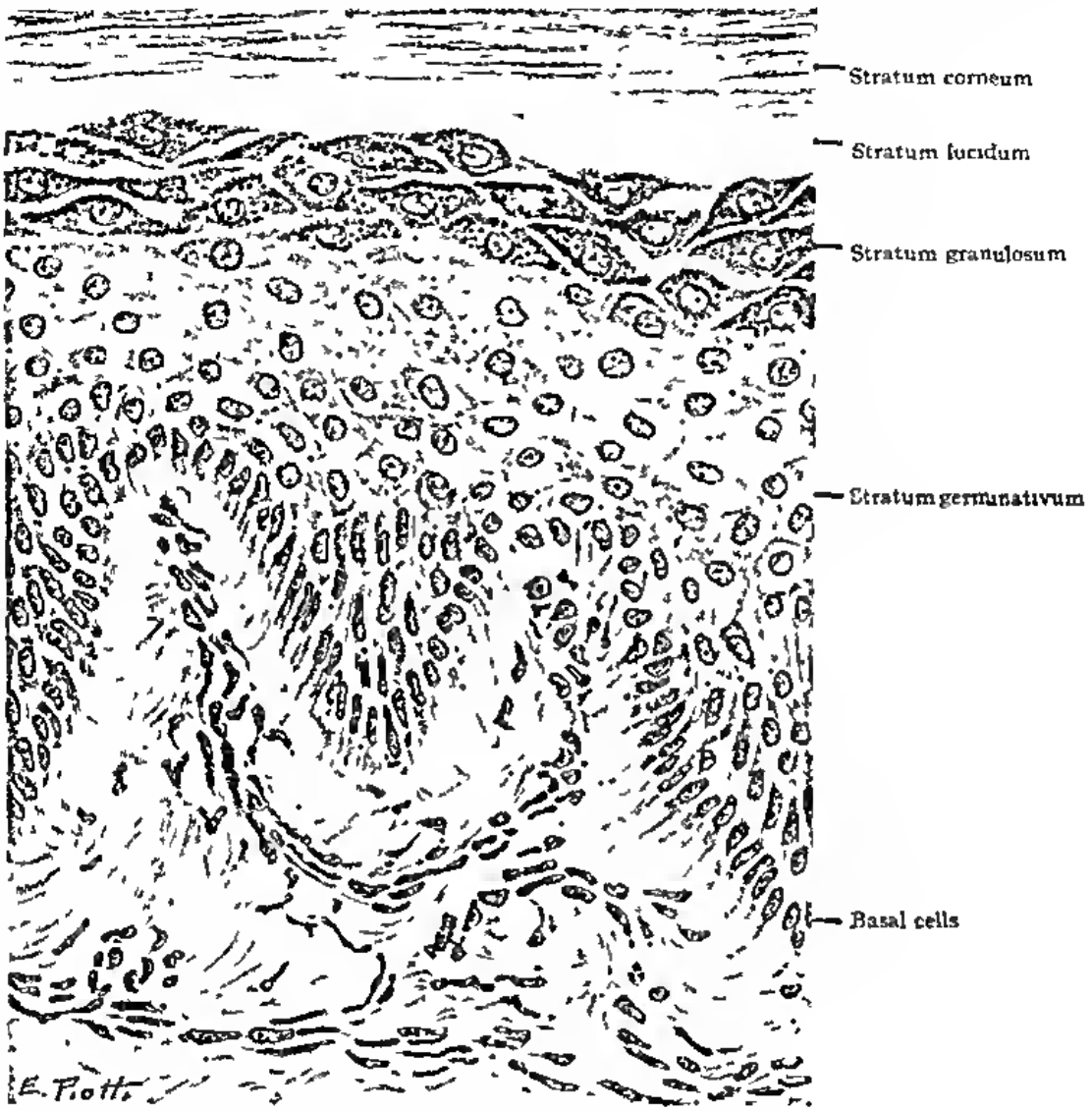


Fig. 499.—Section of a Portion of the Epidermis from the Sole of a Human Foot. Zenker fixation, hæmatoxylin and eosin.

more elaborate picture. In hæmatoxylin and eosin specimens, the granular layer or *stratum granulosum* is followed by a pink and then by a bluish band. The clearness of these two colored bands gives them the name of *stratum lucidum*, a subdivision of the stratum corneum. The rest of this layer in the palms and soles is very thick, composed of heaped-up dead cells, dry and squamous.

Chemically the coarse granules of the stratum granulosum resemble the horny substance *keratin* (from which they differ by dissolving in caustic potash); they are

therefore called *kerato-hyalin* granules. Their diffuse product in the stratum lucidum is named *eleidin*. In the corneum it becomes *pareleidin*, which, like fat, blackens with osmic acid, but the reaction occurs more slowly. The pareleidin is not due to fat entering the skin from oily secretions on its outer surface.

Experimental deprivation of the fat soluble vitamin A results in animals in the substitution of stratified keratinized epithelium for normal epithelium in various parts of the respiratory and alimentary tracts, eyes, and genito-urinary tract.[1] 'Replacement

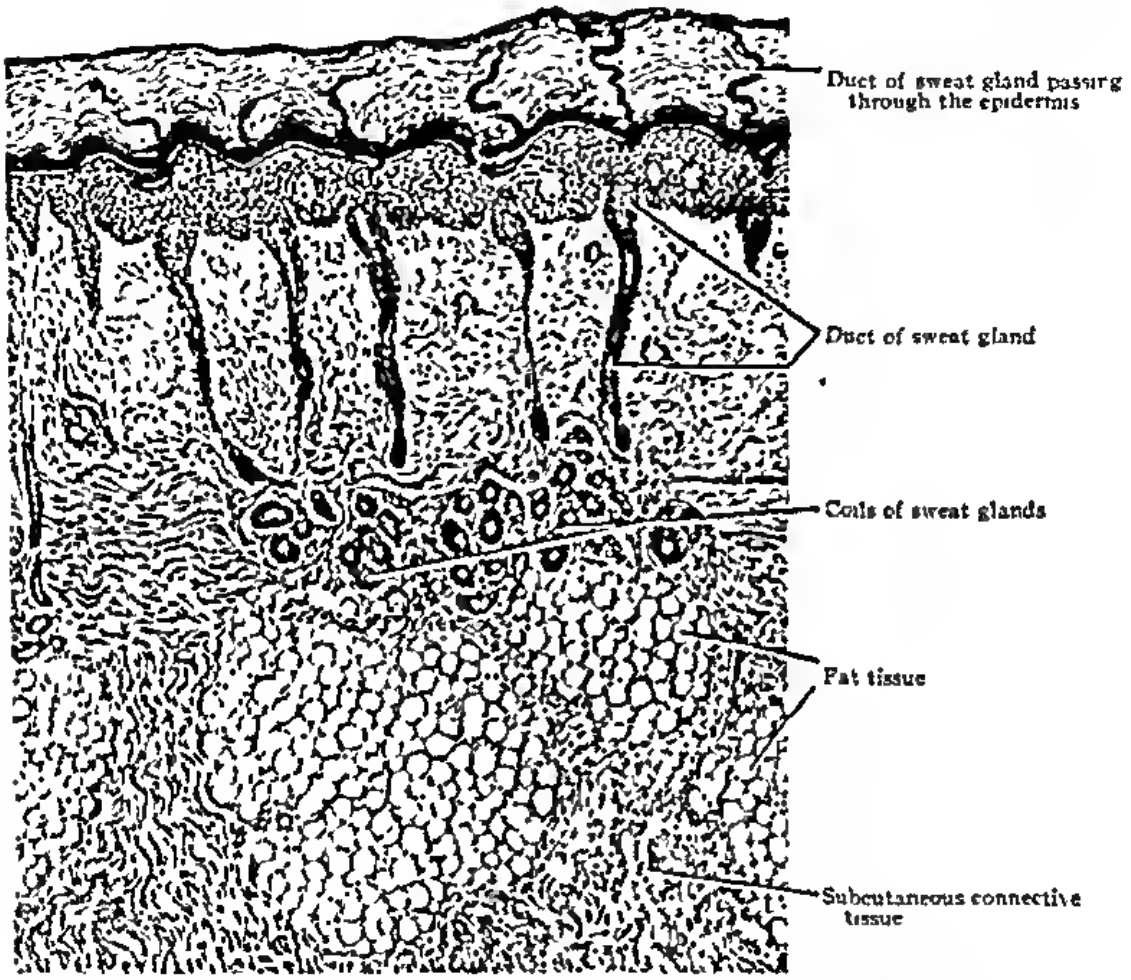


FIG. 500.—SECTION THROUGH THE SOLE OF A NEW-BORN CHILD (Kyrle)

of epithelium arises from focal proliferation of cells arising from the original epithelium and not by differentiation or change of preexisting cells.'

The color of the skin is due partly to fine pigment granules in and between the lowest layers of the epidermal cells and partly to the blood in the vessels beneath the epidermis. Underlying cells of the corium sometimes contain stores of finer pigment granules, but such cells are absent from the palms and soles, and are infrequent elsewhere. They may be found in the deeply pigmented tissue in the eyelids, the axilla, the areolæ of the nipples, the external genitalia and around the anus. According to Edwards and Duntley,[2] the human skin contains five different pig-

[1] WOLBACH AND HOWE, 1925 [2] EDWARDS AND DUNTLEY, 1939.

ments: (1) melanin, (2) melanoid, (3) oxyhæmoglobin, (4) reduced hæmoglobin and (5) carotene. No other pigments are found in the darker races, the differences in color is due only to the amount. 'Tanning' is secondary pigmentation. On exposure to sunlight, there is first an acute reaction resulting in a hyperæmia (more blood to the skin, therefore redness), followed by a venous stagnation in the skin which may last for months. Melanin starts to show an increase in about two days and after reaching a maximum begins to degenerate, producing the allied pigment melanoid which gradually disappears.

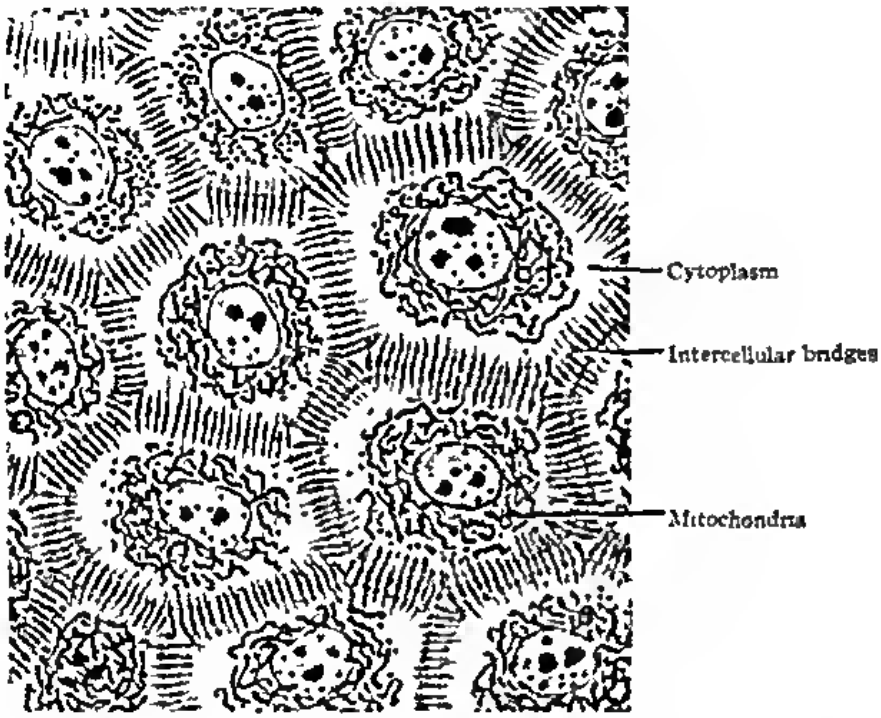


FIG 501.—MITOCHONDRIA IN HUMAN EPIDERMAL CELLS.
Note the intercellular bridges and the edge-canals where three cells meet. (Del Rio Hortega)

Corium. The corium is a layer of densely interwoven bundles of connective tissue extending from the epidermis to the fatty, areolar *subcutaneous tissue.* Toward the epidermis the corium forms *papillæ*, which vary considerably in size and number in different parts of the body. They are tallest (even 0.2 mm. high) and most numerous, often being branched, in the palms and soles. Beneath the epidermal ridges they may occur quite regularly in double rows, as long since observed by Malpighi. In the skin of the face the papillæ are poorly developed, and in advanced age they may wholly disappear. The papillæ are composed of cellular connective tissue, which forms a *lamina propria;* and each papilla contains a terminal knot of capillary blood vessels, or a tactile corpuscle. The corpuscles are most numerous in the sensitive finger tips, where they may be found in one papilla in every four.

The entire corium is somewhat arbitrarily subdivided into an outer *stratum papillare* and an inner *stratum reticulare*. These layers blend with one another, but the outer portion consists of finer bundles of connective tissue, more closely interwoven than those in the coarse network characteristic of the stratum reticulare. Beneath the skin, but inseparable

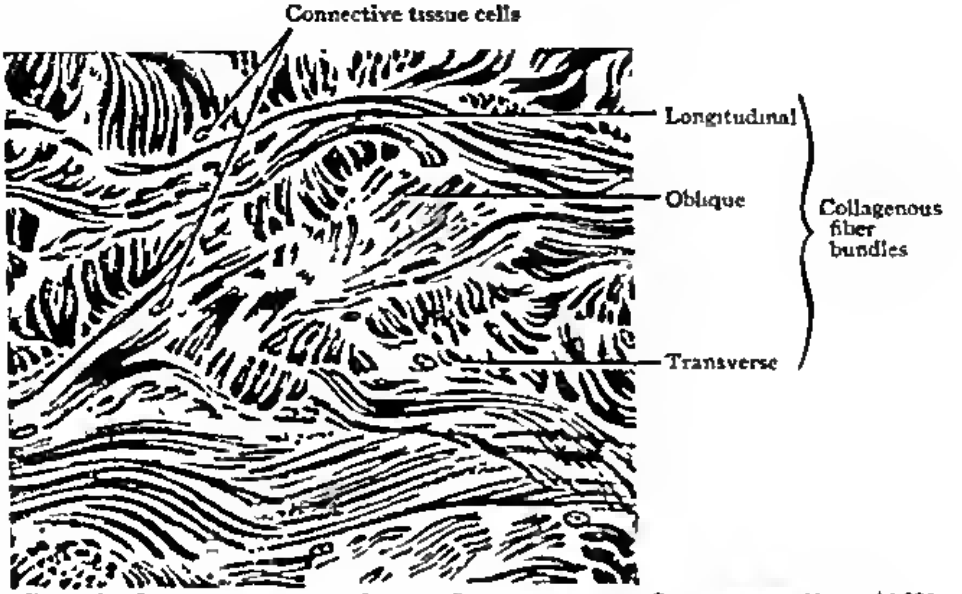


Fig. 502.—Section through the Stratum Reticulare of the Corium of the Hand. × 500. Alcohol fixation, eosin, phosphomolybdic acid, methyl blue. (von Möllendorff.)

from it, is the *stratum subcutaneum*, which is composed of areolar tissue with large areas of fat cells; where the fat forms a continuous layer, it is known as the *panniculus adiposus*. Finally the bundles of the stratum subcutaneum connect more or less intimately with the fascia around the muscles, or, in places, with the periosteum.

Fig. 503.—Model of the Coiled Part of a Sweat Gland from the Sole of the Foot (Huber.)

The elastic fibers of the corium form evenly distributed networks, which are finer in the stratum papillare and coarser in the stratum reticulare. There is said to be a subepithelial network, and a layer of numerous coarse fibers immediately above the general layer of fascia. In old age a notable decrease in the elastic fibers has been recorded. The muscle fibers of the corium are chiefly the small bundles of smooth muscle attached to the sheaths of the hairs, forming the *arrectores pilorum*. Smooth muscle is diffusely distributed in the nipple, and in the scrotum it forms a layer pervaded by elastic tissue, known as the *tunica dartos*. Striated muscle fibers derived from the muscles of expression terminate in the skin of the face. The vessels and nerves of the corium are described on page 587.

Sweat Glands. The *glandulæ sudoriparæ* are long, unbranched tubes terminating in a simple coil (described by Oliver Wendell Holmes as

resembling a fairy's intestine, Fig. 503). The coil is found in the deep part of the corium or in the subcutaneous tissue. The duct pursues a straight or somewhat tortuous course to the epidermis which it enters between the connective tissue papillæ. Within the epidermis its spiral windings are pronounced; it ends in a pore which may be detected macroscopically.

The epithelium of the ducts consists of two or three layers of cuboidal cells; it has an inner cuticula, and an outer basement membrane covered

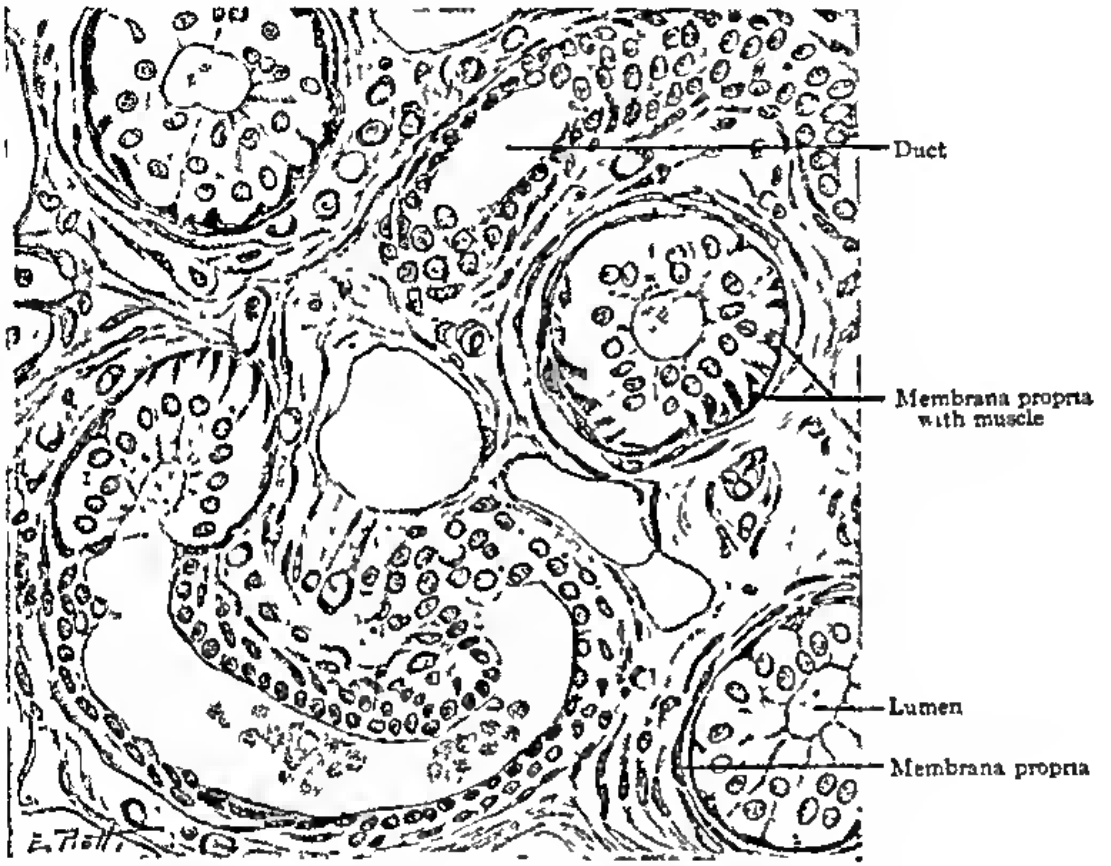


FIG. 504.—SECTION OF SWEAT GLANDS AND DUCTS HUMAN FINGER TIP
Note the smooth muscle at the bases of the cells in the secretory portion and the secretory droplets in the ducts. Formaldehyde fixation, hæmatoxylin and eosin

by longitudinal connective tissue fibers. Within the epidermis its walls are made of the cells of the strata through which it passes. The secretory portion of the gland (3.0 mm. long according to Huber) forms about three-fourths of the coil, the duct constituting the remainder. The secretory epithelium is a simple layer of cells, varying from low cuboidal to columnar, according to the amount of secretion which they contain. Those filled with secretion present granules, some of which are pigment and fat. The product is eliminated through intra- and intercellular secretory capillaries. It is ordinarily a fatty fluid for oiling the skin, but it becomes the watery sweat under the influence of the nerves. The gland cells are not destroyed by either form of activity. The secretory tubule is

surrounded by a distinct basement membrane, within which there is a row of small, longitudinally elongated cells described as muscle fibers.

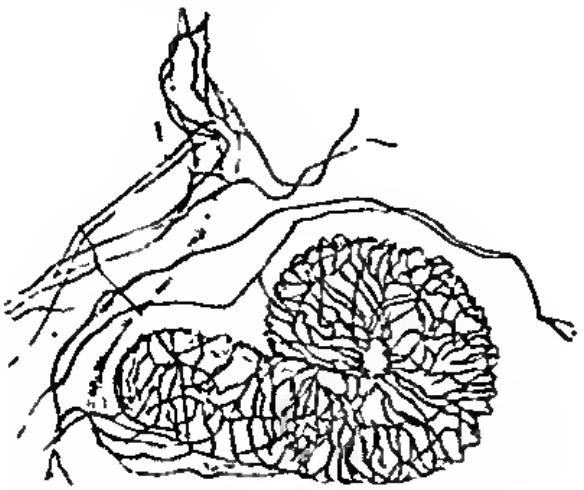


Fig. 505.—Nerve Fibers on the Ampulla of a Sweat Gland with Excretory Duct. Human. Methylene Blue Preparation. (Arnstein.)

They do not form a complete membrane, and they appear as a continuation of the basal layer of cells of the ducts.

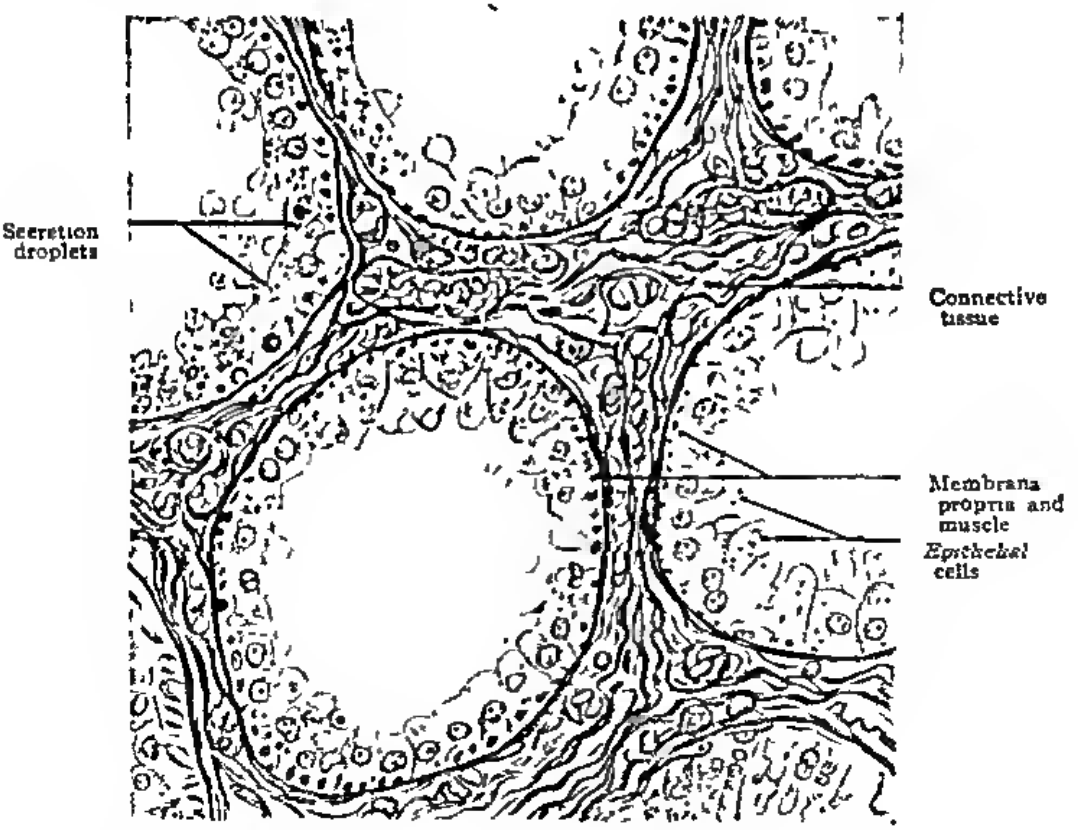


Fig. 506.—Section of an Axillary Gland from a Human Female. Apocrine type of secretion. Zenker fixation, Azan.

Sweat glands are distributed over the entire skin, except that of the glans and the inner layer of the præputium penis. They are most numerous in the palms and soles

Another type of sweat gland is present in the axilla, around the nipple, on the scrotum, labia majora and pubic regions, and surrounding the anus. They are large and branched, sometimes with 30 mm. of coiled tube, and are characterized, according to Schiefferdecker,[1] by having an apocrine method of secretion and by their constant relation to hair follicles. The secretion is stored in the cells as droplets in the upper poles which then bulge, become stalked, and cast off. The cell thus loses the rim of its cytoplasm which encloses the droplet. Ordinary serous secretion may be going on at the same time in neighboring cells. The process is similar to the secretion of milk, and the mammary gland is possibly a modified sweat gland of this type. It will be noted that their distribution coincides with the course of the embryonic milk-line. They acquire their large size at puberty, and have been considered as sexual 'odoriferous' glands, and are said to enlarge at the menstrual periods. All these glands are associated with smooth muscle—the *muscularis sexualis.*

Sebaceous Glands. The sebaceous glands are simple, branched or unbranched alveolar structures situated in the superficial layer of the corium and usually appended to the sheath of a hair. In some regions of the body a large gland may be associated with a very small hair, and in exceptional cases, as at the margin of the lip or on the labia minora, they occur independently of hairs. They vary in size from 0.2 to 2.2 mm., the largest being found in the skin of the nose where the ducts are macroscopic. None are found in the palms or soles, where hairs also are absent.

The short duct is a prolongation of the outer epithelial sheath of the hair and is formed of stratified epithelium, the number of layers of which decreases toward the alveoli. The alveoli consist of small cuboidal basal cells, and of large rounded inner cells in all stages of fatty metamorphosis. As the cell becomes full of small fat droplets, the nucleus degenerates, and the whole cell is cast off with its contained secretion. Thus the process may be considered as the desquamation of cells from the upper layers of a stratified epithelium, and differs from the secretion of the sweat glands; the glands are actually cytogenic. In life the product of the glands is a semi-fluid material, composed of fat and broken-down cells.

Glandulæ præputiales are sebaceous glands without hairs which are sometimes, but not always, found on the glans and præputium penis. The designation 'Tyson's glands' is not justified since Tyson described the epithelial pockets ½ to 1 cm. long which regularly occur near the frenulum præputii. Præputial glands and crypts are not found in the embryo. The præputium is united to the outer surface of the glans by an

[1] SCHIEFFERDECKER, 1917.

epithelial mass, which often persists after birth and is broken up by the formation of concentric epithelial pearls. Glands and crypts are absent from the præputium and glans of the clitoris.

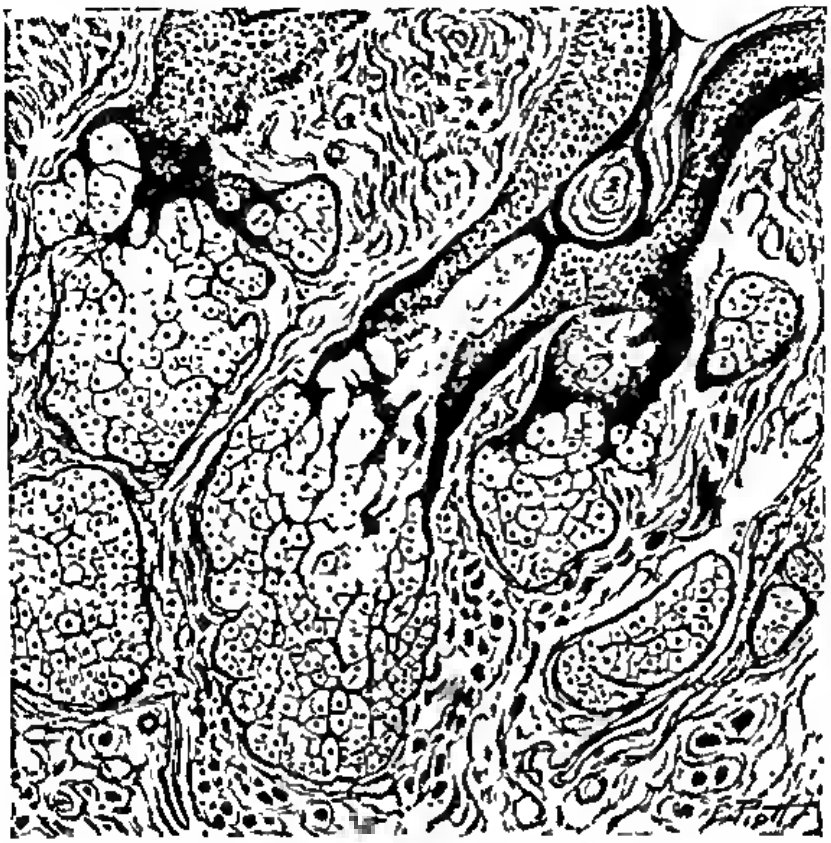


Fig. 507.—Vertical Section through a Sebaceous Gland from the Wing of the Nose. Susa fixation, Azan. (Drawn from a preparation by Prof. M. Heidenhain.)

MAMMARY GLANDS

In young mammalian embryos generally, the mammary glands are first indicated by a thickened line of ectoderm extending from the axilla

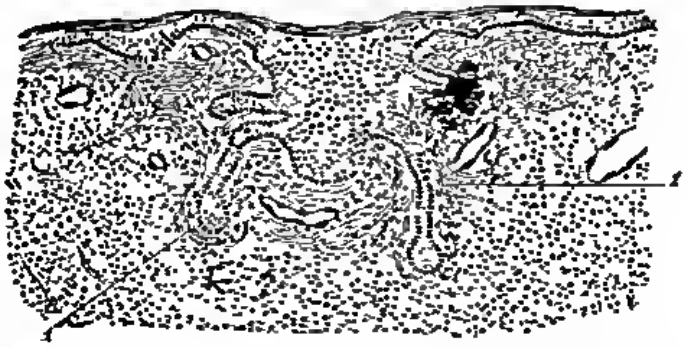


Fig. 508.—Section through the Mammary Gland of an Embryo of 25 cm. 1, Connective tissue of the gland. (After Basch, from McMurrich.)

to the groin. Later much of the line disappears, leaving a succession of nodular thickenings corresponding with the nipples. In some mammals this row of nipples remains, in others only the inguinal thickenings, and

in still others only those toward the axilla. Thus in man there is normally only one nipple on each side, but structures interpreted as accessory nipples are frequent; they are not always situated along the mammary line. In an embryo of 25 cm. (Fig. 508) several solid cords have grown out from the ectodermal proliferation. There are ultimately from 15 to 20 of these in each breast, and they branch as they extend through the connective tissue. At birth the nipple has become everted, making an elevation, and at that time the glands in either sex may discharge a

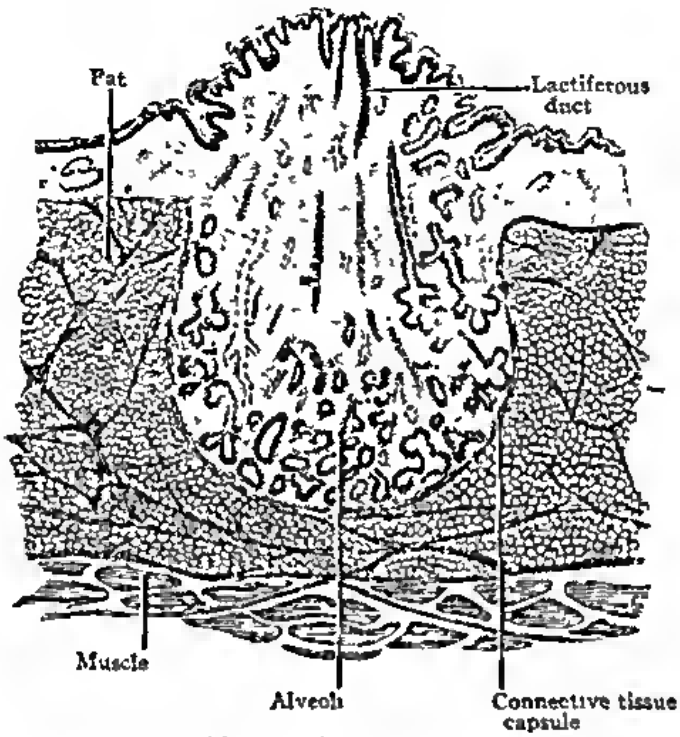


FIG. 509.—SECTION THROUGH THE MAMMARY GLAND OF A NEW-BORN FEMALE CHILD (Corning)

little milky secretion similar to the *colostrum* which precedes lactation. The glands grow in both sexes until puberty, when those in the male atrophy and only the main ducts persist. In the female enlarged terminal alveoli are scarcely evident until pregnancy. The glands until then are discoid masses of connective tissue and fat cells, showing in sections small scattered groups of duct-like tubes.

At the beginning of pregnancy a great growth of new end-pieces begins, accompanied by a rearrangement of the surrounding connective tissue. Numerous mitotic figures appear in the epithelium. This change is apparently due to the influence of an ovarian hormone, found in the liquor folliculi of ripe follicles; a placental hormone may also be active in this respect. The interesting reactions of these hormones in spayed monkeys and their further influence on other sexual organs are described by Allen.[1]

[1] ALLEN, E., 1927.

Toward the end of pregnancy each of the fifteen or twenty branched glands forms a mammary lobe, and its alveolo-tubular end-pieces are grouped in lobules. The secretory epithelium is a simple cuboidal or flattened layer, in which fat accumulates at the seventh or eighth month of pregnancy. The fat first appears as small granules at the basal ends of the cells. It is apparently the result of an active synthesis by the cell protoplasm, and not of cell degeneration as in sebaceous glands. The granules increase in size and number, and accumulate in the form of droplets or a single large drop of fat in the upper or apical pole of each cell, where they may cause the cell membrane to bulge, as is seen also

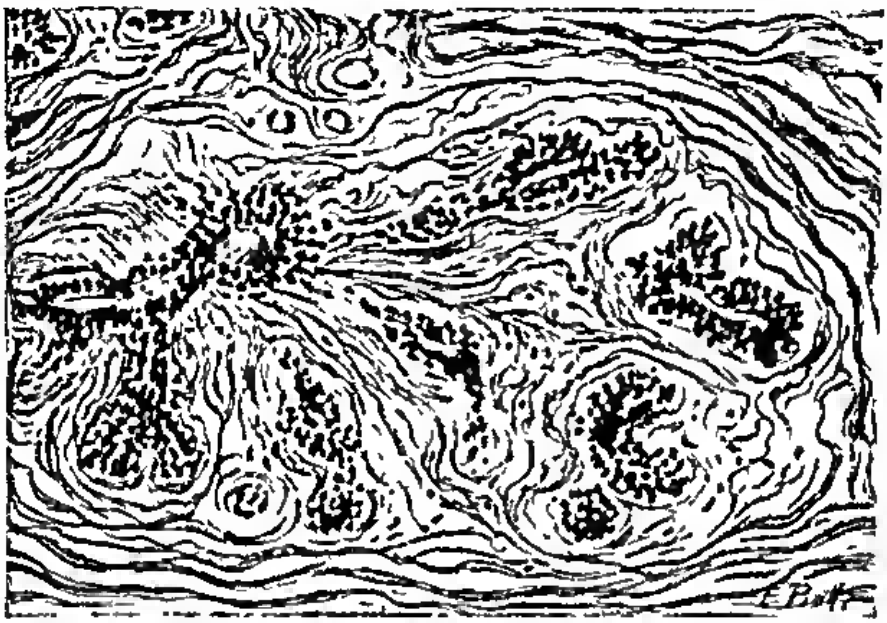


FIG. 510.—SECTION FROM THE MAMMARY GLAND OF A 20 YR. OLD WOMAN. RESTING STAGE. Zenker fixation, Azan.

in the large sweat glands. Phagocytic cells, derived from the connective tissue, make their way between the epithelial cells of the alveoli and enter the gland lumen, where some of them degenerate; others receive fat from the gland cells, either in solution, or in drops which are devoured by phagocytic action. These fat-containing cells may grow to considerable size and are called *colostrum corpuscles*. Beneath the alveolar epithelium there are basal or basket cells, which have been compared with the muscle fibers of sweat glands. A basement membrane separates them from the connective tissue, which contains many lymphocytes and eosinophilic cells.

After the birth of the child, the first milk delivered is that found in the lactiferous ducts and sinuses, and consists of a watery fluid containing many colostrum corpuscles. At the same time the fat droplets are released from the gland cells either by rupture of the cell membrane or by the loss of the whole upper pole of the cell, the fat droplet being

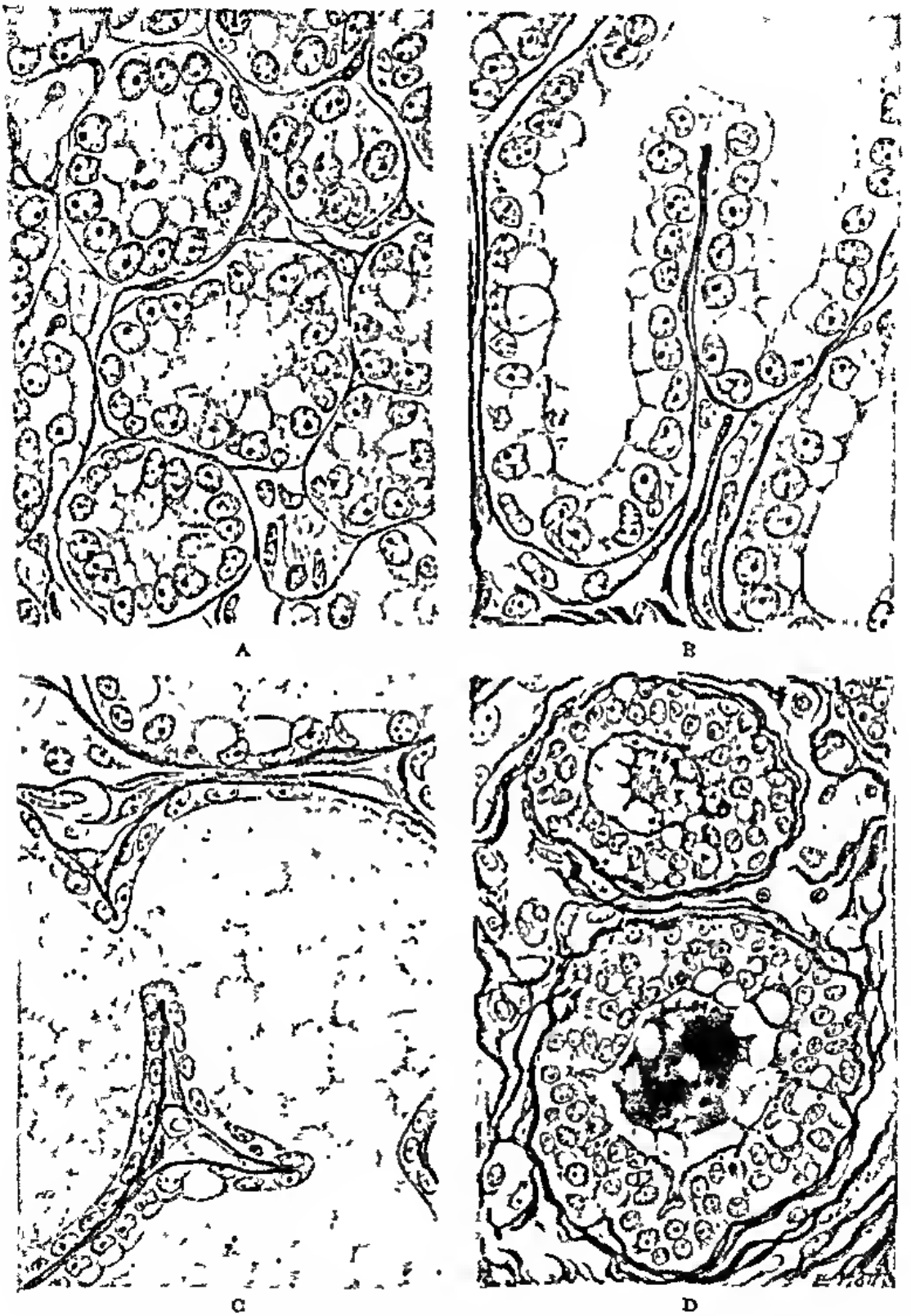


FIG. 511.—HUMAN MAMMARY GLAND IN DIFFERENT FUNCTIONAL STATES

A, late pregnancy; B, lactation, active phase; C, lactation, storage phase; D, beginning of regression. Note the heights of the epithelial cells. Zenker-formaldehyde fixation; Azan.

enclosed in a film of protoplasm. The empty cells are flat, with irregular surface; they begin again the formation of fat granules and fat droplets. Morphological changes have been observed in the mitochondria and Golgi apparatus corresponding to the functional states.[1] Few gland cells are cast off and few mitoses are seen. The relations of the hormones to the

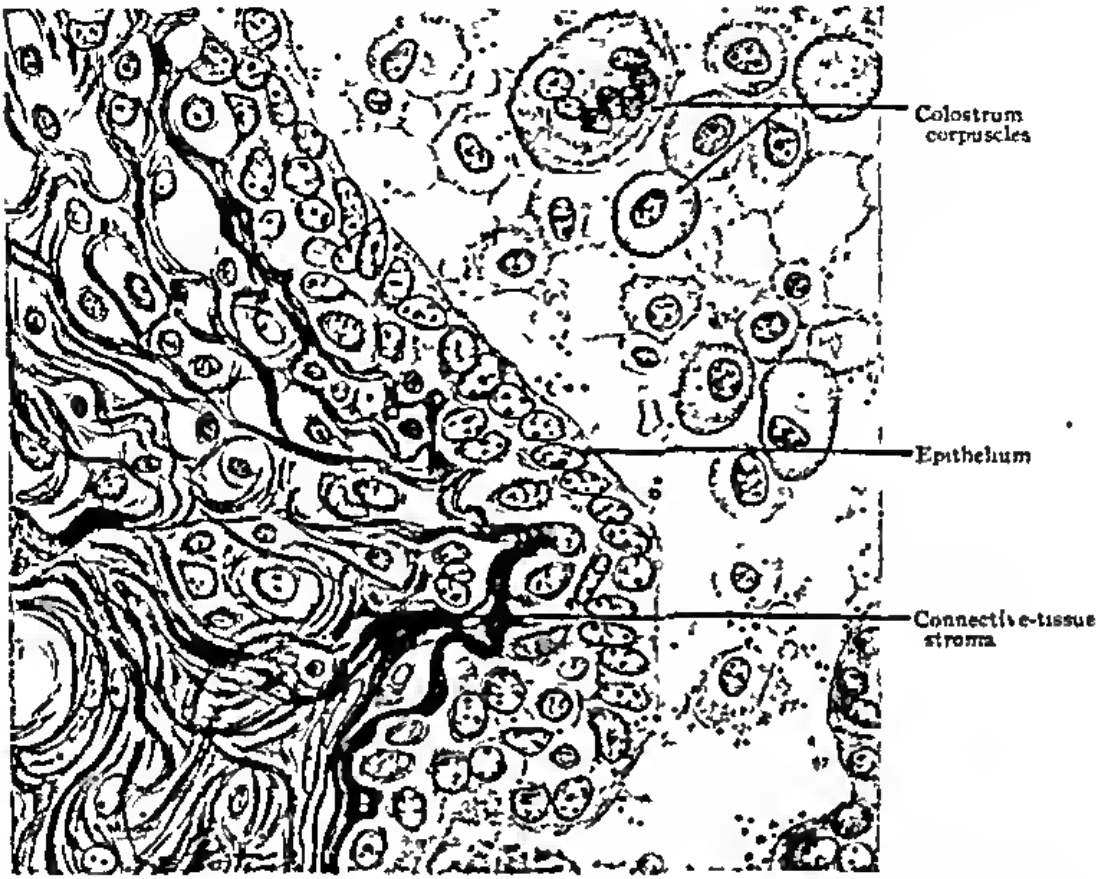


FIG. 512.—PORTION OF A SECTION OF A MAMMARY GLAND OF AN 18 YR. OLD WOMAN, DYING A FEW DAYS AFTER GIVING BIRTH TO A CHILD

Colostrum corpuscles in the lumen, 'lymphoid' cells in the connective tissue stroma and in the epithelial wall. Zenker fixation, Azan

phenomenon of lactation are given on p. 536 and are discussed by W. O. Nelson, Physiol. Revs., 16, 1936.

Milk consists of fat droplets, 2–5 μ in diameter, floating in a clear fluid which contains nuclein derived from degenerating nuclei, and occasionally a leucocyte or colostrum corpuscle. Free nuclei may be found, and some cells which undoubtedly are to be interpreted as detached from the alveoli of the gland. Emmel[2] shows that leucocytes contribute a definite cellular constituent to milk.

At the end of lactation, the connective tissue, which has become greatly reduced owing to the enlargement of the glands, increases in quantity and the leucocytes reappear; as during pregnancy, they form colostrum corpuscles. The lobules become smaller and the alveoli begin

[1] WEATHERFORD, 1929. [2] EMMEL, WEATHERFORD AND STREICHER, 1926.

to degenerate. In old persons all the end-pieces and lobules have gone and only the ducts remain.

The *ducts* are lined with simple columnar epithelium, surrounded by a basement membrane and generally by circular connective tissue bundles. Toward the nipple each duct forms a considerable spindle-shaped dilatation, the *sinus lactiferus*. The epithelium near the outlet of the ducts is stratified and squamous.

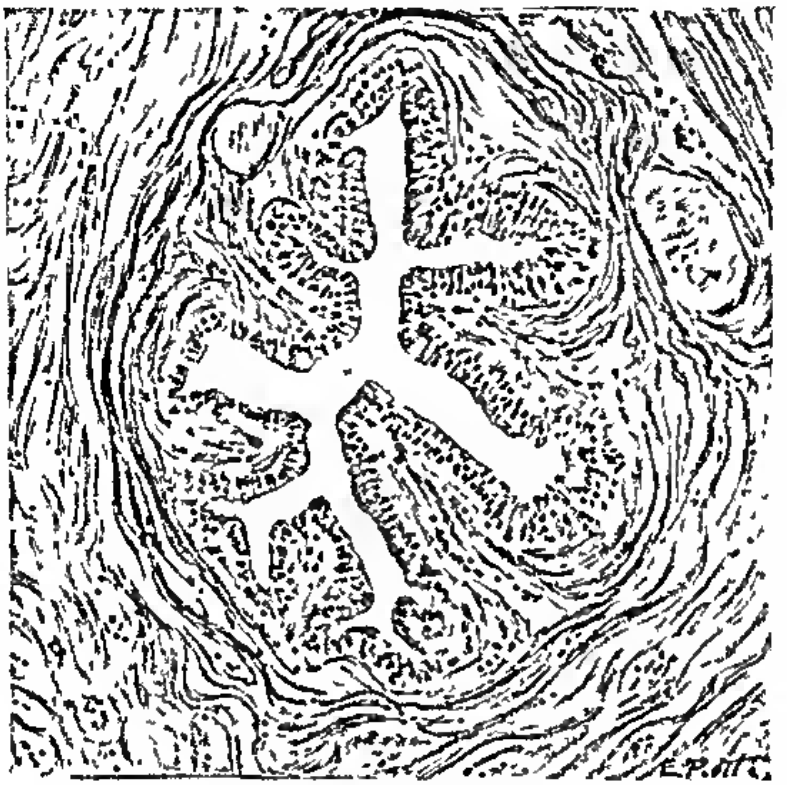


FIG 513.—CROSS-SECTION OF A LACTIFEROUS DUCT—HUMAN NIPPLE
Zenker fixation, hæmatoxylin and eosin

The skin of the nipple, and of the *areola* at its base, contains abundant pigment in the deepest layers of its epidermis. The corium forms tall papillæ and contains smooth muscle fibers, some of which extend vertically through the nipple and others are circularly arranged around the ducts. There are tactile corpuscles in the nipple, and lamellar corpuscles have been found beneath its areola. It is particularly sensitive, and upon irritation becomes rapidly elevated, due both to muscular and vascular activity. There are many sweat and sebaceous glands in the areola, and occasional rudimentary hairs. The *areolar glands* (of Montgomery) are branched tubular glands having a lactiferous sinus and otherwise resembling the constituent mammary glands. Their funnel-shaped outlets are surrounded by large sebaceous glands. The areolar glands are regarded as transitions between sweat glands and mammary glands.

Blood vessels enter the breast from several sources and form capillaries around the alveoli. Lymphatic vessels are found in the areola,

around the sinuses, and in the interlobular tissue. The collecting lymphatics pass chiefly toward the axilla; a few penetrate the intercostal spaces toward the sternum. The nerves are mostly those which supply the blood vessels, but fibers are said to extend to the glandular epithelium.

NAILS

The nails are areas of modified skin consisting of corium and epithelium. The corium is composed of fibrous and elastic tissue, the bundles of which in part extend vertically between the periosteum of the phalanx and the epithelium, and in part run lengthwise of the finger. In place of

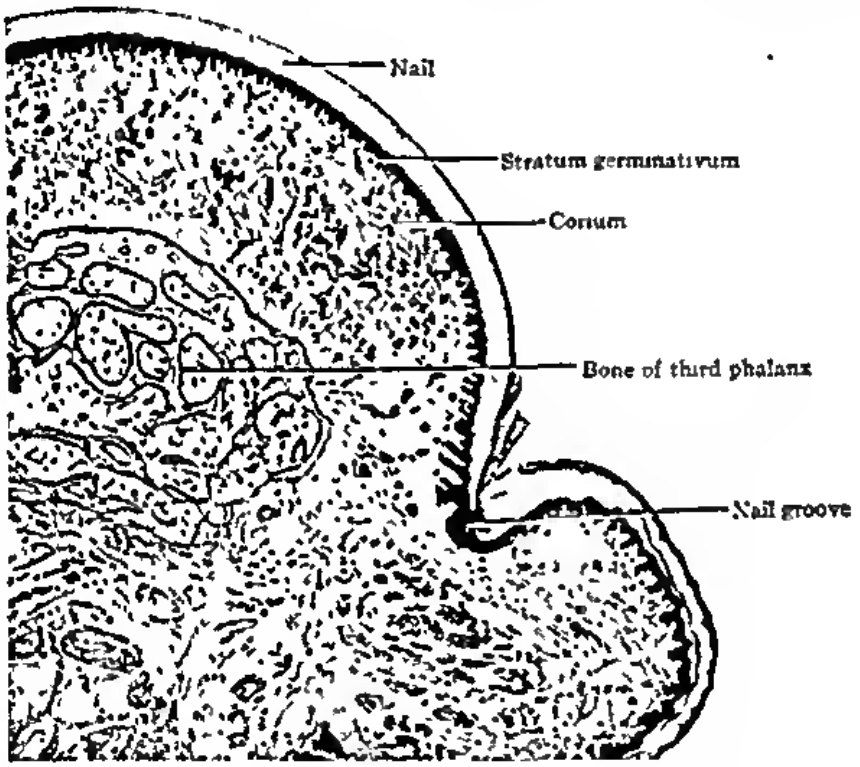


FIG. 514.—CROSS-SECTION THROUGH A HUMAN FINGER TIP. × 10
Hæmatoxylin and eosin (von Möllendorff)

papillæ, the corium of the nail forms narrow longitudinal ridges, which are low near the root of the nail but increase in height toward its free distal border; there they abruptly give place to the papillæ of the skin. The epithelium consists of a *stratum germinativum* and a *stratum corneum*. The latter, according to Bowen,[1] represents a greatly thickened stratum lucidum, but this opinion requires confirmation. In the embryo the horny substance is entirely covered by a looser layer, the *eponychium*, and this name is applied in the adult to the skin-like tissue which overlaps the root and sides of the nail. The eponychium is the stratum corneum of the adjoining skin.

[1] BOWEN, J. T., 1889.

It is now generally considered that the cells of the stratum germinativum covering the greater part of the 'nail bed' do not produce any of the overlying horny material. This function is reserved for the germinative cells at the root of the nail, beneath the crescentic white area, the *lunula*, and its extension backward under the *nail fold*. The latter is a fold of skin which is deep at the root of the nail, but becomes shallower as it extends forward on each side, bounded by the nail wall. It is now stated that cornification in the nails takes place without the formation of keratohyalin granules, and a fibrillar arrangement of the keratin has been thought to account for the whiteness and opacity of the lunula. The cornified cells of the nail may be separated by placing a fragment in a strong solution of caustic potash and heating to boiling. The cells differ from those in the outer layers of the skin by retaining their nuclei.

HAIR

Development. The hairs arise as local thickenings of the epidermis. They soon become round columns of ectodermal cells extending obliquely downward into the corium (Fig. 515). As the columns elongate, the terminal portion becomes enlarged, forming the *bulb* of the hair, and a mesodermal *papilla* occupies the center of the bulb. On that side of the epithelial column which from its obliquity may be called the lower surface, there are found two swellings (Figs. 516–518). The upper is to become a *sebaceous gland*, discharging its secretion into the epithelial column; the lower or deeper swelling is called the 'epithelial bed,' and its cells, which increase by mitosis, contribute to the growth of the column. (The lower swelling is often described as the place of insertion of the arrector pili muscle.) Beginning near the bulb, the core of the column separates from the peripheral cells; the latter becomes the *outer sheath* of the hair. The core forms the *inner sheath* and the *shaft* of the hair. The cells of the shaft become cornified just above the bulb, and they are surrounded by the inner sheath as far as the sebaceous gland. Beyond this point the inner sheath degenerates, so that in later stages the distal part of the shaft is immediately surrounded by the outer sheath. As new cells are added to the hair from below, the shaft is pushed toward the surface. The central cells of the outer end of the column degenerate, thus producing a 'hair canal' which is prolonged laterally in the epidermis

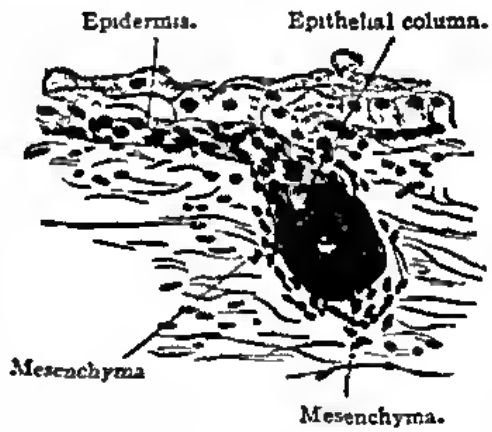


FIG. 515.—VERTICAL SECTION OF THE SKIN OF THE BACK OF A HUMAN EMBRYO OF FIVE MONTHS. × 230

FIG 516.—VERTICAL SECTION OF THE SKIN OF THE GLUTEAL REGION OF A HUMAN EMBRYO OF FIVE MONTHS × 230

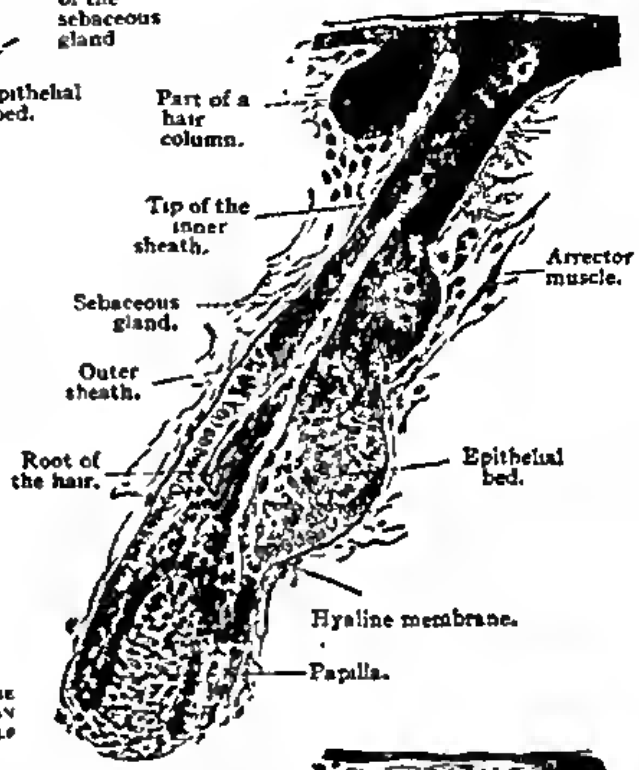


FIG 517.—VERTICAL SECTION OF THE SKIN OF THE BACK OF A HUMAN EMBRYO OF FIVE AND A HALF MONTHS × 230

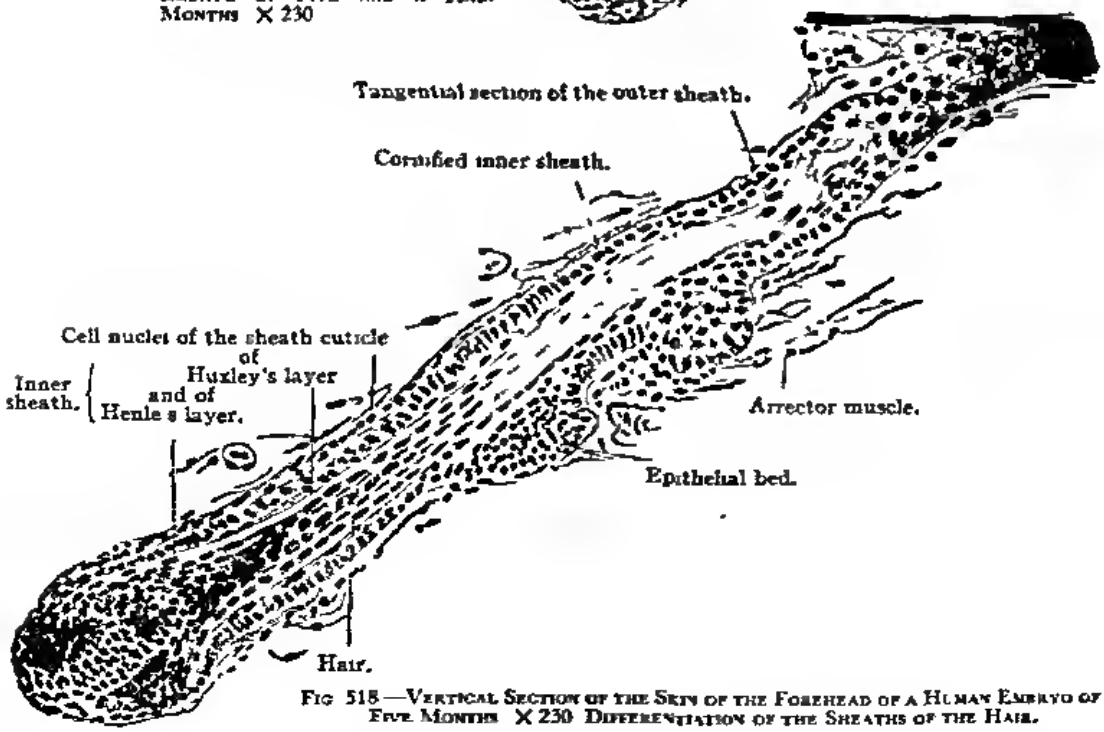


FIG 518.—VERTICAL SECTION OF THE SKIN OF THE FOREHEAD OF A HUMAN EMBRYO OF FIVE MONTHS × 230 DIFFERENTIATION OF THE SHEATHS OF THE HAIR.

(Fig. 519). The shaft enters the canal, breaks up the overlying epitrichium, and projects from the surface of the body. That portion of the hair which remains beneath the epidermis is called its *root*. In addition to the epithelial sheaths, the root in all large hairs possesses a *connective tissue sheath*, derived from the corium. This serves for the insertion of a bundle of smooth muscle fibers, the other end of which is connected with the elastic and fibrous elements in the superficial part of the corium. Since this muscle by contraction causes the hair to stand on end, it is

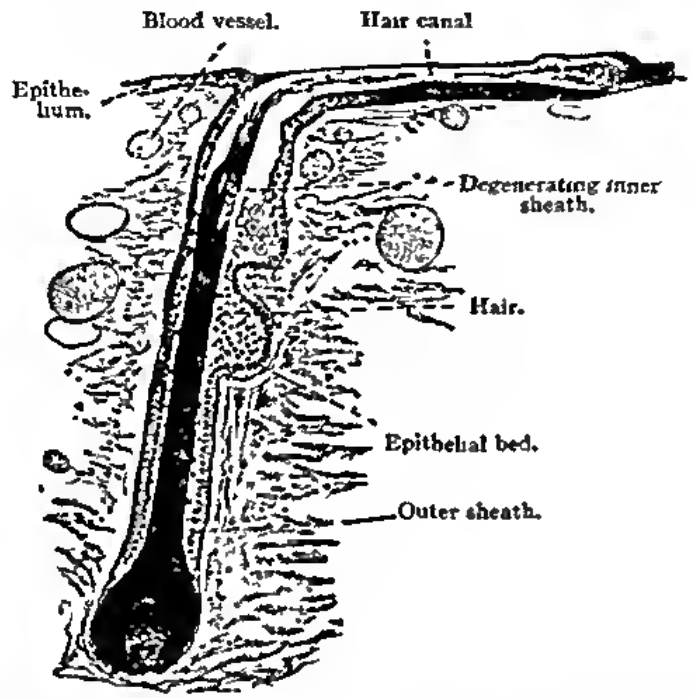


FIG 519.—VERTICAL SECTION OF THE SKIN OF THE BACK OF A HUMAN EMBRYO OF FIVE AND A HALF MONTHS × 120
The staining with iron hæmatoxylin has made the horny parts so black that their details are invisible

called the *arrector pili*. Its insertion is always below the sebaceous gland and on the lower surface of the hair, as shown in Fig. 520. The hairs which cover the body of the embryo, persisting after birth to a variable extent, are soft and downy, and are known as *lanugo*. Arrector muscles are absent from the lanugo of the nose, cheeks and lips, and also from the eyelashes (cilia) and nasal hairs (vibrissæ).

Adult Structure. The general appearance of hairs in sections of the adult skin is shown in Fig. 520, which includes also the sebaceous glands emptying into the sheaths of the hairs, and sweat glands which are usually entirely separate structures. Occasionally a sweat gland opens into the sheath of a hair near its outlet. Each hair consists of a papilla, bulb and shaft, together with sheaths around the root, namely an inner and outer epithelial sheath and, external to these, a connective tissue sheath. These structures, together with the arrector pili muscle which is inserted into the connective tissue sheath, are indicated in Fig. 520, but

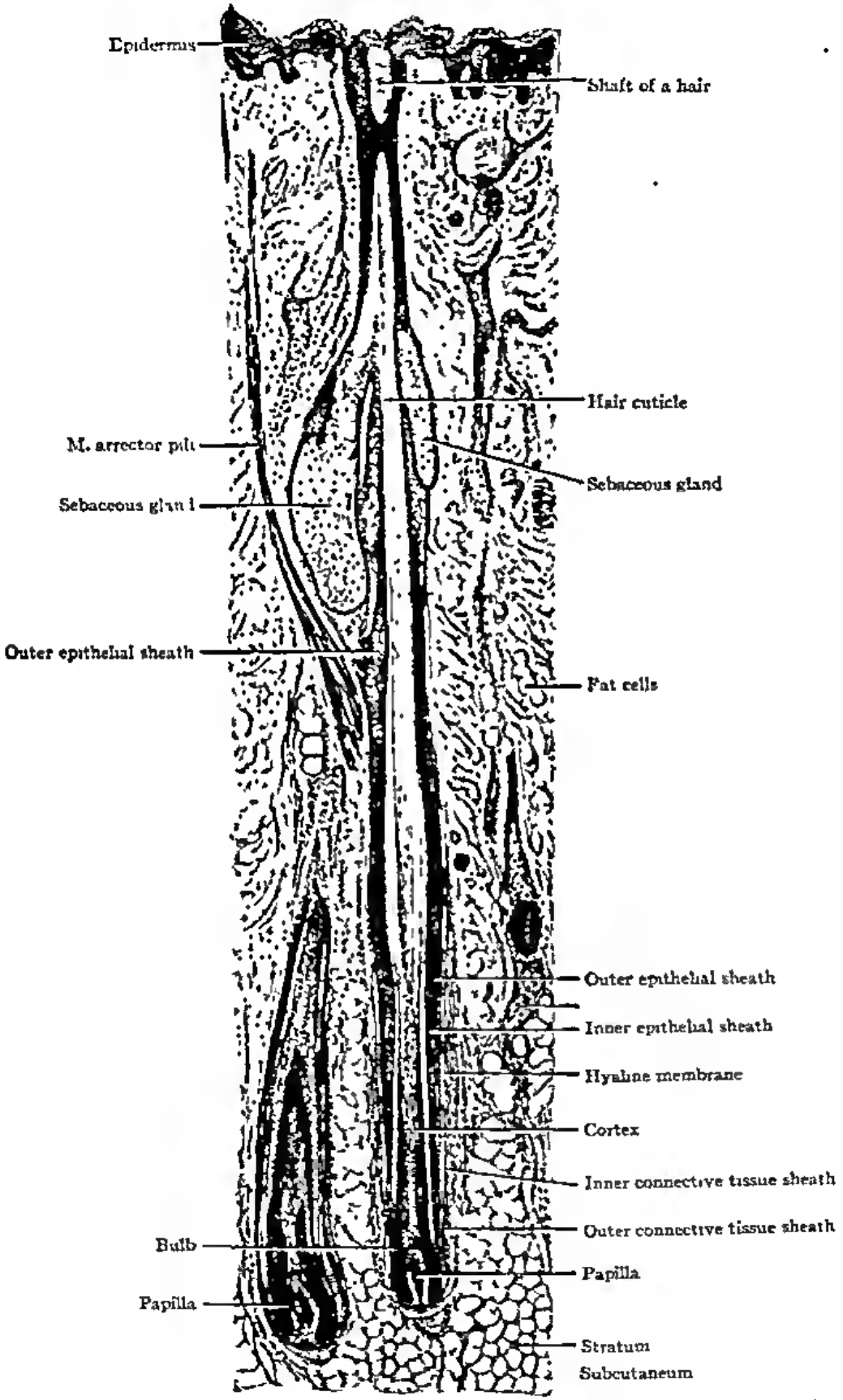


FIG. 520.—VERTICAL SECTION OF THE SKIN OF THE SCALP. THE SECTION PASSES THROUGH ONE COMPLETE HAIR WITH ITS FOLLICLE. (Piersol.)

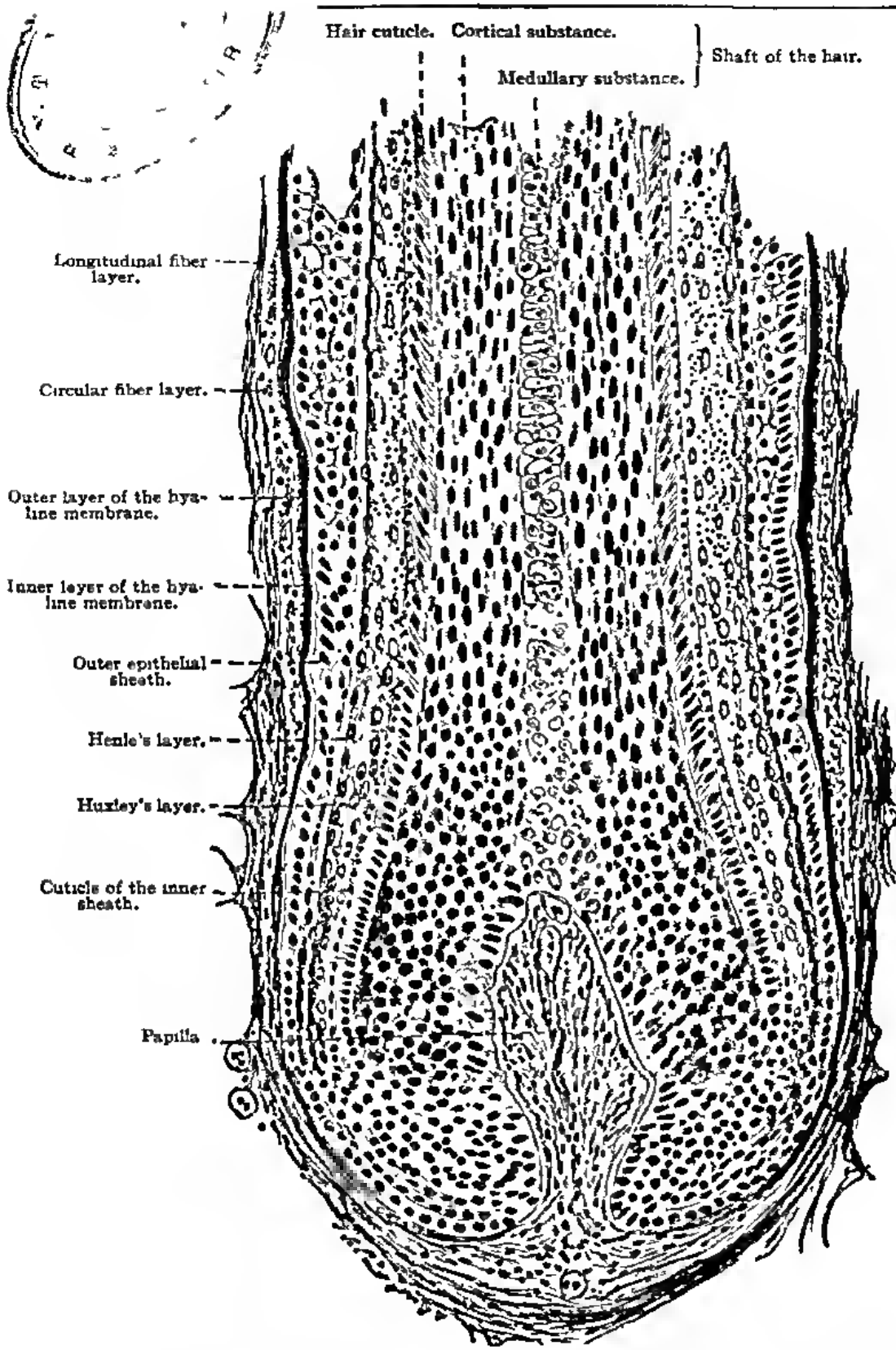
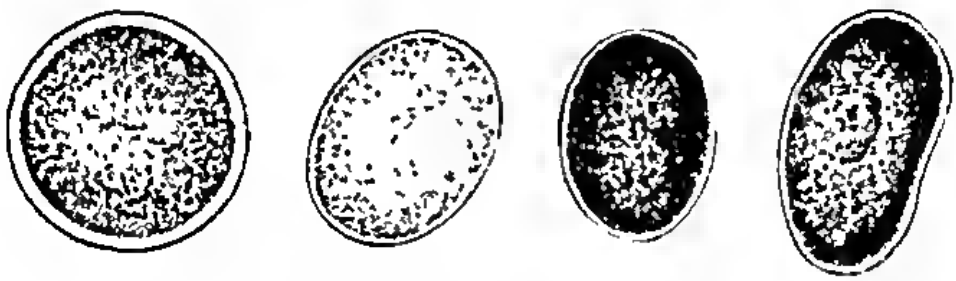


FIG. 521.—LONGITUDINAL SECTION OF THE LOWEST PART OF THE ROOT OF A HAIR. (From a section of the human scalp.) × 200

The kerato-hyalin granules are colored red

they are shown in detail in the longitudinal section, Fig. 521, and in the transverse sections, Figs. 524–527. They may be described as follows:

The *connective tissue sheath*, derived from the corium, is found around the roots of the coarser hairs, but is absent from the lanugo. It may be subdivided into three concentric layers. The outermost consists of loose connective tissue with longitudinal fibers, and contains elastic tissue and numerous vessels and nerves. The middle layer, which is thicker, consists of circular bundles of connective tissue without elastic fibers. The inner layer, also free from elastic tissue, is sometimes longitudinally fibrous, and sometimes homogeneous. It forms the outer stratum of the *hyaline* (or *vitreous*) *membrane*, and is continuous below with the thin but distinct layer which covers the papilla (Fig. 521). An inner stratum of the hyaline



A B C D

Fig. 522.—Cross-section Form and Pigment Difference in Racial Hairs
A, Chinese, B, European, C, Negro and D, Solomon Islander (Martin, Anthropologie.)

membrane is formed, according to Stöhr, from the epithelial cells of the root sheath. This inner stratum is provided with fine pores, and is always clear and homogeneous. It may unite with the connective tissue stratum so that both may appear as a single membrane. The connective tissue sheath is found fully developed only around the lower half of the root.

The *outer epithelial sheath* is an inpocketing of the epidermis. The stratum corneum extends to the sebaceous gland; the stratum granulosum continues somewhat deeper, but only a thinned stratum germinativum can be followed to the bulb. All of these are included in the outer epithelial sheath (Figs. 523–527, I, II, and 5).

The *inner epithelial sheath* extends from the sebaceous gland to the bulb. It begins as a layer of cornified cells below the termination of the stratum granulosum, but it is not a continuation of that layer. Toward the bulb the inner sheath is divisible into two layers. The outer or *Henle's layer* consists of one or two rows of cells with occasional atrophic nuclei; for the most part they are non-nucleated. The inner or *Huxley's layer* is a row of nucleated cells. The inner surface of Huxley's layer is covered by a membrane, the cuticula of the sheath, composed of non-nucleated cornified scales. Traced downward, the elements of the inner epithelial sheath and its cuticula all become nucleated cells, but the

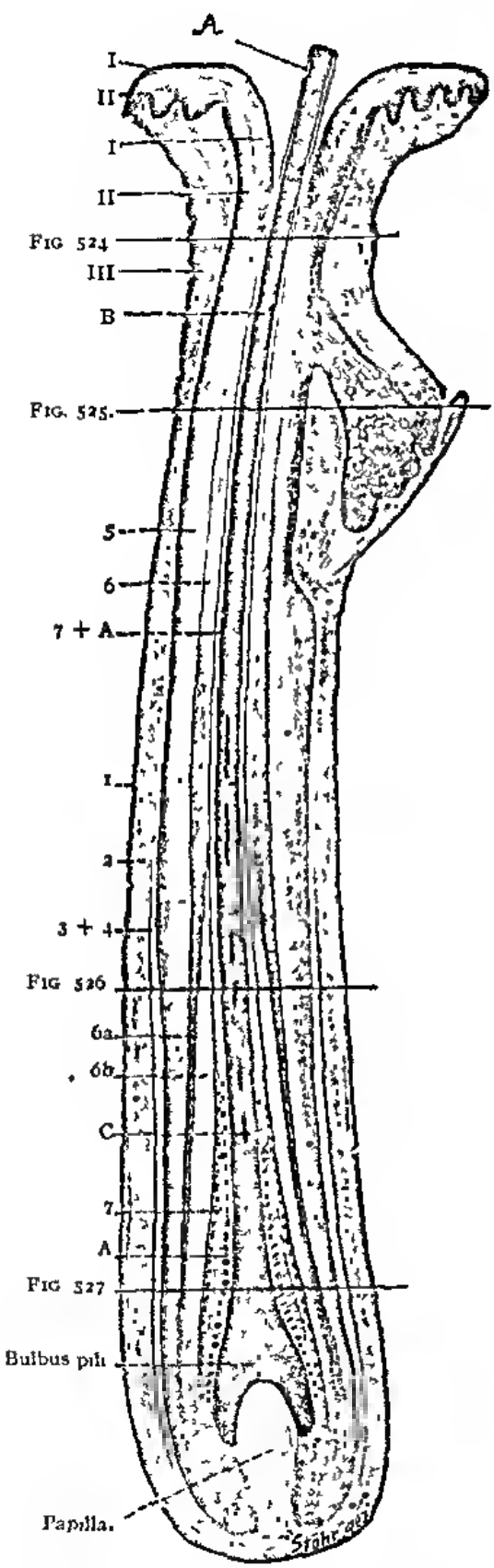


Fig 523.

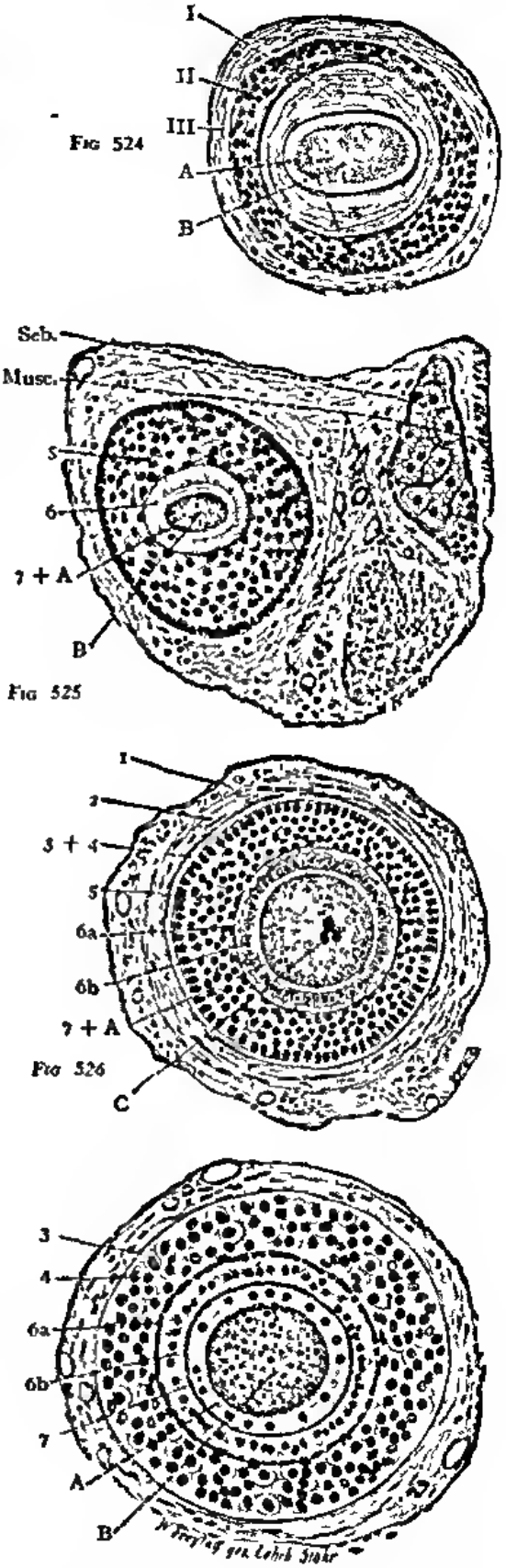


Fig 527

Figs. 523–527.—*Four Cross Sections of a Hair of the Head (X 160), with a Diagrammatic Longitudinal View for Orientation.*

A, Cuticula, B, cortex; C, medulla I, Str corneum; II, str germinativum; III, corium. 1–3, Connective tissue sheath; 1, longitudinal fiber layer; 2, circular fiber layer, 3, conn. tiss. hyaline membrane, 4; epithelial hyaline membrane, 5, *outer epithelial sheath*; 6, *inner epithelial sheath*, 6a, *Henle's layer* 6b, *Huxley's layer*, 7, *cuticula of* the sheath, Musc., arrector pili, Seb., sebaceous gland.

layers may be distinguished almost to the neck of the papilla. There they lose their sharp boundaries, but may still be distinguished from the pigmented cells of the bulb. Traced upward, it is found that kerato-hyalin granules appear in Henle's layer at the level of the papilla, and in Huxley's layer somewhat higher (Fig. 521); still higher these granules disappear and the cells of the inner sheath become cornified.

The *shaft* of the hair is entirely epithelial; it consists of cuticula, cortex and medulla. The *cuticula*, which covers its surface, is a thin layer formed of transparent scales directed from the center of the shaft outward and upward, thus overlapping like inverted shingles. This arrangement is readily seen in wool and the hairs of various mammals, but is much less evident in human hair. The cuticula is composed of non-nucleated cornified cells.

The greater portion of the shaft is included in the *cortex*. Toward the bulb, the cortex consists of soft round cells; distally these cells become cornified, elongated and very closely joined together. Their nuclei are then linear. The cortex of colored hairs contains pigment both in solution and in the form of granules. These granules are partly within the cells, and partly between them. Moreover every fully developed hair contains minute intercellular air-spaces, found within both cortex and medulla. But a *medulla* is lacking in many hairs, and when present, in the thicker hairs, it does not extend their whole length. It consists of cuboidal cells containing kerato-hyalin (Fig. 521), and generally arranged in a double row. Their nuclei are degenerating.

Growth and Replacement of Hairs. The growth of the shaft, and of the inner epithelial sheath with its cuticula, takes place through continued mitotic division of the epithelial *matrix cells* of the bulb of the hair. These become cornified, and are added from below to the cells previously cornified. Accordingly the oldest cells are at the tip of the hair and the youngest are immediately above the bulb. The outer epithelial sheath grows in a radial direction from the inner surface of the hyaline membrane toward the shaft. A certain rhythmic activity of the follicles is described by Trotter.[1]

Shortly before and after birth, there is a general shedding of hair, subsequent to which the loss and replacement of individual hairs are constantly taking place. A hair of the scalp is said to last 1600 days, but the duration of other hairs has not been definitely determined. The process of removal begins with a thickening of the hyaline membrane and circular fiber sheath. The matrix cells cease to produce, first the inner epithelial sheath, and then the cuticulæ and shaft. The hollow bulb becomes a solid cornified 'club.' The matrix cells increase without

[1] TROTTER, 1932.

differentiating into hair cells or sheath cells, and the clubbed hair, with its inner sheath, is forced outward to the level of the orifice of the sebaceous gland, where it may remain for some time. The lower part of the outer epithelial sheath, which has become empty, forms an epithelial strand which shortens and draws the papilla upward; but the connective tissue sheath remains behind, forming the 'hair stalk.' After

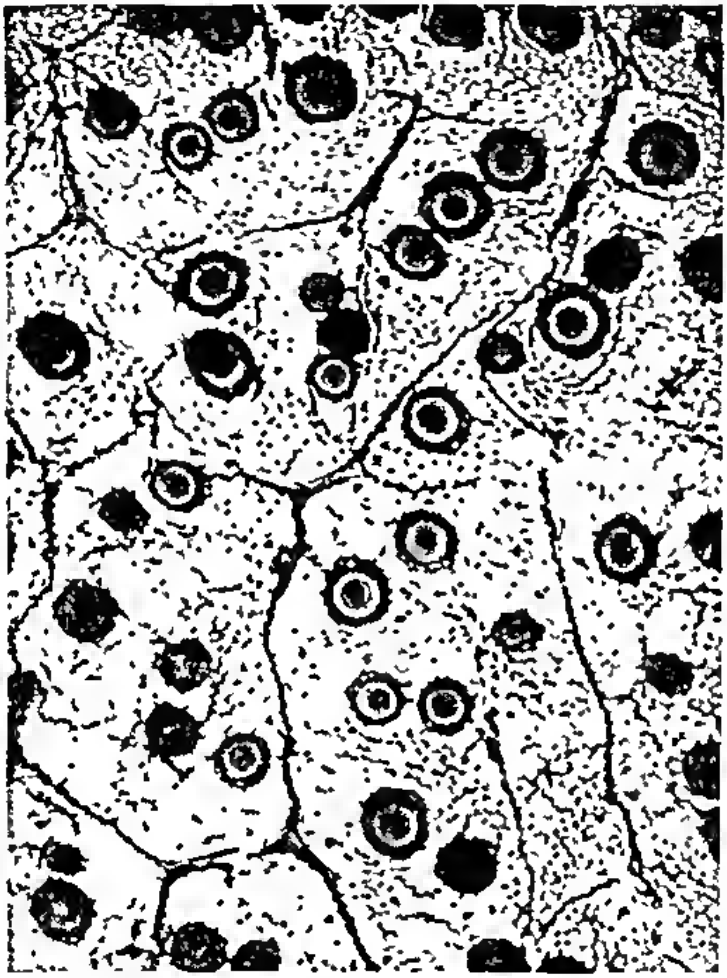


FIG 528.—A TANGENTIAL SECTION THROUGH THE SCALP OF A JAVANESE, IN THE REGION OF THE HAIR PAPILLÆ. THE HAIR FOLLICLES LIE IN THE SUBCUTANEOUS FATTY TISSUE, MOSTLY IN GROUPS OF TWO OR THREE. (Fritsch.)

some time, the columnar cells of the epithelial bed proliferate, causing the epithelial cord to return to its former depth, and a new hair develops in the old sheath upon the old papilla. The new hair in growing toward the surface completes the expulsion of its predecessor, which is dislodged together with cells of the adjacent epithelial bed.

VESSELS AND NERVES OF THE SKIN

The arteries proceed from a network above the fascia, and branch as they ascend toward the surface of the skin. Their branches anastomose, forming a *cutaneous plexus* in the lower portion of the corium. From this plexus branches extend to the lobules of fat and to the coils of the

sweat glands, about which they form 'baskets' of capillaries. Other branches pass to the superficial part of the corium where they again anastomose, forming a *subpapillary plexus*, before sending terminal arteries into the papillæ. The subpapillary plexus sends branches also to the

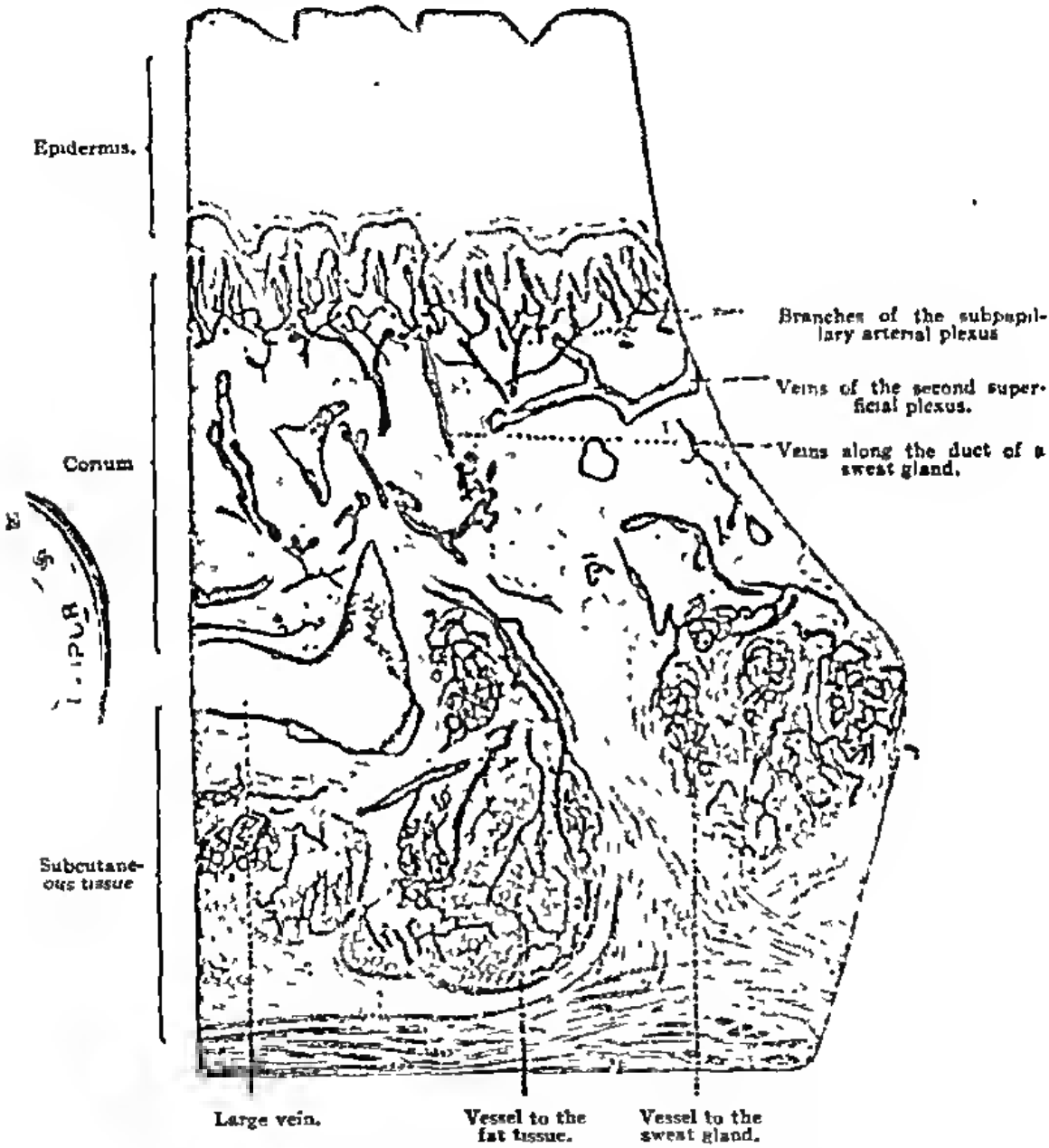


FIG. 529.—PART OF A VERTICAL SECTION OF THE INJECTED SKIN OF THE SOLE OF THE FOOT. × 20.
The veins are not completely filled by the injection

sebaceous glands and hair sheaths, but the papilla of a hair receives an independent artery. The veins which receive the blood from the superficial capillaries form a plexus immediately beneath the papillæ, and sometimes another just below the first and connected with it. The veins from these plexuses accompany the arteries and the ducts of the sweat

glands to the deeper part of the corium, where they branch freely, receiving the veins from the fat lobules and sweat glands. Larger veins continue into the subcutaneous tissue where the main channels receive specific names. In certain favorable areas, such as the base of the nail, the epithelium can be made so transparent by oil that the capillary loops, usually one to a papilla, can be studied directly.[1] They vary greatly in size and in sinuosity in different individuals, and enlarge or contract in different physiological states. Arterio-venous connections are also recognizable.

The lymphatics form a very rich superficial fine capillary plexus in the papillary layer of the corium, with branches running up into the papillæ, and connecting with a deeper plexus of larger vessels in the lower layer of the corium which drains in company with the blood vessels to the subcutaneous tissue. They can be studied by vital injections and direct examinations.[2] In the papillæ the lymphatics are described as ending blindly or as forming periendothelial lymph spaces around the capillary loops.[3] There are special lymphatics for the glands and hair follicles.

The *nerves* form a wide-meshed plexus in the deep subcutaneous tissue, and secondary plexuses as they ascend through the skin. Sympathetic, non-medullated nerves supply the numerous vessels, the arrector pili muscles, and the sweat glands; an epilamellar plexus outside of the basement membrane sends branches through the membrane to terminate in contact with the gland cells. Medullated sensory nerves end in the various corpuscles already described, and in free terminations, some being intraepithelial. Medullated fibers to the hairs lose their myelin and form elongated free endings with terminal enlargements in contact with the hyaline membrane. (The nerves to the tactile hairs of some animals penetrate the hyaline membrane and terminate in tactile *menisci among the cells of the outer epithelial sheath.*) *Small, round or* discoid elevations of the epidermis, visible with the naked eye, occur close to the hairs as they emerge from the skin, being on the side toward which the hairs slope. These 'hair discs' (Pinkus[4]) are said to be abundantly supplied with nerves. The corium beneath the nails is rich in medullated nerves, the non-medullated endings of which enter the Golgi-Mazzoni type of lamellar corpuscle (having a large core and few lamellæ), or they form knots which are without capsules. Elsewhere the skin contains tactile corpuscles in its papillæ and lamellar corpuscles in the subcutaneous tissue, together with free endings in the corium and epidermis (as far out as the stratum granulosum).

[1] Wright and Duryee, 1933. [2] Hudack and McMaster, 1933.
[3] Heimberger, 1927. [4] Pinkus, 1927.

In the skin of the palms and soles the vessels and nerves pass through the corium in tunnels of cellular connective tissue which pass into the papillæ. Thus pressure, as in standing, is borne by the dense fibrous tissues, while the vessels and nerves escape.

TEETH

The teeth are the masticatory organs of the digestive system, located at the entrance of the alimentary canal. There are two sets of these

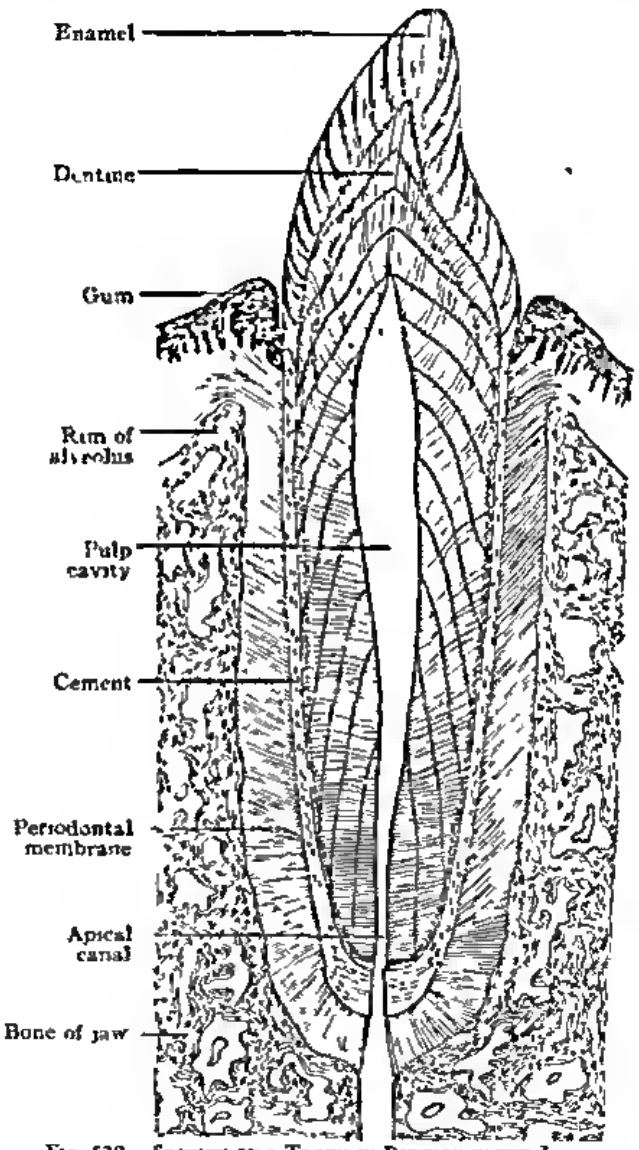


Fig. 530.—Section of a Tooth in Position in the Jaw

organs in man: the deciduous set, consisting of ten teeth in each jaw, which erupts after birth and is replaced at various periods during childhood by the permanent set of sixteen teeth in each jaw, the last of which, the posterior molars or 'wisdom teeth,' do not erupt till after puberty. The central teeth are shaped like chisels for cutting, with single broad edges, the molars have multiple, irregular surfaces for grinding the food.

A tooth consists of three parts, *crown*, *neck*, and *root* or *roots*. The crown is that portion which projects above the gums; the root is the part inserted into the *alveolus* or socket in the bone of the jaw; and the neck, which is covered by the gums, is the connecting portion between the root and crown. A tooth contains a *dental cavity* filled with *pulp*. The cavity is prolonged through the *canal of the root* to the *apex of the root*, where it opens to the exterior of the tooth at the *foramen apicis dentis*. The solid portion of the tooth consists of three calcified substances, the *dentine* or ivory (*substantia eburnea*), the *enamel* (*substantia adamantina*), and the *cement* or cementum (*substantia ossea*). Of these the dentine is the most abundant. It forms a broad layer around the dental cavity and root canal, and is interrupted only at the foramen. Nowhere does the dentine reach the outer surface of the tooth. In the crown it is covered by a thick layer of the enamel cap, which becomes thin and ends at the neck; in the root it is enclosed by the layer of cement, which begins at the neck, where it sometimes overlaps the enamel, and increases in thickness toward the apex. The cement, and therefore the tooth, is attached to the socket by the periodontal membrane. The pulp, dentine, and cement are of mesenchymal origin, the dentine and cement being varieties of bone. *The enamel is an ectodermal formation*, but so intimately associated with the others that it may be described with them.

The Development of the Teeth. The first indication of tooth development in human embryos is a thickening of the oral epithelium, which has been observed in specimens measuring 11–12 mm. At this stage the oral plate, which marks the boundary between ectoderm and entoderm, has wholly disappeared, but it is evident that the thickening takes place in ectodermal territory. The tongue is well developed, but the upper and lower lips are not as yet separated by depressions from the structures within the mouth. Soon after the thickening has appeared, it grows upward in the upper jaw, and downward in the lower jaw, into the adjacent mesenchyma, thus forming an epithelial plate which follows the circumference of each jaw. It undergoes the same sort of transformation in both the maxilla and mandible, and the following description of the conditions in the mandible is therefore applicable to both. As the plate descends into the mesenchyma, it divides into a *labial lamina* in front, which brings about the separation of the lip from the gum, and a *dental lamina* behind, which is concerned with the production of the teeth. The dental lamina, taken as a whole, is a crescentic plate of cells following the line of the gums, along which the teeth will later appear.

The further development of the dental lamina is shown diagrammatically in Fig. 531, A–D, each drawing representing a part of the oral epithelium above and dental lamina below, free from the surrounding

mesenchyma. The labial side is toward the left and the lingual side toward the right. Almost as soon as the dental lamina has formed, it produces a series of inverted cup-shaped enlargements along its labial surface (Fig. 531, B) and these become the *enamel organs*. There is a separate enamel organ for each of the ten deciduous teeth in each jaw, and they are all present in embryos of two and one-half months (40 mm.). They not only produce the enamel but extend over the roots, so that they are described as forming moulds for the teeth which develop within their

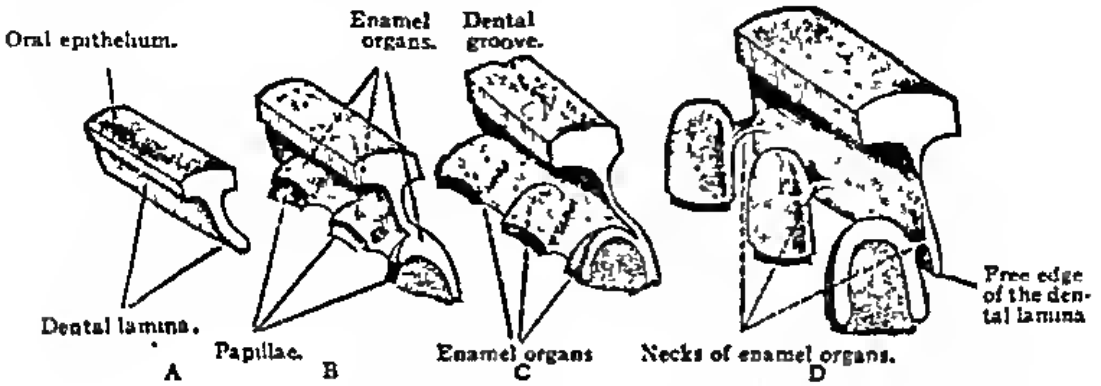


FIG. 531.—DIAGRAMS SHOWING THE EARLY DEVELOPMENT OF THREE TEETH (One of the teeth is shown in vertical section.)

concavities. The tissue enclosed by the enamel organ is a denser mesenchyma, constituting the *dental papilla*. It becomes the pulp of the tooth, and produces, at its periphery, the layer of dentine. As the tooth develops, the connection between its enamel organ and the dental lamina becomes reduced to a flattened strand or neck of epithelial tissue, which subsequently disintegrates.

In order to produce enamel organs for the three permanent molars, which develop behind the temporary teeth on each side of the jaws, the dental lamina grows backward, free from the oral epithelium. This backward extension becomes thickened and then is inpocketed by a papilla, thus forming the enemal organ for the first permanent molar in embryos of 17 weeks (180 mm.). It grows further back, and gives rise to the enamel organ for the second molar at about six months after birth, and for the third or *late molar* (wisdom tooth) at five years. In rare cases, several of which have been reported, there is a fourth molar behind the wisdom tooth, and it is assumed that in these cases the dental lamina continued its backward growth beyond the normal limits.[1]

The permanent front teeth develop from enamel organs on the labial side of the deep portion of the dental lamina. Owing to the obliquity of the lamina the permanent teeth are on the lingual side of the deciduous teeth. The enamel organs for the incisors develop slighdy in advance of

[1] WILSON, J. T., 1905.

those for the canines, but all of these are indicated in an embryo of 24 weeks (30 cm.) described by Röse. He found the enamel organs for the first premolars in an embryo of 29 weeks (36 cm.) and for the second premolars at 33 weeks (40 cm.). Each front tooth develops in the alveolus occupied by the corresponding deciduous tooth, but later a bony septum forms between the two teeth and subdivides the alveolus. When the deciduous teeth are shed, the partitions are resorbed, together with the

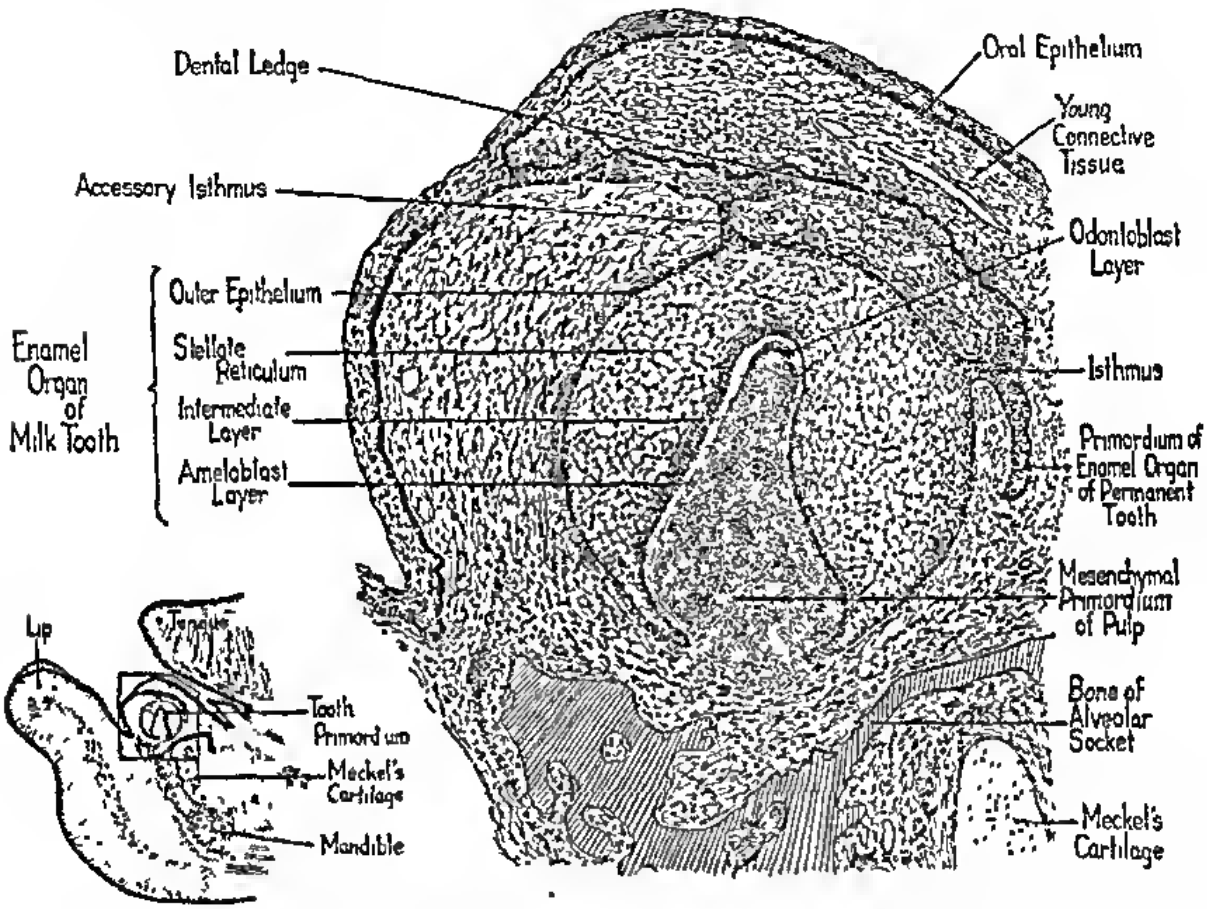


FIG 532.—PROJECTION DRAWING × 50 OF PARASAGITTAL SECTION OF THE LOWER JAW OF A HUMAN EMBRYO OF THE 14TH WEEK PASSING THROUGH THE PRIMORDIUM OF A LOWER INCISOR. (From a forthcoming "Human Embryology," By Bradley M. Patten. Embryo of 104 mm. C. R. Length, University of Michigan Collection.)
The small sketch on lower left indicates the relations of the area represented.

dentine and cement of the roots of the deciduous teeth. This resorption is accompanied, as in bone, by the production of osteoclasts.

The portion of the dental lamina which is not utilized in producing enamel organs becomes perforated and forms irregular outgrowths. This disintegration begins in the front of the mouth and spreads laterally. Epithelial remnants from the lamina have been found in the gums at birth and have been mistaken for glands. Like other epithelial remains they occasionally develop abnormally, forming cysts and other tumors. The deepest part of the lamina, below the enamel organs of the permanent teeth, is considered by Röse to be a possible source of a third set, and he states that a case has been reported to him in which such a set,

consisting of thirty-two teeth, developed on the lingual side of the permanent teeth. The models which Röse prepared, showing the enamel organs in various stages of development, form the basis of present accounts of tooth development.[1]

ENAMEL ORGAN AND ENAMEL

The basal cells of the oral epithelium may be followed as a distinct layer over the dental lamina and enamel organ, as shown in Fig. 532.

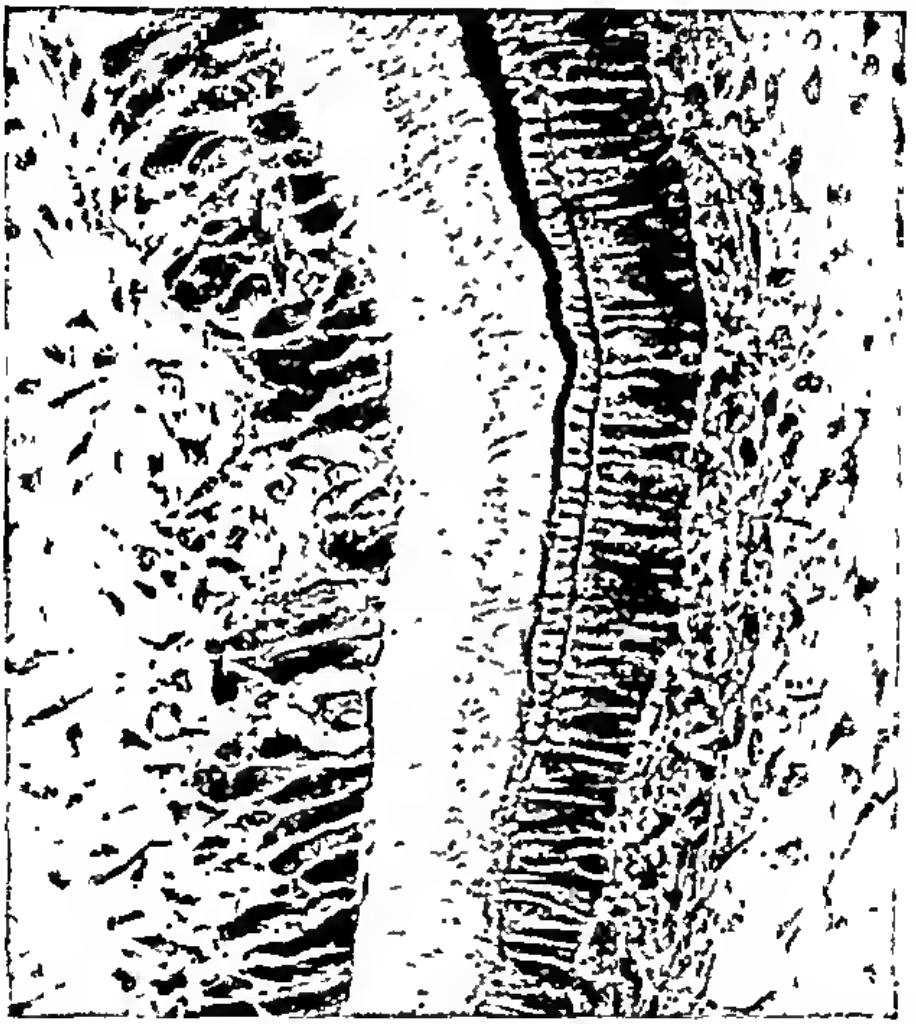


Fig. 533.—Formation of Dentine and Enamel, Fetal Pig

From right to left are shown the stellate reticulum of the enamel organ, the cellular layer, the layer of ameloblasts with Tomes's processes, and the thin layer of enamel (black) thicker toward the crown, then dentine, predentine, and the layer of odontoblasts with Tomes's fibers extending into the dentine; finally the mesenchyma of the pulp

This suggests that the enamel organ should be regarded as an infolding of the oral epithelium, and the occurrence of a transient *dental groove* immediately above the lamina (Fig. 531, C) favors this interpretation. The basal surface of the epithelium of the enamel organ is therefore directed toward the surrounding mesenchyma, and the superficial cells are found in the interior of the organ. At first these internal cells are in close contact, like those of ordinary epithelium, but later, through an

[1] Rose, 1891.

accumulation of gelatinous intercellular substance they constitute a protoplasmic reticulum which resembles mesenchyma, and is known as the *enamel pulp* or stellate reticulum (Fig. 532). On the side away from the dental papilla the enamel pulp is covered by the *outer enamel epithelium.* At first the single layer of cells are cuboidal, but later they become flattened. Toward the dental papilla the enamel pulp is bounded by the *inner enamel epithelium;* the cells develop differently over the upper and lower parts of the tooth respectively. Over the lower portion of the dental papilla they remain as cuboidal or low columnar cells. Here, through a thinning of the pulp they are brought into contact with the cells of the outer enamel epithelium and the two layers together form the *epithelial sheath* of the root. This sheath, often referred to as Hertwig's sheath determines the extent of downward growth of the root; the outer enamel epithelium is believed to contribute most to its formation. Over the upper part of the dental papilla, the cells of the inner enamel epithelium elongate and become enamel-producing cells or *ameloblasts* (syn. adamantoblasts, ganoblasts). The enamel organ is usually devoid of blood vessels, but during enamel formation capillaries against its outer surface may push the thin epithelium papilla-like into the soft pulp, bringing the vessels closer to the forming enamel. This is a constant occurrence in some marsupials, rodents and certain other mammals.

The ameloblasts produce enamel along their basal surfaces, which are toward the dental papilla, but they become so transformed that their basal surfaces appear like free surfaces, and the entire cells seem inverted. In columnar epithelial cells the nuclei are generally basal, and the secretion gathers near the free surface, but in the ameloblasts these conditions are reversed. The nuclei are toward the enamel pulp, and the latter forms a dense layer over the ameloblasts. Near the center of each cell, and therefore on the basal side of the nucleus, Cohn[1] has described typical centrosomes or diplosomes. The Golgi apparatus which was originally on the side of the nucleus toward the enamel pulp shifts to the opposite side toward the dental papilla.[2] Granules and globules, the *enamel droplets,* which reduce osmic acid appear in the same region of the cytoplasm and presumably indicate secretory activity.

The basal surface of each ameloblast presents a cuticular border and gives rise to a tapering projection known as *Tomes's process.* Around these processes minute globules are deposited, which resemble and stain similar to the granules within the cells, since they blacken with osmic acid. They are described as composed of a horny substance similar to that found in the epidermis. This material may become fibrillar, and Tomes's processes also readily break up into fibrils. There is, therefore,

[1] Cohn, 1897. [2] Beams and Kino, 1933.

an unealcified fibrillar layer next to the ameloblasts. Further from the ameloblasts the enamel is ealcified and consists of rods known as *enamel*

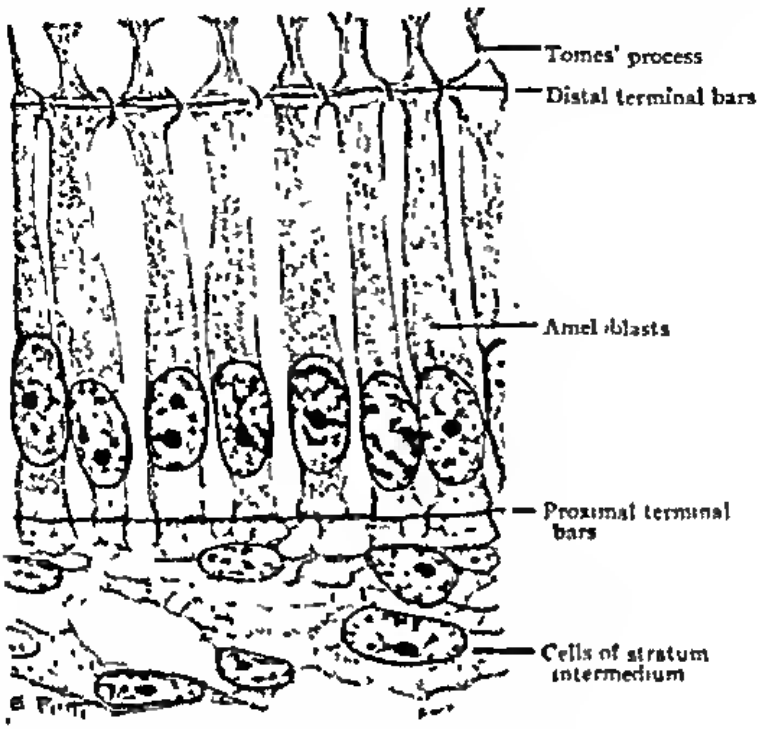


Fig 534 —Ameloblasts and Cells of the Stratum Intermedium Enamel Organ of a Cat Fetus-mandible Susa fixation; Azan.

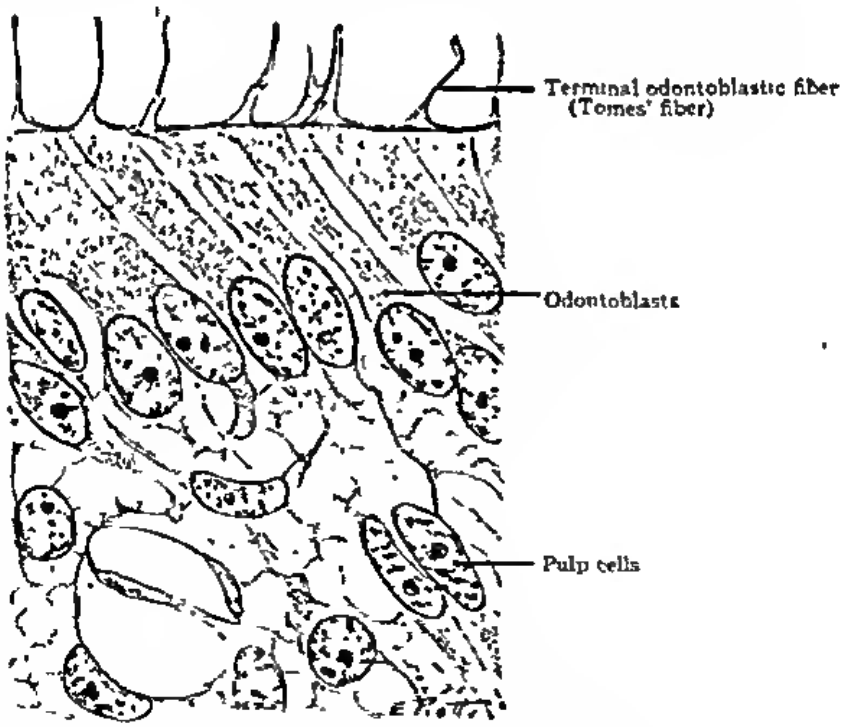


Fig 535 —Odontoblasts in the Dental Papilla of a Cat Fetus. Susa fixation, Azan

prisms, which are bound together by a calcified enamel cement or *interprismatic substance*.

The formation of enamel begins at the top of the crown of each tooth and spreads downward over its sides. If the tooth has several cusps, a cap of enamel forms over each, and these caps coalesce. The enamel increases in thickness by the elongation of the prisms, the ameloblasts receding in the direction of the enamel pulp. To accommodate themselves to the greater area of the outer surface of the layer, the prisms become broader as they develop radially. The ratio of average width of the prisms at the inner and outer surfaces has been given as 5.5 to 10.[1] There may also be some increase in the interprismatic substance. The supposition that new

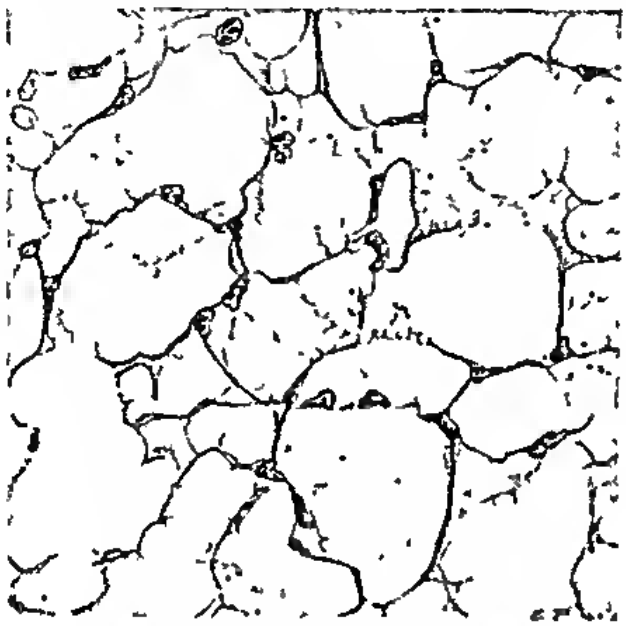


Fig 536.—Section of Enamel Pulp.—Pig Embryo. Susa fixation; Azan

cells are added to the ameloblast layer to form interpolated prisms is not warranted.

When the tooth erupts the enamel organ is broken through and the cells degenerate. For a time the enamel is covered by 'Nasmyth's membrane' (cuticula dentis), which is formed by the fused, modified cuticular borders of the ameloblasts and a few of the cells of the enamel organ and of the gum.[2] It is soon worn away, remaining longest in the depressions between tooth cusps.

The fully developed enamel is the hardest substance in the body. Both the prisms and the interprismatic substance are calcified and contain from 96% to 98% inorganic material, 90% of which is acid calcium phosphate. Calcium carbonate, acid magnesium phosphate, calcium fluoride and traces of other salts form the remainder. This composition precludes the study of enamel in histological sections, because decalcification dissolves practically the entire mass. Enamel contains no cells or

[1] Williams, J. L., 1923. [2] Chase, 1926.

other protoplasmic structures, but it exhibits various markings which can be seen on the surface or in ground sections. The outer surface of the enamel of the permanent teeth, especially on the sides of the crown and on young teeth, presents a succession of circular ridges and depressions, which may be seen with a hand lens. These *ripple marks* were discovered by Leeuwenhoek (1687). The enamel, as seen in ground sections passing lengthwise through the tooth, shows numerous brownish bands which are broadest and most distinct toward the free surface. These are the *contour lines* or *lines of Retzius*, first described in Müller's Archiv, 1837 (pp. 486–566). The coarsest of them may be seen with the naked eye, but upon magnification they are resolved into many finer lines. They arch over the apex of the crown, and on its sides tend to be parallel with the long axis of the tooth. Thus they cross the enamel prisms, and are not the lines along which the enamel most readily fractures. Apparently they indicate the shape of the entire enamel at successive stages in its development, and for this reason they are called contour lines. When Leeuwenhoek's ridges or ripple lines are present, the lines of Retzius end in furrows between them. It was once supposed that their brown color was due to pigment, and it is well known that the enamel of certain teeth in rodents is deeply pigmented and brown. But when the lines are highly magnified, no pigment granules are found. It then appears that the lines are due to imperfect calcification of the enamel cement, which is often vacuolated where a line crosses it.

FIG. 537.—GROUND SECTION OF A TOOTH PHOTOGRAPHED IN REFLECTED LIGHT

The light enamel layer is clearly distinguishable from the dentine. (Meyer-Churchill.)

Another set of lines crosses the enamel radially, taking the shortest course from the dentine to the free surface. These *radial lines* are due to the arrangement of the enamel prisms, and fractures of the enamel tend to follow them. As seen in reflected light, under low magnification, they appear as alternating light and dark bands, often called *Schreger's lines*. The prisms in crossing the enamel are bent in such a way that they are cut in alternating zones of cross and longitudinal sections, respectively. These zones vary in shape and sometimes the prisms in cross section form an island surrounded by longitudinal sections. Since an entire prism cannot be isolated or included within the limits of a single

section, the course which they take is difficult to determine, and the shifting position of groups of ameloblasts that might explain this disposition of the prisms has not been recognized. Even the more radially directed prisms may be deflected in certain regions, assuming sharp curves, and between the cusps of the larger teeth the confusion of direction forms '*gnarled enamel*.'

The individual enamel prisms, when seen lengthwise, exhibit transverse markings usually, but not always, aligned to form continuous

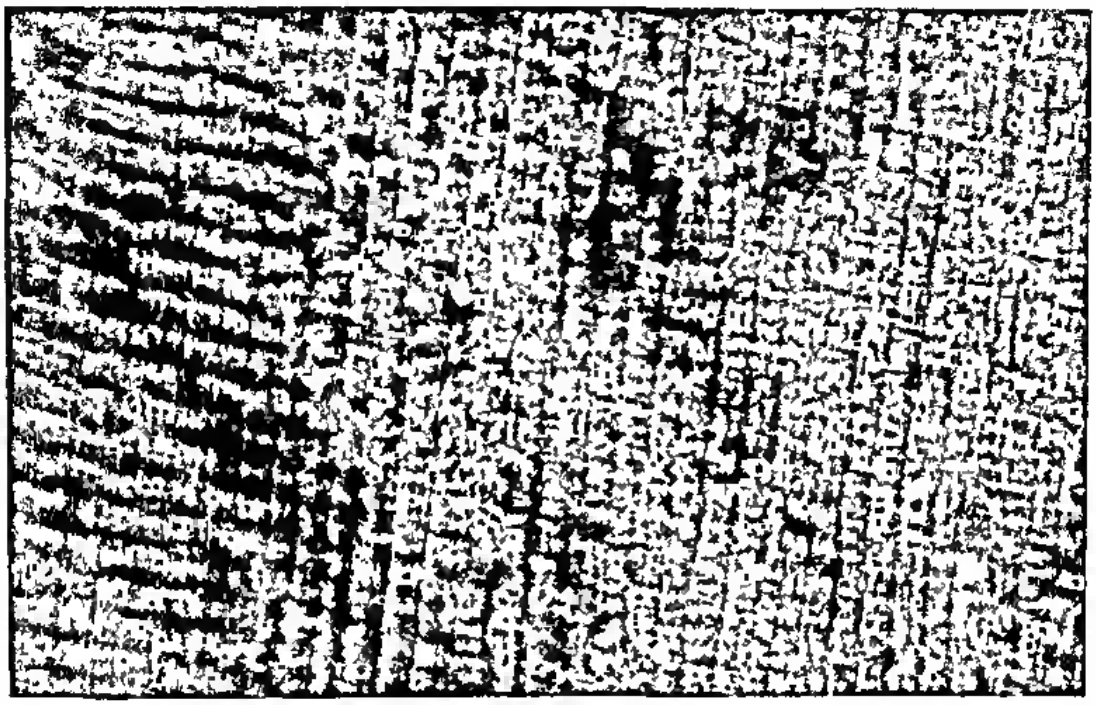


FIG 538.—ENAMEL PRISMS, TIP OF TOOTH, SHOWING CONTOUR LINES AND STRIATIONS

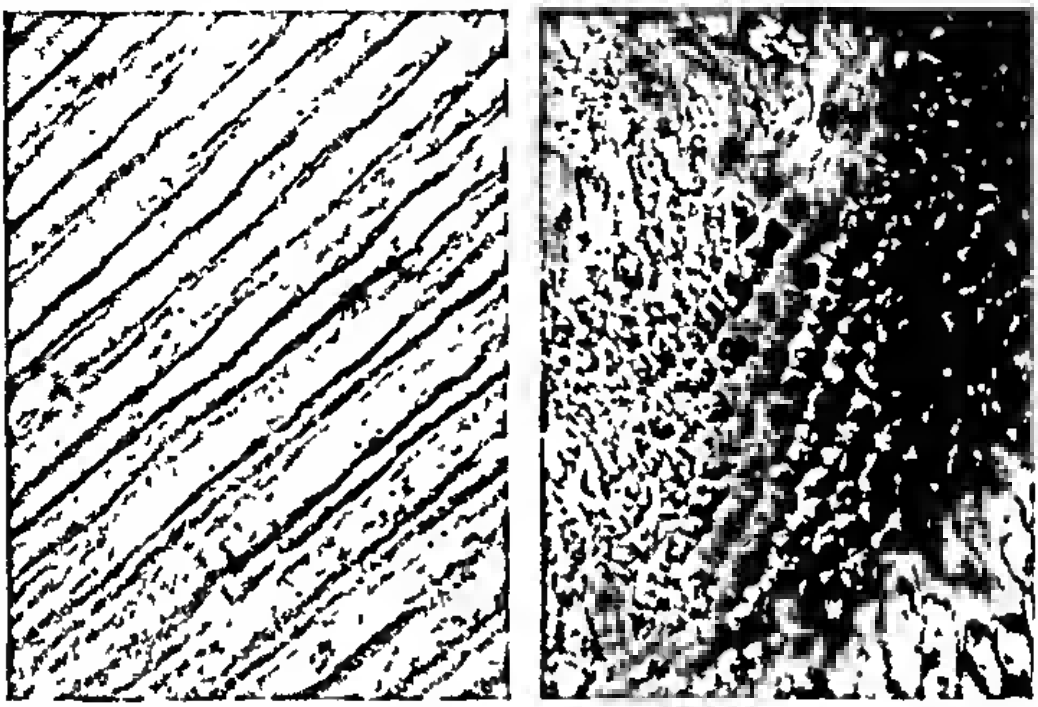
bands across many prisms. They are more intense in certain areas than in others, and represent segments of less complete calcification. It is surmised that they correspond with certain resting stages of the individual, as during sleep. 'As a matter of fact the number of cross striations of the prisms approximates the number of days necessary for their development.'[1]

When seen in cross section the prisms have refractive outlines and are from 3 μ to 6 μ in diameter. They are primarily hexagonal in shape, but may assume rounded or even indented crescentic forms.[2] Such appearances can be explained often as the result of oblique lighting.

In the enamel at the dentino-enamel border, especially near the neck of the tooth, certain structures called *enamel tufts* often appear, and extend for shorter or longer distances between the enamel prisms. They have been considered as inclusions of collagenous fibers projecting from the dentine, but probably represent local areas of poorly calcified interprismatic substance, the branching appearance being due to its extension

[1] CHURCHILL, 1935. [2] SMREKER, 1905.

into several laminæ between the prisms. A few of these structures extend to the surface of the enamel, and are then called *enamel lamellæ*. Other structures extend for short distances in directions not necessarily parallel to the prisms; these are called *enamel spindles*, and are thought to represent misplaced processes of odontoblasts.



A B

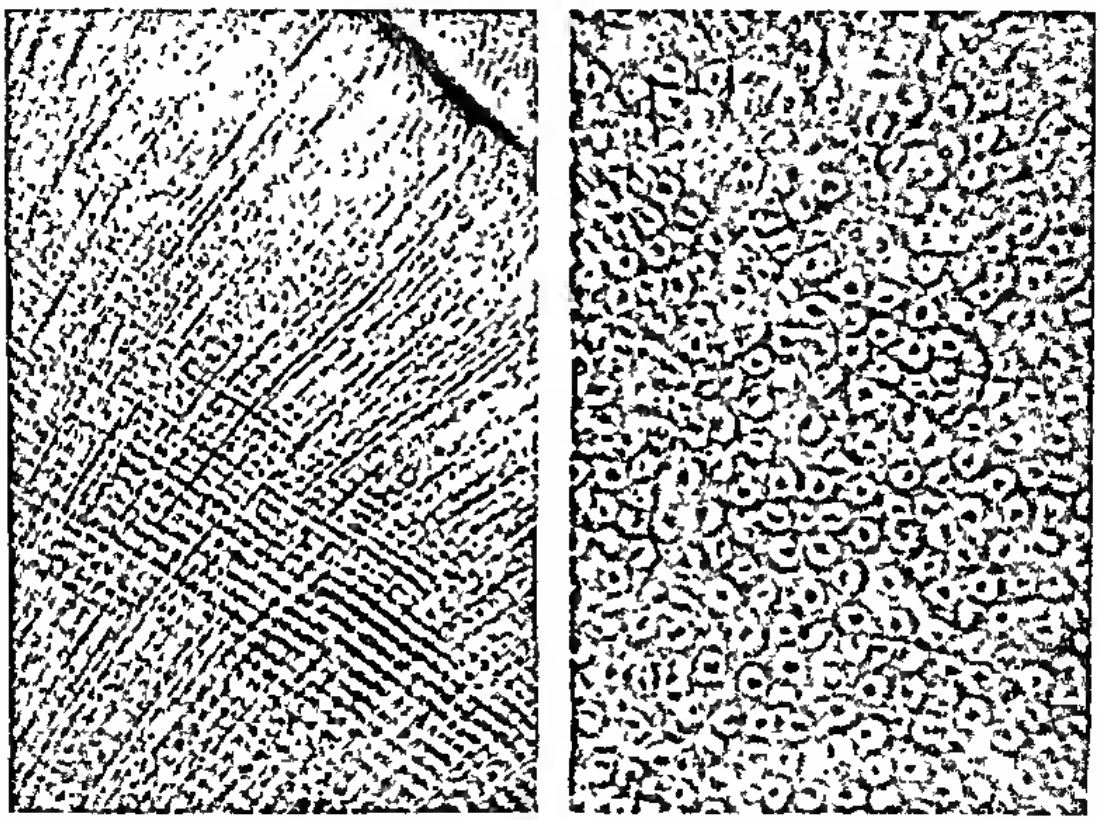
FIG. 539.—ENAMEL PRISMS, IN LONGITUDINAL AND TRANSVERSE SECTION. B, SHOWS A SCHREGER'S BAND

DENTINE AND PULP

The dental papilla is composed of condensed mesenchyma, enclosed and probably moulded by the enamel organ. At the end of the fourth month, shortly before the formation of enamel has begun, the outermost cells of the papilla become elongated and arranged in an epithelioid layer. These cells, once thought to produce dentine, are known as *odontoblasts*. They are tall columnar cells, with one or more thin processes directed toward the enamel, which branch dichotomously at the tips and bear smaller side branches. Each process occupies a canaliculus in the dentinal matrix; but the odontoblasts remain always at the inner border of the mass and do not become buried. They are in contact with adjacent cells and with cells more central in the pulp through fine protoplasmic processes and therefore, may be regarded as part of a mesenchymal syncytium. The existence of the dentinal canaliculi and the fact that they open into the pulp cavity, were recorded by Leeuwenhoek in 1687. 'The presence of fibrils of soft tissue within the dentinal tubes' was established by Tomes in 1856. He found that if a section of a fresh

tooth is placed in dilute hydrochloric acid and then torn across the tubules, fibrils will be seen projecting from the broken edges; and that if the pulp is pulled away from the dentine, fibrils can be drawn out of the tubes. The fibers within the dentinal canaliculi are called *dentinal* or *Tomes's fibers;* they should not be confused with the Tomes's processes of the ameloblasts.

Fine argyrophil fibers arising in the pulp form spirally twisted bundles between the odontoblasts and then fan out into the uncalcified dentine



A B

FIG 540.—DENTINE, LONGITUDINAL SECTION TO DENTINO-ENAMEL JUNCTION B, TRANSVERSE SECTION, HIGHER MAGNIFICATION

or *predentine* in a delicate meshwork. These fibers which are often spoken of as von Korff's[1] fibers form the fibrillar framework for the dentine. They are buried in a jelly-like matrix and both become calcified. It is presumed that this jelly-like organic matrix is secreted by the odontoblasts because when dentine is being formed granules and droplets accumulate in the part of the cell between the nucleus and the predentine. A Golgi apparatus in the same region becomes more open-meshed and spread out in the same direction. Calcareous granules are deposited in the matrix and the predentine becomes calcified.

Calcification proceeds by the formation of large spherules, called *calcospheres* which may remain of a different density from the intervening material. In the layer of dentine broadly parallel bands are seen, called

[1] VON KORFF, 1907*a*, AND 1907*b*.

contour lines, which take oblique directions. These are caused by areas in which complete calcification is lacking, the spherules only being fully calcified. The intervening spaces are called *interglobular spaces*, which appear black in ground sections. Their presence in successive layers represents periodical arrests of development, and they are said to be particularly abundant in poorly developed teeth. Frequently Tomes's fibers can be seen passing through the spaces. The position of the lines

FIG. 541.—TERMINAL ARBORIZATIONS OF THE DENTINAL TUBULI IN A GROUND SECTION OF A HUMAN TOOTH. Fuchsin staining. × 800 (Meyer-Churchill.)

indicates that the root of the tooth forms after the crown is essentially complete. Dentine continues to be formed slowly through life and the pulp cavity becomes reduced in size with age. Injury causes an increased activity and the deposit of new or secondary dentine.

Dentine when fully developed is not so hard as enamel and contains a much larger amount of organic matter (approximately 25%). When the inorganic substances are removed from enamel, the remaining tissue scarcely holds together, but dentine and bone, when so treated, leave a gelatinous matrix which preserves the form of the original object. The dentinal canaliculi pass radially through the dentine, often following a somewhat S-shaped course. In addition to these primary curves, they

may show spiral twists and secondary curves. As they cross the dentine, they divide dichotomously a few times and give off many slender lateral branches, some of which anastomose with those from adjacent canaliculi. They finally become very slender and end blindly. Each canal is surrounded by a resistant uncalcified layer known as *Neumann's sheath*. This sheath may be isolated with acids, and thus it is comparable with

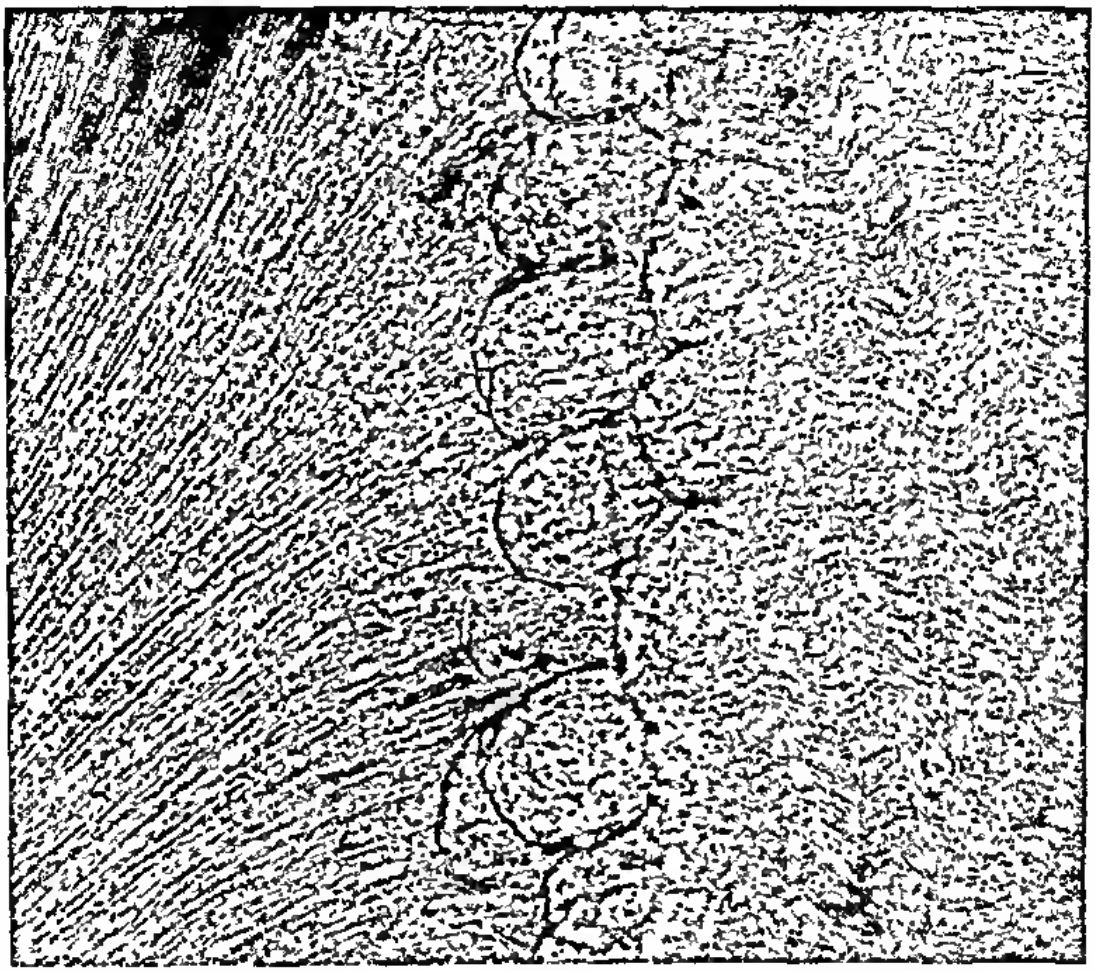


FIG. 542.—DENTINO-ENAMEL JUNCTION

the 'corpuseles' of bone and the capsules of cartilage. This appearance has also been attributed to refractive illusions, and the presence of an actual sheath denied.[1]

The contact between the dentine and the enamel may be quite smooth, but often the enamel surface takes the form of irregular festoons (Fig. 542). Toward the root of the tooth, where the dentine is in contact with the cement, it exhibits a more or less continuous layer of especially small interglobular spaces, known as the granular layer.

The Pulp. The pulp consists of a syncytium of undifferentiated mesenchymal cells together with the peripherial layer of odontoblasts already described. The intercellular spaces are filled with a viscid fluid

[1] CHURCHILL, 1935.

staining with basic dyes and a fine network of reticular fibers. The pulp tissue is free from elastic fibers and ordinarily from bundles of collagenous fibers. Most of the latter being found in the walls of the blood vessels. It is very vascular. Small arteries entering the apical foramina branch and give rise to numerous capillaries which may pass between the odon-

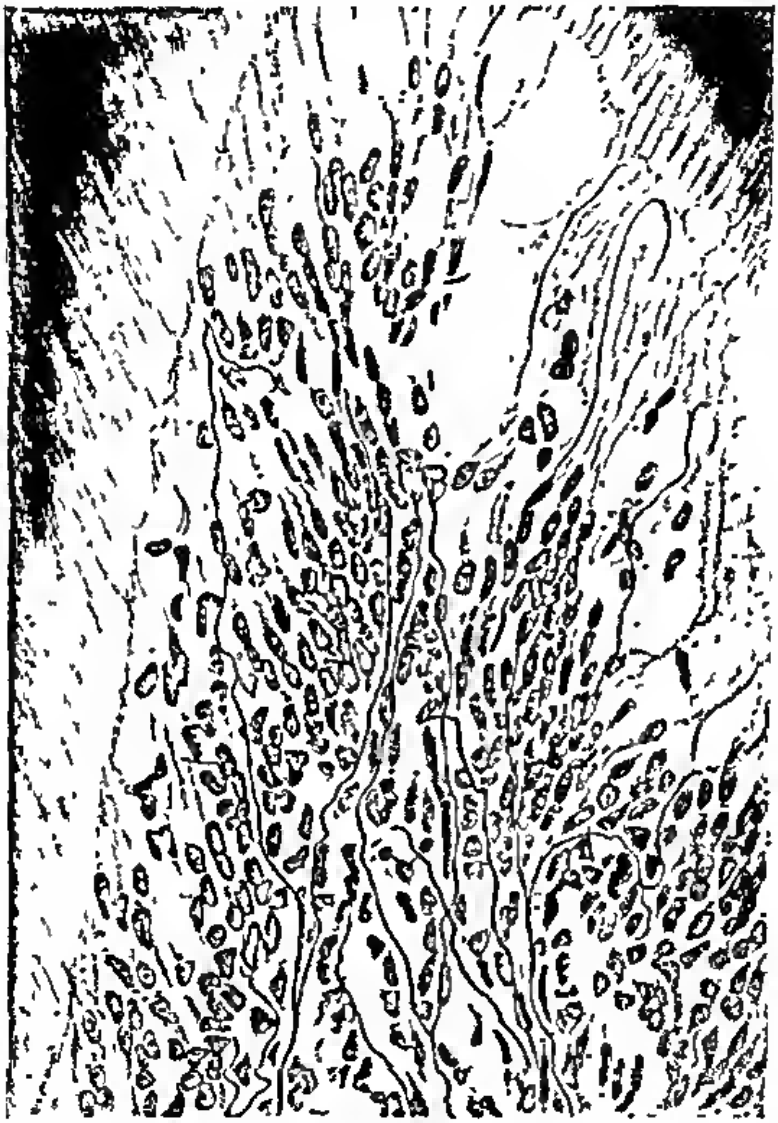


FIG. 343.—NERVE FIBERS IN THE ANTERIOR CORNUA OF A MANDIBULAR MOLAR I TOOTH OF A TEN DAY OLD RAT. Varicose fibers end in the layer of odontoblasts, some bend abruptly or loop back. Bouin fixation, Bodian impregnation.

toblasts but normally do not enter the predentine. The capillaries empty into very thin-walled veins which are larger in diameter than the arteries. The veins have often been regarded as having no smooth muscle fibers in their walls but by appropriate staining one or two layers of circularly disposed muscle can be brought out. They become smaller and leave the pulp in company with the entering arteries. There has been much discussion whether or not lymphatic vessels are present in the pulp. Attempts to inject lymphatics either directly or indirectly

sometimes shows the injection mass in tiny capillary-like vessels within the pulp but these have not been seen passing through the apical foramina. Until such vessels can be shown leaving a tooth the presence of lymphatics must be questioned. The nerves of the pulp are derived from medullated branches of the aveolar nerves which enter through the apical foramina in company with the arteries. Within the pulp they often twist spirally around the vessels or lie embedded in loose connective tissue on one side of them. Their branching follows in general that of the arteries. Toward the odontoblasts they lose their medullary sheaths and form loose interlacing plexuses from which the fine fibers leave to terminate in free endings at different levels between the odontoblasts. Some fibers on reaching the predentine may loop back and terminate more centrally. Individual fibers may enter the mouth of a dentinal tubule but it is doubtful if any nerve fibers ever pass into the calcified dentine. Non-medullated nerve fibers accompanying the medullated fibers seem destined to supply the musculature of the blood vessels.

CEMENT AND PERIODONTAL MEMBRANE

Each embryonic tooth, consisting of its enamel organ and papilla, is completely surrounded by mesenchyma, of which the papilla itself is a modification. Against the layer of inner enamel cells the odontoblasts form dentine, and this is true also in the neck and root of the tooth which is enclosed by a deeper extension of the enamel organ in which no enamel pulp is present and no ameloblasts develop. After the dentine of the root is thus formed, the ectodermal layers degenerate, at first in the region of the neck, leaving the outer surface of the root exposed to the surrounding mesenchyma. The mesenchymal cells become osteoblasts and form appositional bone on the dentinal surface. This modified true bone is the cement or cementum. The ectodermal sheet near the apex persists and elongates for some time, so that the dentine of the deeper part of the root develops and the root lengthens after the eruption of the crown.

The thin layer of cement which grows before the tooth erupts is non-cellular, since none of its osteoblasts become bone cells; it is designated *primary cement.* After eruption a new set of osteoblasts form bone against its surface, and this time, since no further movement of the tooth disturbs the osteoblasts, the *secondary cement* becomes much thicker and contains several layers of bone cells. A peculiarity of this bone is that the bone cells seem to have their largest and most numerous protoplasmic processes directed away from the dentine, and this figure is reflected in the shape of the lacunae and canaliculi. The lamellæ of the cement, which are seldom well marked, are concentrically placed around

the root. In young teeth Haversian canals are absent, but in old teeth they occur in the outer layers near the apex of the root.

Beside supplying osteoblasts (cementoblasts or petroblasts) to form the secondary cement, the mesenchyma also produces thick collagenous fibrils which become embedded in the cemental matrix. These are called Sharpey's fibers, as are the similar structures in bone (cf. p. 142). They extend across the periodontal membrane and are inserted into the bone of the alveolar process which forms the tooth socket, and serve to support the tooth sufficiently rigidly, while allowing a certain amount of motion to withstand the shock of biting and chewing. Their direction varies at

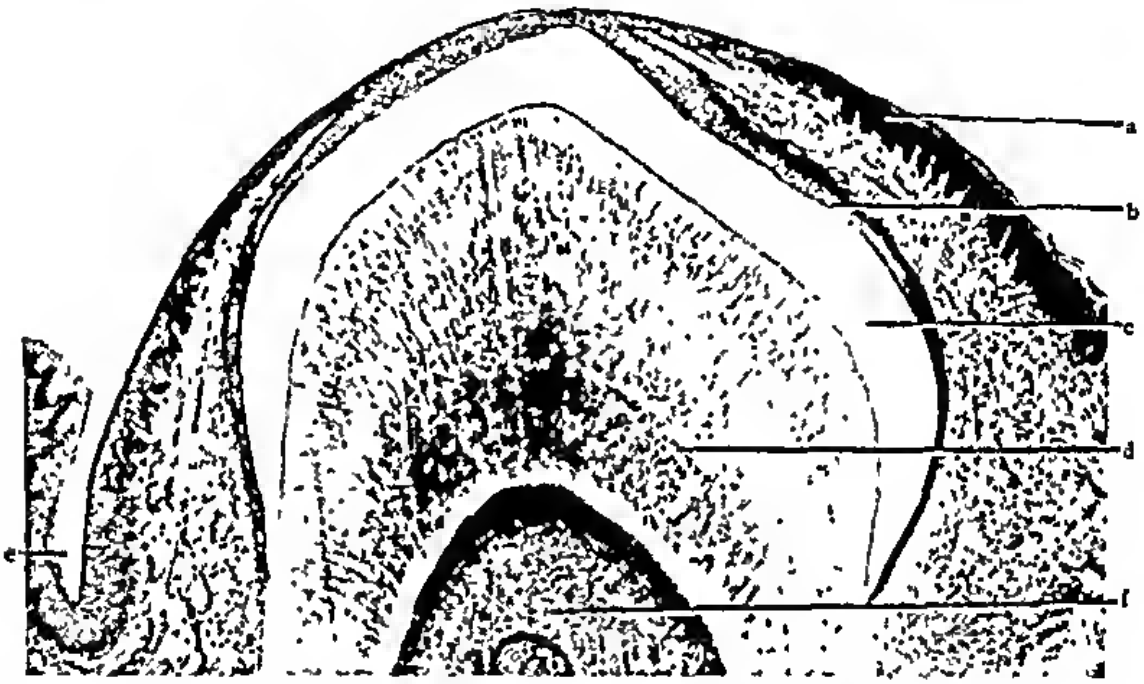


Fig. 544.—Tooth in Eruption

United enamel epithelium and oral epithelium above the apex of the tooth. Carmine staining. × 20. (Meyer-Churchill.) a. Oral epithelium. b. Fusion of inner and outer enamel epithelium. c. Space occupied by enamel lost through decalcification. d. Dentine. e. Labial-gingival groove. f. Dental pulp.

different regions of the tooth, as is shown in Fig. 530. Above the alveolar processes they are attached to the dense connective tissue of the gum.

Periodontal Membrane and Gingiva. The periodontal membrane includes all the structures lying in the narrow space between the cement and the bony alveolar process. In addition to the cementoblasts and Sharpey's fibers already mentioned, it contains the usual constituents of dense connective tissue and also occasional epithelial nests or cords, remnants of the enamel organ. Toward the mouth it is continuous with the gum or gingiva. The latter contains groups of Sharpey's fibers, the transseptal and gingival fibers, and is capped by a thick layer of stratified squamous epithelium, with many long basal papillæ. The layers become fewer as the gum dips down to enclose the enamel. The blood vessels in the periodontal membrane pass in a direction parallel to the tooth but

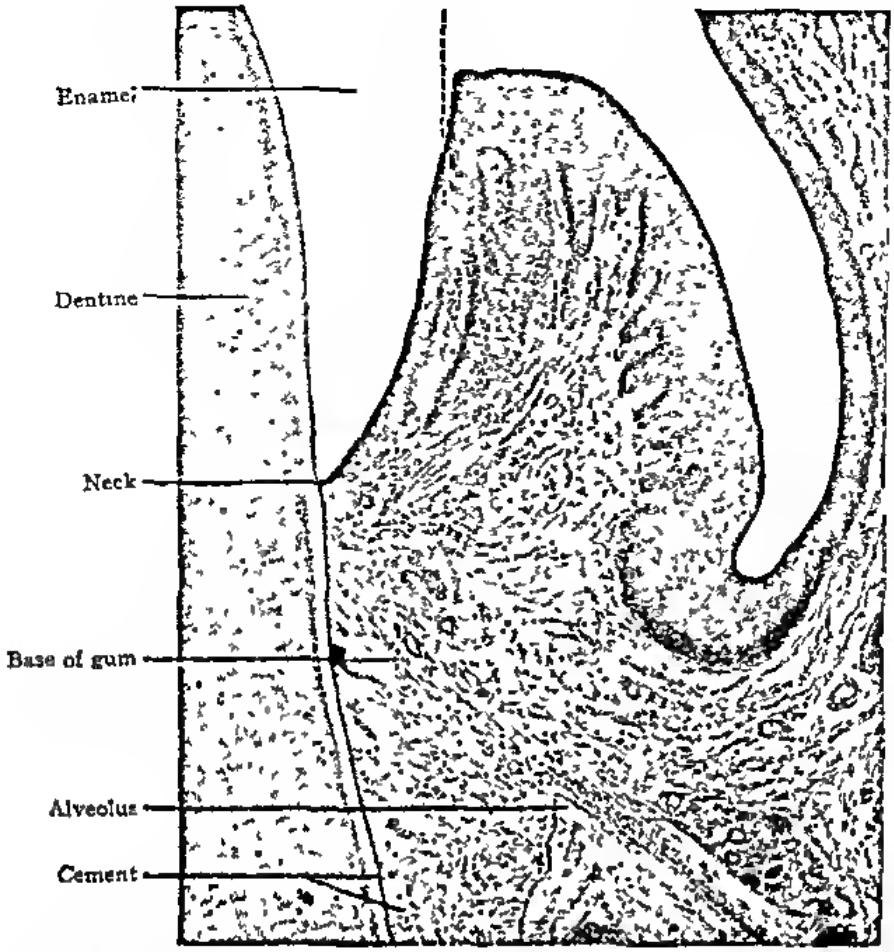


FIG. 545.—TOOTH, PERIODONTAL MEMBRANE, AND GUM. DECALCIFIED SECTION, THE ENAMEL HAS THEREFORE BEEN DESTROYED, BUT ONCE OCCUPIED THE WEDGE-SHAPED SPACE BETWEEN DENTINE, GUM AND THE DOTTED LINE.

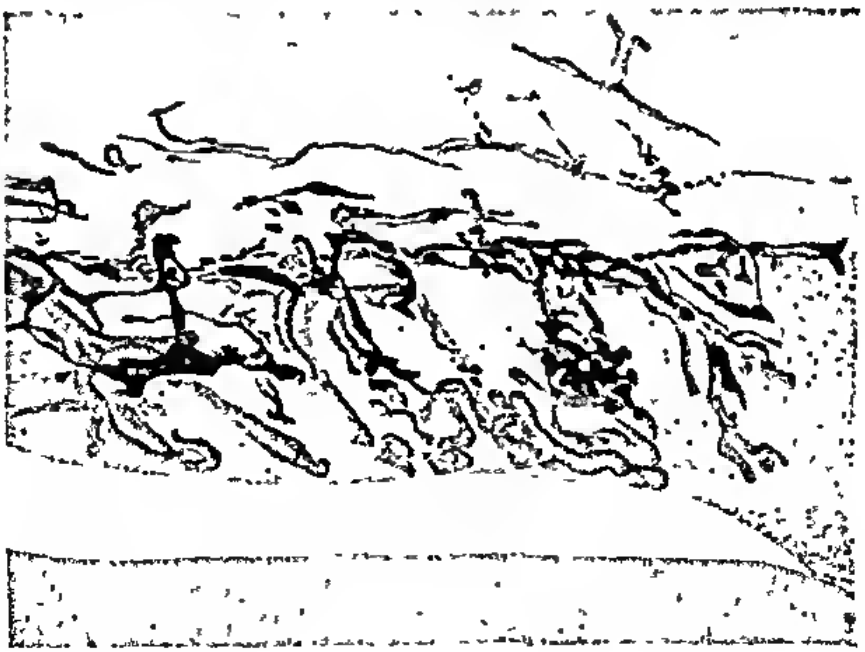


FIG. 546.—LYMPHATIC VESSELS AT THE TRANSITION BETWEEN GINGIVAL AND PERIODONTAL TISSUE. THE LYMPHATIC VESSELS ARE BLACK AND THE BLOOD CAPILLARIES GRAY. DOG, × 115. (Schweitzer.)

nearer to the alveolar bone. They form fine capillary loops toward the tooth. The lymphatic vessels also form loops toward the tooth but they are more tortuous (Fig. 546). In the gum there are found elongated loops of blood and lymphatic capillaries in the tall connective tissue papillæ under the epithelium. Because the papillæ are not always perpendicular to the surface these capillary loops may appear in sections as if they had penetrated the stratified squamous epithelium of the gum. The gum is richly innervated and has in addition to free endings some of which pass into the epithelium between the cells, several different types of encapsulated and non-encapsulated terminal bulbs and coils.

BRAIN

MENINGES

The spinal cord and brain are surrounded by two membranes or *meninges*, of which the outer is dense and fibrous, and is known as the *dura mater;* and the inner is thin and vascular, forming the *pia mater.*

Curiously they are not called membranes, and the term *meninx* (in the singular) is not employed in anatomy. They retain the ancient Arabic designation of 'mother of the brain,' following, according to Hyrtl, a general Arabian tendency to name things 'mothers,' 'fathers,' etc. (The vena cava was the *mater venorum*, and the pupil, the *filia oculi*.) Carrying the figure further, the adjectives of double meaning, dura and pia, were substituted for dense and thin. In the fifteenth century it was said that these membranes were called *matres* because they produce the membranes surrounding the nerves, the coats of the eye, and the periosteum of the skull, with which they are continuous; but Hyrtl denies that the term has any such significance.[1]

The *dura mater spinalis*, or dura mater of the cord, consists of compact fibrous connective tissue with many elastic fibers, flat connective tissue cells and plasma cells. Its inner surface is covered by a layer of flat cells forming a mesenchymal epithelium. It has few nerves and blood vessels. Anteriorly it is continuous with the dura mater of the brain at the foramen magnum. It does not fill the vertebral canal, and is not continuous with the vertebral periosteum. Around it externally there is a layer of vascular fatty connective tissue; and internal to it there is a capillary cleft containing a very small amount of fluid. This *subdural space* connects with tissue spaces in the dura and with those which extend out in the perineurium of the peripheral nerves. It communicates freely, but probably indirectly, with the lymphatic vessels.

The *dura mater cerebralis*, or dura mater of the brain, includes the periosteum of the inner surface of the cranium and consists, therefore, of two lamellæ. The inner is like the dura mater of the cord but contains more elastic fibers; the outer corresponds with the periosteum of the vertebral canal. It contains the same elements as the inner layer, but its

[1] Hyrtl, 1879.

fibers run in a different direction. In order that the dura of the brain and cord may be strictly comparable, some anatomists count the vertebral periosteum and the considerable layer of vascular fatty tissue beneath it as a part of the dura of the cord. In relation with the brain, the dura forms reduplications extending between the cerebellum and the hemispheres, and between the right and left hemispheres. Its two layers separate to enclose large, thin-walled veins, the *sinuses* of the dura. These receive veins from the substance of the brain, but the arteries of the dura, or *meningeal arteries*, supply the cranial periosteum. The dura has many nerves, some with free endings, and others supplying the musculature of the vessels.

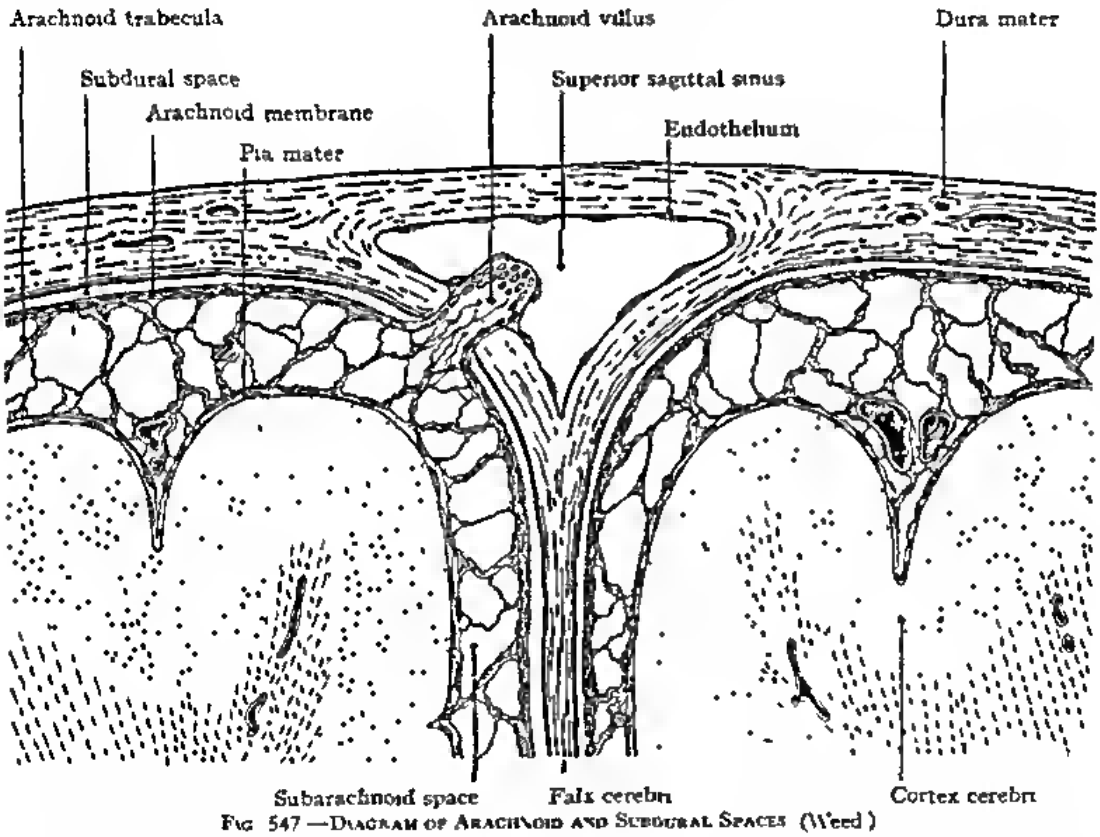


FIG. 547.—DIAGRAM OF ARACHNOID AND SUBDURAL SPACES (Weed.)

The *pia mater spinalis*, as described by Stöhr, is a two-layered sac. The outer layer is covered on its free outer surface with a simple layer of flat cells, which is lightly connected with the dura, and forms the inner wall of the subdural space. The inner layer, or pia proper, is a delicate and very vascular connective tissue, closely connected with the spinal cord, into which it sends prolongations accompanying the blood vessels. The arteries of the spinal cord are primarily two pairs, situated as shown in Fig. 142, E (p. 176). One pair is ventral to the dorsal roots, and the other is near the mid-ventral fissure; their branches supply both the white and gray substance, and the collecting veins branch freely in the pia mater. Between the two layers of the pia, as described by Stöhr, there

is a wide space filled with *cerebrospinal fluid* and traversed by many strands and membranes which pass from one layer of the pia to the other. These strands constitute the *arachnoid membrane*, so-called from its cobwebby texture. Often the name is restricted to the subdural membrane (following Henle), so that the spaces between the meshes of the arachnoid are described as subarachnoid. They are preferably termed *arachnoid spaces* and they are of great importance. The delicate strands are of connective tissue invested by flattened cells belonging to the reticulo-endothelial system, which are phagocytic and capable of forming free macrophages.[1] The fluid which they contain has free access to the arachnoid spaces of the brain. The spaces do not open directly into lymphatic vessels, but in the spinal region there is an exchange by osmosis through special funnel-shaped, closed prolongations along the emerging roots of the spinal nerves.[2]

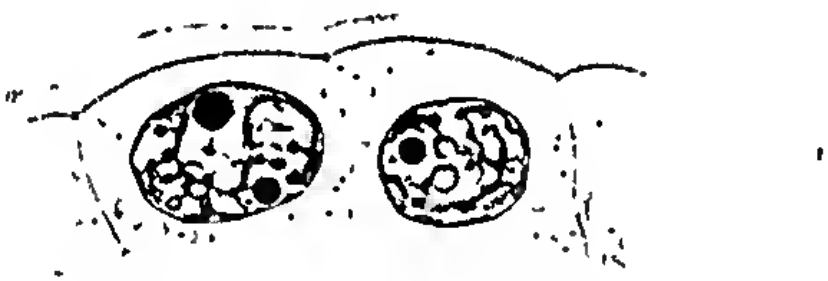


FIG. 548.—TWO EPITHELIAL CELLS FROM THE CHOROID PLEXUS OF THE LATERAL VENTRICLE OF A DOG. Note the 'brush border' and in the cell at the right a single vacuole. Carnoy's fixation, iron-haematoxylin (Szymonowicz).

The *arachnoid membrane*, in the brain, is separated from the dura by a cleft-like subdural space. In certain places, especially along the sides of the superior sagittal sinus, there are found *arachnoid villi* (Pachionian bodies or granulations), which project into the cavity of the venous sinus. They are elevations of the arachnoid covered with a thin portion of the dura and venous endothelium, and possibly facilitate the transfer of fluid between the arachnoid (or subarachnoid) spaces and the veins. These spaces contain the cerebrospinal fluid, and are continuous with the corresponding spaces around the cord. The subarachnoid spaces also communicate with the lymphatic system, and it is possible to demonstrate by x-ray that material (thorium dioxide) introduced into the subarachnoid spaces in the living animal is transferred rather readily into the cervical lymph nodes.[3] The arrangement of the spaces and their relation to brain drainage have been carefully studied by Weed.[4]

[1] ESSICK, 1920. [2] WISLOCKI, 1932. [3] MORTENSEN AND SULLIVAN, 1933.
[4] WEED, 1923

Through apertures in the thin roof of the fourth ventricle, they communicate with the central cavity of the cord and brain.

The *pia* is a delicate and highly vascular layer, containing arteries which send branches into the cortex from all points on its surface. These cortical arteries arise from the anastomoses between the internal carotid and vertebral arteries at the base of the brain, which produce the arterial circle of Willis. Other branches from these vessels enter the substance of the base of the brain, supplying the basal nuclei, thalamus, and internal capsule. Because of the effects of hæmorrhage in relation with the motor

FIG. 549.—SECTIONS OF VILLI FROM THE CHORIOID PLEXUS FROM A 22 YR. OLD MAN 1½ HR. AFTER DEATH. The brush border is no longer seen. Sublimate-acetic acid fixation, hæmatoxylin and Eosin. (Szymonowicz.)

and sensory tracts in this region, these small arteries are of very great importance. The vascular membranes which cover the thin portions of the roof of the third and fourth ventricles are in places invaginated into the ventricles, forming the chorioid plexuses.

The *chorioid plexuses* are essentially irregular invaginations of the pia into the ventricles of the brain, covered by a single layer of ectodermal non-nervous epithelium of special areas where the brain wall normally fails to thicken. The epithelial cells may be both secretory and selectively absorptive, and are in close relation with the underlying vessels. They are supposed to produce the cerebrospinal fluid. They are usually cuboidal in form, may contain fatty droplets or inclusions, and have been described as having a brush border or even cilia. The nerves of the plexuses are of the sensory type and also go to the blood vessels.[1]

[1] CLARK, S. L., 1928.

CEREBRUM

The cerebrum, or hemispheres of the brain, consists of an inner or central portion composed of neuroglia and medullated nerve fibres, covered by a layer of 'gray matter,' the cerebral cortex, in which nerve cells are found. The latter is deeply folded in the sulci and gyri, which form the convolutions of the brain. The arrangement of the cells in the cortex differs slightly in different areas, and the following account is to be taken as somewhat of a generalization. For more detailed knowledge the student is referred to special books on the brain.

The cortex is divided into four ill-defined layers— an outer molecular or neuroglia layer; a layer of small pyramidal cells; a layer of large pyramidal cells; and next the white substance, a layer of polymorphous cells. The layers are shown in Figs. 550 and 551.

The *molecular layer*, which in ordinary sections appears finely punctate or reticular, contains, beside many neuroglia cells, a network of medullated *tangential fibers*, which are parallel with the surface. Other fibers, as shown by the Golgi method, are partly neuroglia, and partly dendrites of pyramidal cells. The 'cells of Retzius' found in this layer have bodies of irregular shape, which send out processes parallel with the surface, and these processes send short branches outward; other processes descend into the deeper layer (Fig. 552). They are probably neuroglia cells.

The *layer of small pyramidal cells* contains a special form of nerve cells, with pyramidal bodies measuring 10–12 μ. Since they taper into a dendritic process, their length cannot be definitely determined. The chief dendrite, after producing small lateral branches, enters the molecular layer where it arborizes freely; its terminal branches often show small irregular projections. Lesser dendrites proceed from the sides and basal surface of the pyramidal cell body. The neuraxon always arises from the basal surface, and after producing branched collaterals, it generally enters the white substance where it may divide in two (Fig. 552, 3). Sometimes the neuraxon turns toward the molecular layer, joining the tangential fibers; infrequently an inverted pyramidal cell is found. The neuraxons and collaterals are medullated.

The *layer of large pyramidal cells* contains those with bodies 20–30 μ long (the 'giant pyramidal cells' of Betz of the anterior central convolution measure even 80 μ). The very large neuraxon always goes to the white substance, after sending out several collaterals in the gray.

The *layer of polymorphous cells* includes oval or polygonal cells which lack a chief dendrite directed toward the surface; their slender neuraxons produce collaterals, and enter the white substance where they may divide into two branches in T-form (Fig. 552, 4). Polymorphous cells, with

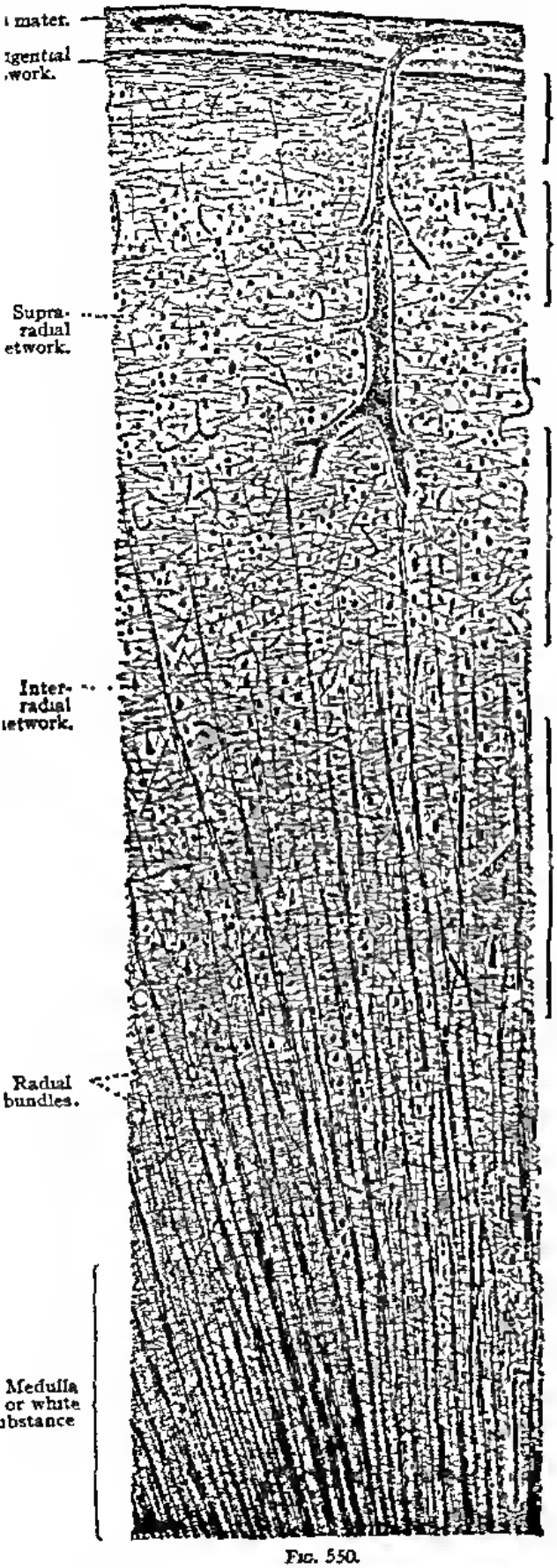


FIG. 550.

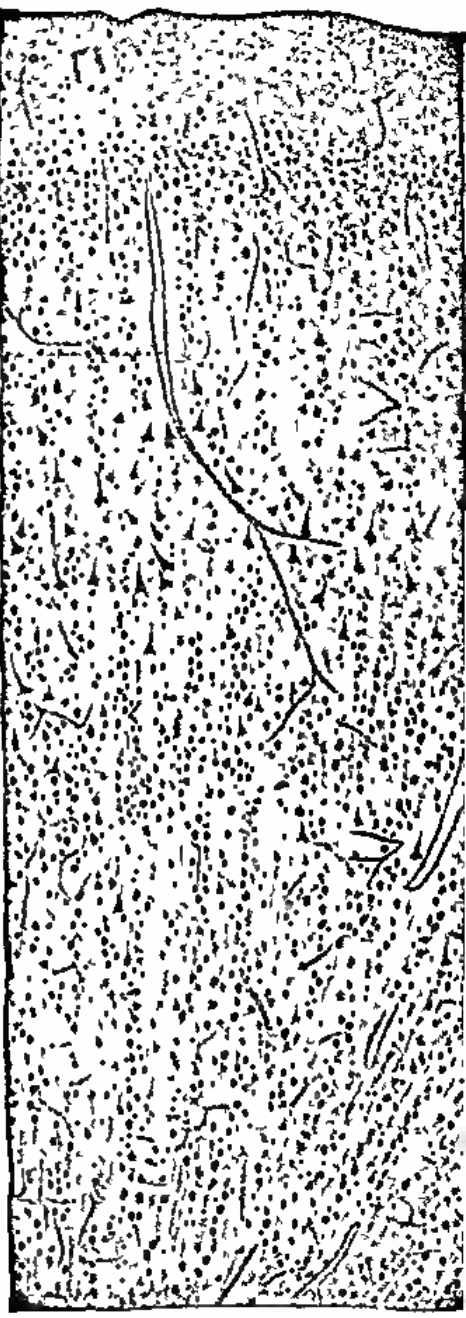


FIG 551.

FIGS 550 and 551 are from vertical section of the cortex (central convolution) of an adult. FIG. 550 is a Weigert preparation, FIG 550 is from a section stained with *hæmatoxylin and eosin*. × 45.

branched neuraxons limited to the vicinity of the cell body, are found in this layer and in the pyramidal layers also. The neuraxon may branch in the molecular layer (Fig. 552, 6).

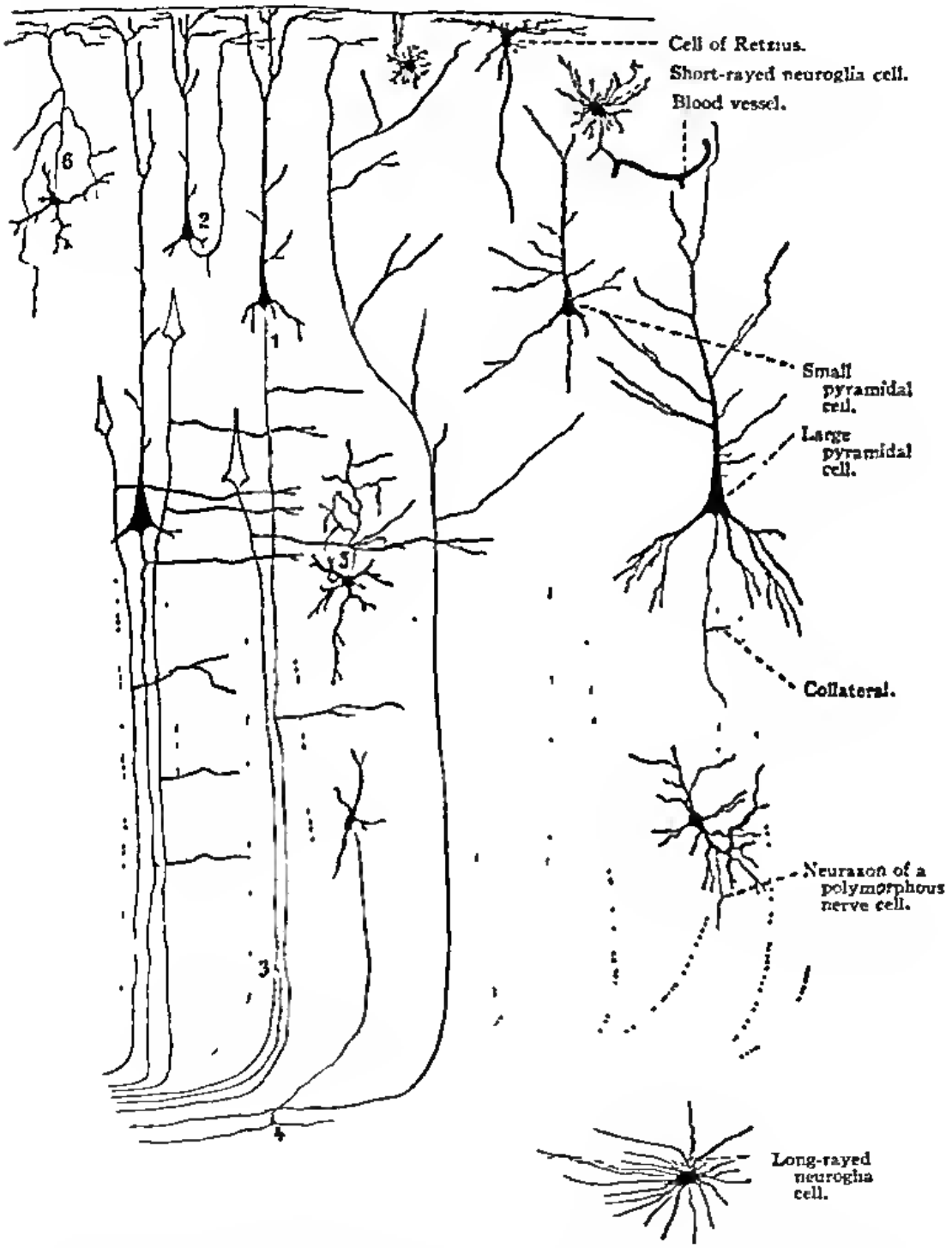


Fig. 552.—Diagram of the Cerebral Cortex. The Cells on the Right Are Drawn from Golgi Preparations of an Adult Man. × 120. The Left Portion of the Diagram is × 60.

Many medullated fibers are found in the deeper layers of pyramidal and polymorphous cells. They are grouped in tapering radial bundles

which terminate toward the layer of small pyramidal cells, as seen in Fig. 550. The bundles include the descending medullated neuraxons of the pyramidal and polymorphous cells, and the ascending medullated sensory fibers from the white substance. The latter branch repeatedly, forming the supra-radial and tangeotial networks. The medullated collaterals of the pyramidal cells run at right angles with the radial bundles; they form an inter-radial network, the outer part of which is so thick in the region of the calcarine fissure that it can be seen without magnification, and is there known as the 'stripe of Vicq d'Azyr.' Similar bands may be detected elsewhere in thick sections (Baillarger's stripes).

The *neuroglia* of the hemispheres, like that of the cord, is at first a syncytium with strands extending from the ventricle to the periphery. Later, the syncytium is divisible into short-rayed neuroglia cells found chiefly in the gray substance, long-rayed cells found chiefly in the white, and ependymal cells lining the ventricles. The ependymal layer is continuous through the aqueduct with that of the fourth ventricles and central canal. In early stages its cells have cilia-like processes which are in part retained in the adult. The short-rayed cells, which are characterized by knotted branching processes, are often in close relation with the blood vessels; they may serve to transfer the nutritive and myelin-forming material from the vessels to the nerve fibers. The outer surface of the cerebral cortex is covered with a feltwork of neuroglia fibers.

CEREBELLUM

The cerebellar cortex also is deeply folded, and everywhere covers the 'white matter.' Unlike the cerebral cortex, the cortex of the cerebellum is throughout its extent of the same structure, and shows the same arrangement of cells. It consists of three strata—an inner *granular stratum*, which is rust-colored in the fresh condition; a middle *ganglionic stratum*, composed of a single row of large cell bodies; and an outer *gray stratum*.

The inner granular stratum consists of many layers of small cells which by ordinary methods show relatively large nuclei and very little cytoplasm. With the Golgi method it appears that, besides neuroglial cells, two sorts of nerve cells are present, the *small* and *large granule cells;* the former (Fig. 554) are multipolar ganglion cells with short dendrites having claw-like terminations, and slender non-medullated neuraxons which ascend perpendicularly to the gray layer and there divide in T-form into two branches. The branches run lengthwise of the transverse folds or convolutions of the cerebellum and have free, unbranched endings. In sagittal sections (Fig. 555) the terminal branches of the neuraxons are cut across. The small granule cells form the bulk of the

granular stratum. The less frequent large granule cells are more than twice the size of the small ones; their branched dendrites penetrate the gray stratum and their neuraxons, going in the opposite direction, are soon resolved into very numerous branches which ramify throughout the granular stratum.

The granular layer contains also a thick network of medullated fibers which enter it chiefly from the white substance. A part of these fibers end in the 'eosin bodies' of the granular stratum, which are heaps of stainable particles found between the small cells. Some of the fibers form bundles

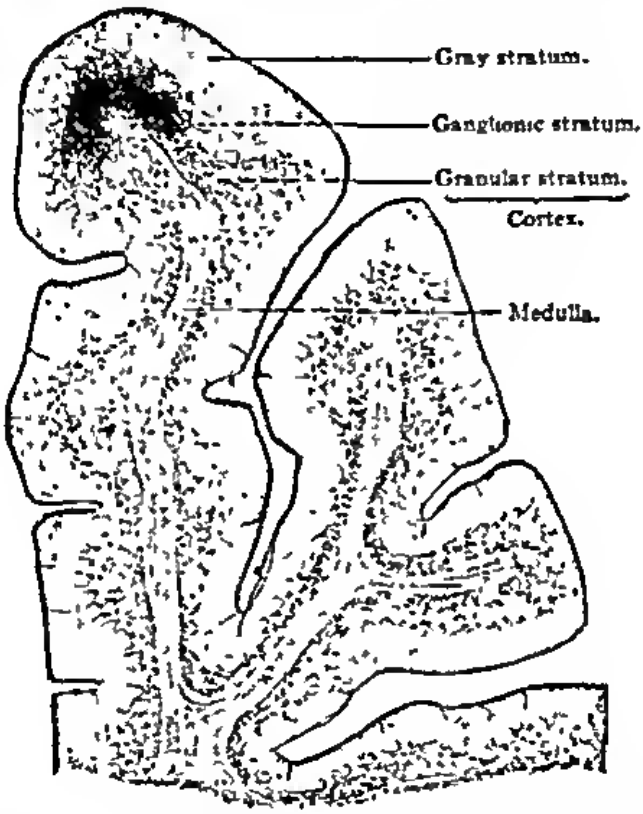


Fig. 553.—From a Sagittal Section of the Cerebellum of an Adult Man. × 12.

parallel with the surface, running between the granular and ganglionic strata in the sagittal direction; they send branches into the gray layer. A small portion of the granular stratum is formed by the medullated neuraxons of the cells of the ganglion layer.

The middle ganglionic stratum consists entirely of a single layer of very large multipolar ganglion cells, called *Purkinje's cells*. Their oval or pear-shaped bodies send two large dendrites into the gray stratum, where they form an extraordinary arborization. Their many branches do not extend in all directions but are confined to the sagittal plane, that is, to a plane at right angles with the long axes of the convolutions. When the convolutions are cut lengthwise, Purkinje's cells appear as in Fig. 554. The neuraxons arise from the deep surface of the cell bodies,

and as medullated fibers they pass through the granular stratum to the white substance. Within the granular layer they produce collateral fibers which branch and in part run back into the ganglionic layer, ending near the bodies of other Purkinje's cells.

The outer gray stratum, of gray color, contains two sorts of nerve cells, the *large* and the *small cortical cells*. The large cortical or *basket cells* are multipolar ganglion cells, the dendrites of which pass chiefly toward the surface. Their long neuraxons, thin at first but later becoming thicker, run parallel with the surface in the sagittal plane. They send occasional collaterals toward the surface, and at intervals produce fine branches which descend and terminate in baskets and around the bodies of Purkinje's cells, often surrounding also the beginning of their neuraxons.

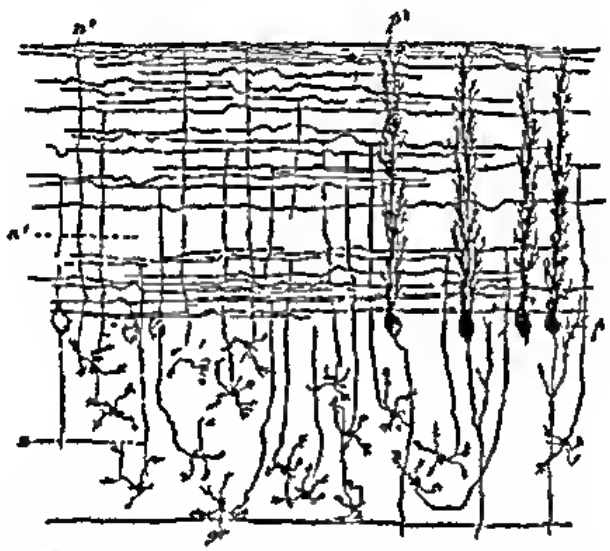


FIG 554.—DIAGRAM OF A SECTION OF THE CEREBELLUM LENGTHWISE OF THE TRANSVERSE CONVOLUTIONS GOLGI'S METHOD (Kölliker)

gr, Cells of the granular stratum, *n*, their neuraxons in the granular layer and *n'*, in the gray stratum, *p p'*, Purkinje's cells (From Bailey's 'Histology')

The small cortical cells, distinguishable from the basket cells since their neuraxons are not in relation with Purkinje's cells, may be divided into two types, connected by intermediate forms. The cell bodies of the first type are nearly or quite as large as those of the basket cells. Their two to five dendrites lie in the sagittal plane like those of Purkinje's cells; the slender neuraxons, 1 mm. long or more, sometimes form loops and are characterized by abundant branches in their proximal parts. The terminal branches are few. Cells of the second type are in general somewhat smaller; their shorter neuraxons branch in the immediate vicinity of the cell bodies. The elements of the first type form the bulk of the relatively numerous small cortical cells, and are found throughout the gray stratum, though they are more abundant in its superficial part. The second type likewise appears throughout the gray stratum.

The medullated nerve fibers found in the gray layer are prolongations of those in the granular stratum. In part they proceed toward the surface, where, after losing their myelin, they end in branches among the den-

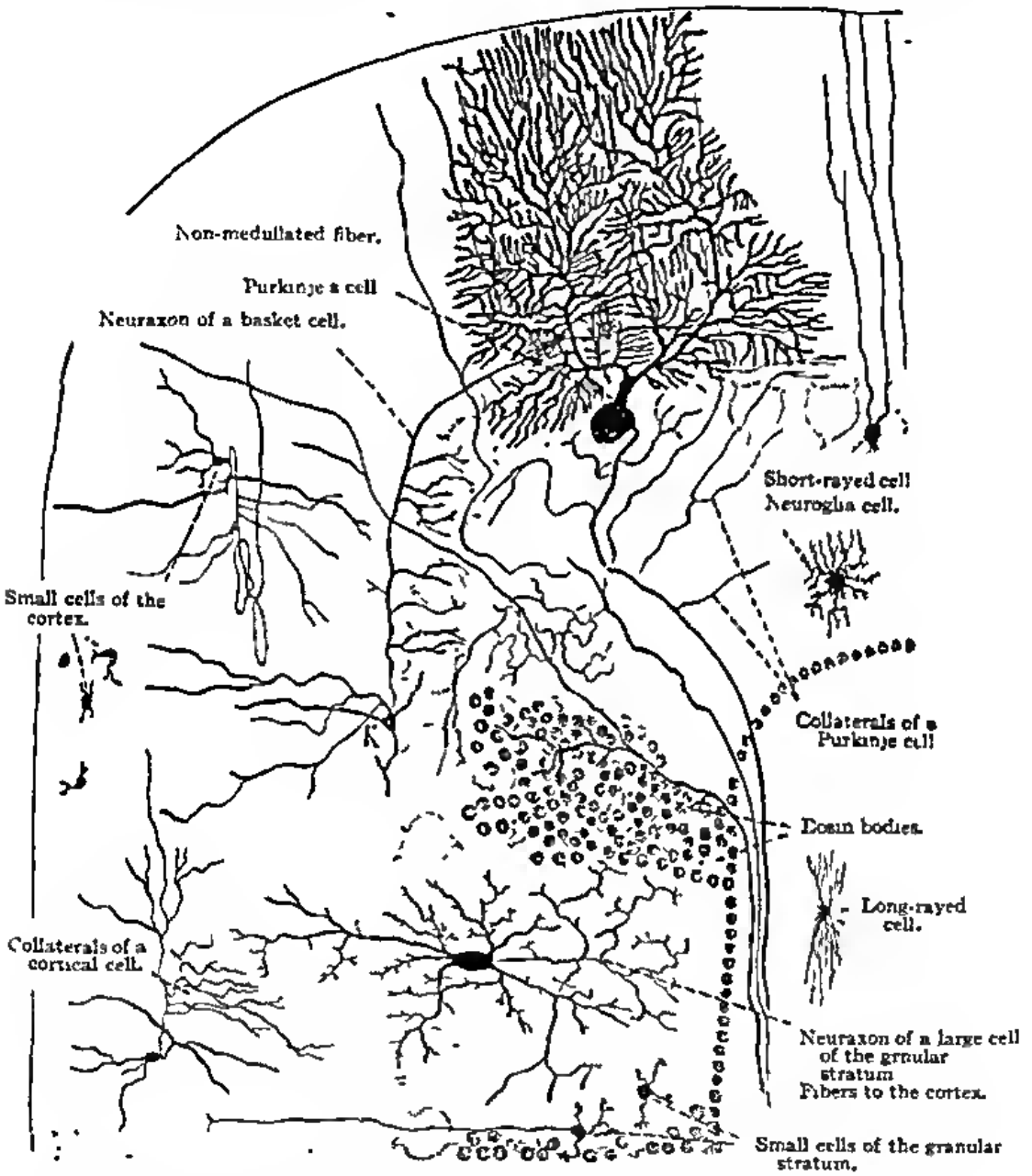


FIG 555.—DIAGRAM OF A SAGITTAL SECTION OF THE CEREBELLUM

Except the large granule cell, which is from a kitten, the cells are drawn from Golgi preparations from an adult man K, large cortical or basket cell.

drites of Purkinje's cells; in part they run between the bodies of Purkinje's cells lengthwise of the convolutions.

The neuroglia of the cerebellum consists of short-rayed stellate cells found in all the layers; of long-rayed cells in the white substance; and of peculiar cells with small bodies at the outer boundary of the granular layer. These send only a few short processes inward, but many long

processes continue out to the free surface, where they end in triangular expansions. In this way a thick peripheral neuroglia layer is produced.

As long as the cerebellar cortex is not fully developed, it presents a series of peculiarities which are lacking in the adult. Thus in embryos and young animals the partly developed gray stratum is covered by a superficial granular layer, the cells of which later become more deeply placed.

ORGANS OF SPECIAL SENSE

These include the eye for sight, the ear for hearing and equilibration, a portion of the nose for smell and of the tongue and epiglottis for taste. In all cases only certain parts of the organ mediate the actual transfer of the sense to the nerves, the rest of the organ serving the purpose of conduction to these special regions, or even some quite unrelated purpose. The sense of touch, with its related feelings of pressure, pain, and temperature, so widespread over the surfaces of the body, is apparently achieved by sensory nerves with special nerve endings, bearing no resemblance to the special sense organs mentioned.

The olfactory sense is perhaps the most primitive, as can be deduced from its nervous connection with the central and most primitive part of the brain, and from the arrangement of its receptive cells. These are neuroepithelial in character, like those of an earthworm—Fig. 141—and, while themselves situated in the epithelial layer, have long nerve processes reaching actually into the brain. Their peripheral ends are provided with special pointed processes reaching to the surface, apparently to receive the impulse which they transmit, and in this is seen the type of all the special sense receptors. In eye, ear, taste-bud, or semicircular ducts the specialized epithelial cell with one or more processes projecting from its free surface is the typical receptor. In these other organs the epithelial cell no longer is provided with a central process running to the brain, but instead is in relation with a sensory nerve fiber coming from some ganglion cell, to which the impulse is transferred. In the same way that the olfactory neuro-epithelial cell is surrounded by other columnar cells of a simple epithelium, so the other sense organs are composed of two types of cell, the receptors and the supporting cells, whose duty is apparently to hold the specialized cells in position. In the taste-bud and the macula of the semicircular ducts these supporting cells are very simple; in the eye, since the whole receptive organ is a portion of the brain, the supporting cells are neuroglia; in the ear they are very much altered in shape and structure, but still to be considered as supporting epithelial cells. From this point of view the organs of special

sense all show a certain basic similarity, though varying greatly in complexity of detail.

EYE

Development and General Anatomy. The eyes first appear as a pair of *optic vesicles*, which are lateral out-pocketings of the fore-brain (Fig. 556, A). They enlarge rapidly, but their connections with the wall

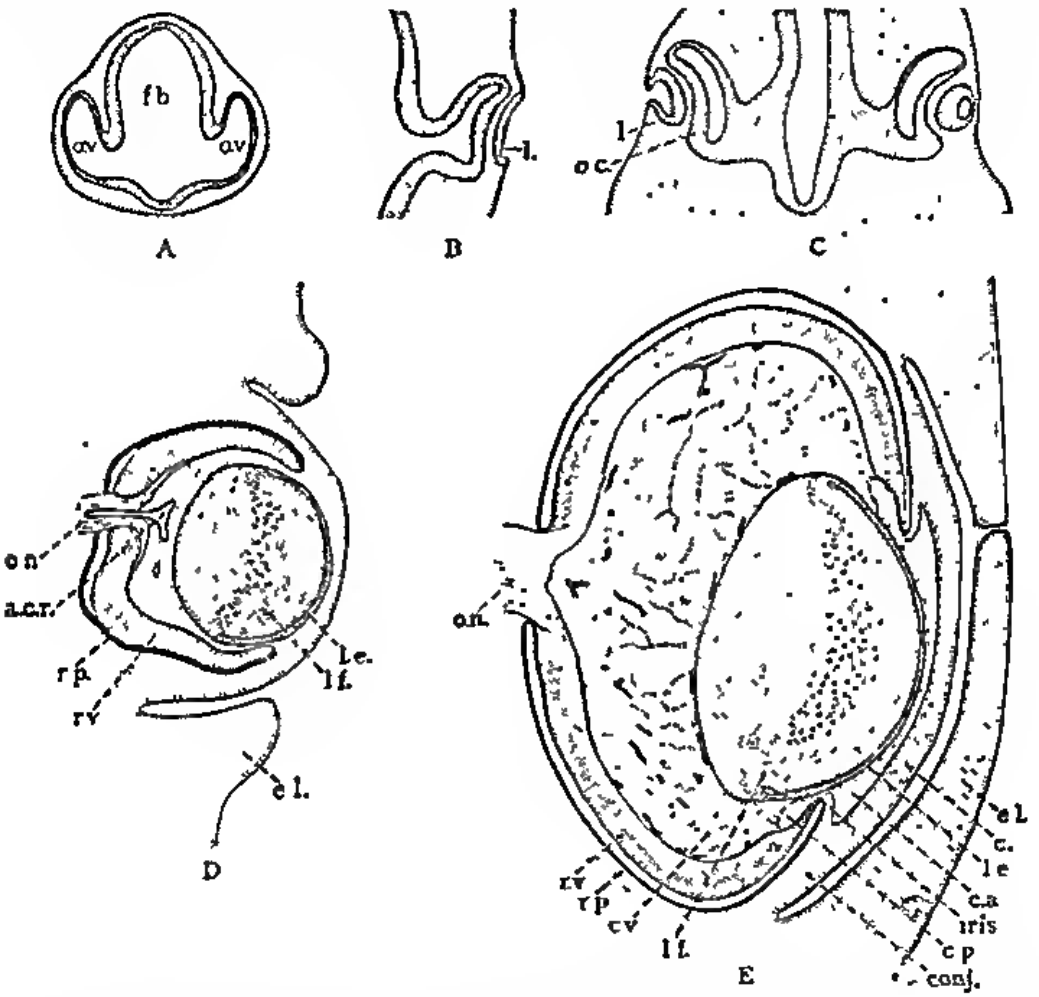


Fig. 556.—Sections of Rabbit Embryos to Show the Development of the Eye. A, 9½ days, 3.0 mm., B, 10½ days, 5.4 mm., C, 11 days, 5.0 mm., D, 14 days, 18 hours, 12.0 mm.; E, 20 days, 29 mm.

a. c. r., Arteria centralis retinæ; c., cornea; c. a., anterior chamber; conj., conjunctiva; c. p., posterior chamber; c. v., corpus vitreum; e. l., eyelid; f. b., fore-brain; l., lens; l. e., lens epithelium; l. f., lens fibers; o. c., optic cup; o. n., optic nerve; o. v., optic vesicle; r. p., pigmented layer of the retina; r. v., visual layer of the retina.

of the brain remain relatively slender, forming the *optic stalks*. The epidermal ectoderm immediately overlying the vesicles thickens and becomes invaginated (B and C). The invaginated portion is then detached in the form of a vesicle, the inner wall of which is distinctly thicker than the outer; this 'lentic vesicle' becomes the lens of the eye. Meanwhile, as seen in B and C, that layer of the optic vesicle which is toward the epidermis sinks in upon the deeper layer, transforming the vesicle into the *optic cup*. At first the cup is not complete, being deficient

on its lower side (Fig. 557). The *arteria centralis retinæ* is seen passing through this indentation, which begins on the lower surface of the stalk and extends to the free margin of the cup; the cleft is called the fetal or 'chorioid fissure.' Distal to the point of entrance of the artery into the optic cup, the edges of the fissure fuse; the artery then appears to perforate the base of the cup, and it retains this relation in the adult. The artery is shown in section in Fig. 556, D.

The older term chorioid or chorioidal fissure, indicating that the contents is connected with the chorioid coat of the eye, should be replaced by the term fetal fissure, or fetal ocular fissure.[1] The vessel which enters the cleft becomes at first the hyaloid artery, quite distinct from the ciliary arteries to the chorioid. The fissure begins to close in its central part at about 11.0 mm. in man, and the notch at the margin of the cup disappears soon after the 15.0 mm. stage. Toward the brain, the fusion carries the artery well back along the optic stalk.

In a series of experiments upon tadpoles, W. H. Lewis[2] has shown that 'the lens is dependent for its origin on the contact influence or stimulus of the optic vesicle.' If the optic vesicle is removed, the epithelium in the region of the normal lens does not become thickened or invaginated; but if an optic vesicle is transplanted by detaching it from its stalk and pushing it caudally through the mesenchyma, it will cause the formation of a lens from any portion of the epidermal epithelium which happens to be above it. Moreover, if an area of skin from the abdomen of a frog of one species is grafted over the optic vesicle of another species, a lens may be produced from the grafted epithelium. Thus there is no predetermined area for lens formation, and its development depends upon the presence of the vesicle beneath.

The two layers of the optic cup, the inner of which is toward the lens, are normally in contact with one another, although in sections they have often become more or less separate. This potential space is maintained throughout life, a point of clinical importance in consideration of possible 'separation of the retina.' The two walls together form the retina, which includes a thin outer *pigmented layer,* and a thick inner *visual layer;* the latter is composed of several strata of nerve cells and fibers. Light passes through this visual layer, which is transparent, and acts upon the pigment layer. This in turn transmits stimuli to numerous tapering projections extending from the outer surface of the visual layer and embedded in the pigment layer. Separation of the two layers causes blindness. In explanation of the fact that the sensory processes are turned away from the light, it may be said that the outer surface of the skin ordinarily receives stimuli, and that, through the infolding which makes the medullary tube and the out-pocketing which makes the optic vesicle, the sensory

Fig. 557.—Optic Cup and Stalk of a Human Embryo of 6 9 mm (Kollmann)

[1] Mann, 1928. [2] Lewis, W. H., 1904.

The retinal cup is surrounded by two layers of mesenchymal origin. The inner *tunica vasculosa* corresponds with the pia mater and forms the *chorioid* coat of the eye; the outer *tunica fibrosa* corresponds with the dura mater and forms the *sclera*, into which the muscles of the eye are inserted. The portion of the retinal cup which forms a curtain, circular in front view, between the anterior and posterior chambers, is called the *iris*. It consists of tunica vasculosa with a thin pigmented prolongation of the retina over its posterior surface (Figs. 556, E, and 559). This *pars iridica retinæ* is rudimentary and without visual function. At the attached border of the iris the vascular coat contains important muscle fibers, and is there thickened to form the *ciliary body*. This is also covered by a rudimentary pigment layer on its inner surface, the *pars ciliaris retinæ*. At the *ora serrata* (Fig. 574) an abrupt thickening of the visual layer of the retina marks the boundary between its ciliary and optic portions. The *pars optica retinæ* extends from the ora to the optic nerve, covered externally by the chorioid and sclera.

As a relatively frequent congenital anomaly, the fetal fissure fails to close normally and the resulting defect is known as *coloboma*. If the closure has been nearly complete, so that there is merely a notch at the free margin of the optic cup, it appears in the adult as a median ventral cleft in the iris, so that the pupil is shaped like an inverted pear. If the deeper parts of the chorioid fissure fail to unite, there will be a median ventral gap in the optic portion of the retina, which may seriously interfere with vision.

The *cornea* is the tissue in front of the anterior chamber, consisting of a non-vascular mesenchymal tissue, bounded posteriorly by mesenchymal epithelium and anteriorly by the epidermal ectoderm. It is extremely transparent. The epidermal ectoderm extends from the cornea and front of the eye over two folds which form the eyelids. They have met in Fig. 556, D, and fused temporarily. Externally the lids are covered by skin, and internally by the *conjunctiva palpebrarum*, or conjunctiva of the lids. The latter is continuous with the *conjunctiva bulbi* which forms the opaque vascular 'white of the eye.' It surrounds the cornea, the epithelium of the two structures being continuous.

The parts of the eye to be examined histologically are therefore the retina, optic nerve, lens, and vitreous body, all of which are ectodermal; then the tunica vasculosa, including the chorioid, the ciliary body, and iris; next the tunica fibrosa, including the sclera and cornea; and finally the accessory structures—the lids, conjunctivæ and glands.

Retina. The retina extends from the papilla of the optic nerve to the pupillary border of the iris, and is divisible into three parts; the *pars optica retinæ* includes all which is actually connected with the optic nerve and which therefore is sensitive to light. It covers the deeper portion of the optic cup, ending near the ciliary body in a macroscopic, sharp, irregular

line bounding the ora serrata. The *pars ciliaris* and the *pars iridica retinæ* are the rudimentary layers covering the ciliary body and iris respectively.

The pars optica retinæ in a fresh condition is a transparent layer colored reddish after dark adaptation by the 'visual purple' (or rhodopsin) in many of the vertebrates, possibly including man.[1] In sections it presents many layers arranged as seen in Fig. 560, the cells of which are related to one another as in the diagram, Fig. 561. The outer layer of the optic cup forms the *pigmented epithelium* of the retina, which consists of a simple layer of six-sided cells. Toward their outer surface (that next the chorioid, where the nucleus lies) they are poor in pigment,

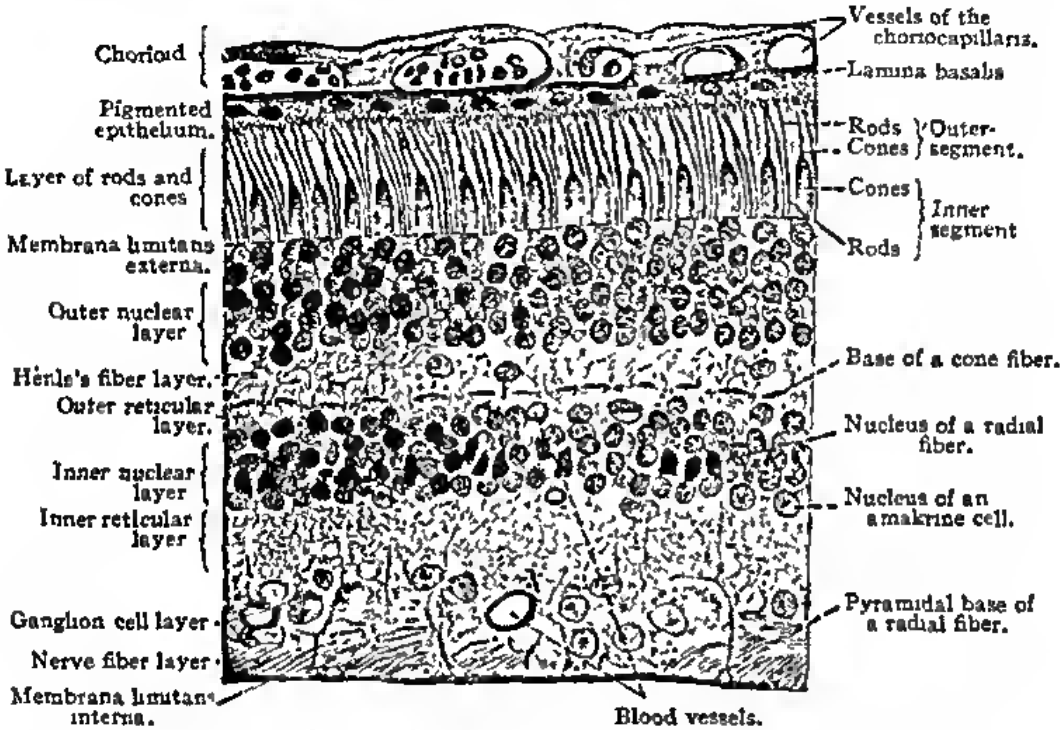


Fig. 560.—Vertical Section of a Human Retina. × 36

whereas in their inner portion they contain numerous rod-shaped (1–5 μ long) brown granules of the pigment 'melanin.'[2] In albinos the pigment is lacking. From the inner surface of the pigmented epithelium, numerous processes extend between the rods and cones of the visual layer. In lower vertebrates these processes react to light by expansion and the migration of the pigment content, and the rods and cones change in length. In mammals the photomechanical changes are very slight, if they occur at all.[3] The visual receptors, which are found along the outer surface of the inner retinal layer, are of two sorts, *rod cells* and *cone cells*. In both, the nucleus is found in the basal half of the cell, and the outer non-nucleated half projects through a membrane, the *membrana limitans externa*. This causes the visual cells to appear divided into layers, their

[1] Duke-Elder, 1931. [2] Jacobsen, 1934. [3] Detwiler, 1924.

nucleated parts beneath the limiting membrane constituting the *outer nuclear layer* (or outer granular layer), and the non-nucleated parts outside of the membrane forming the *layer of rods and cones.*

The rods are in general about four times as numerous as the cones. The latter decrease in frequency at greater distances from the macula, with the exception of a secondary increase of cones at the very periphery of the retina. The rods are elongated cylinders (60 μ long and 2 μ thick) consisting of a homogeneous outer segment and a finely granular inner segment. In the outer third of the inner segment there is said to be an ellipsoidal, vertically striated structure (which in some lower vertebrates is very distinct). The portion of the rod cells below the limiting mem-

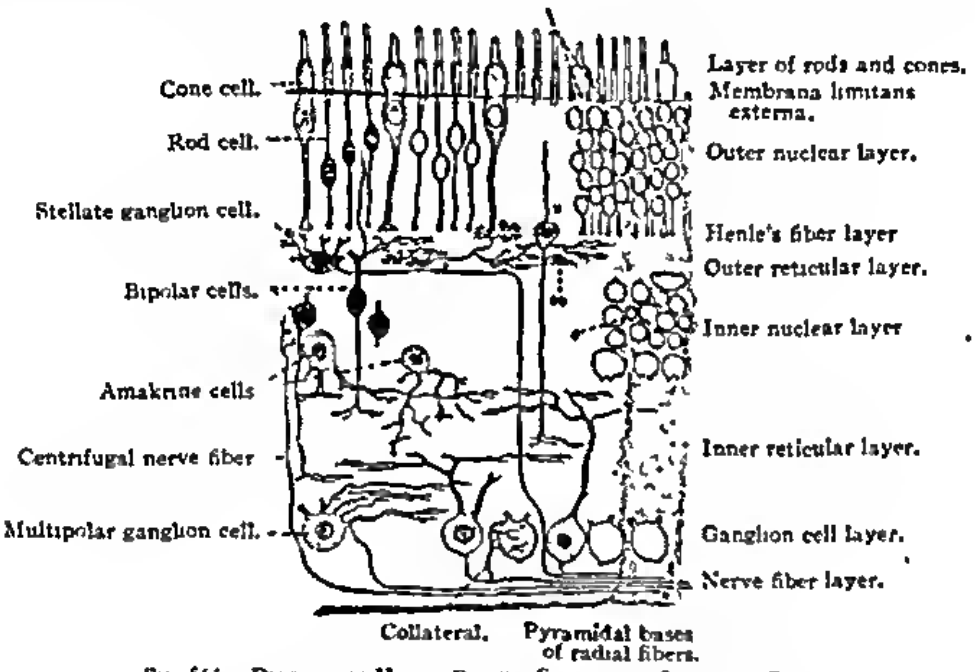


FIG. 561.—DIAGRAM OF HUMAN RETINA. SUPPORTING SUBSTANCE RED.

brane is a slender thread, expanding to surround the nucleus which is characterized by from one to three transverse bands. Beneath the nucleus the protoplasm again becomes thread-like, and this basal prolongation of the cell terminates in a small club-shaped enlargement, without processes.

The cones likewise consist of an outer and an inner segment. The conical outer segments are shorter than those of the rods. The inner segments are thick and somewhat dilated so that the entire cone is flask-shaped. Moreover, the inner segment contains a vertically striated 'fiber apparatus.' The nuclei of the cone cells are situated just beneath the limiting membrane; below the nuclei the protoplasm forms a fiber, ending in an expanded pyramidal base.

The entire visual cells, therefore, form three layers of the retina, namely, (1) the *layer of rods and cones;* (2) the *outer nuclear layer*, contain-

ing the nuclei of the rod and cone cells; and (3) *Henle's fiber layer*, composed of the basal processes of these cells. The three layers next beneath are formed essentially of superposed parts of the radially arranged bipolar nerve cells, which constitute the *ganglion retinæ*. Immediately beneath Henle's fiber layer, dendritic processes of these cells form an *outer reticular layer*, whereas their nuclei are situated in an *inner nuclear layer*, and their centripetal processes, or neuraxons, enter an *inner reticular layer*. There they terminate in relation with dendrites and cell bodies of large ganglion cells which constitute the *ganglion of the optic nerve*. Cell bodies of this ganglion form the *ganglion cell layer*, and their neuraxons, traveling toward the papilla of the optic nerve, are the principal elements

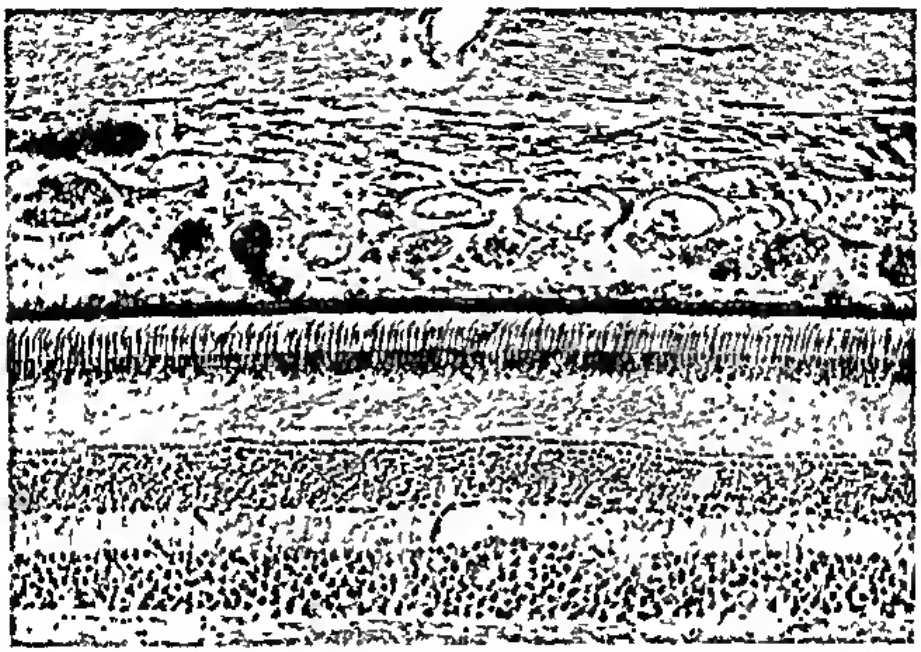


Fig 562.—Vertical Section of a Human Retina Near Macular Region (Verhoeff)
The ganglion cells are very numerous near the fovea centralis

in the *nerve fiber layer*. The latter is separated from the vitreous body by an *internal limiting membrane*. Thus visual stimuli, received by the rods and cones, are transferred by means of the bipolar cells of the ganglion retinæ to the ganglion cells of the optic nerve, through the neuraxons of which they proceed to the brain. These layers may be described in further detail as follows:

Henle's fiber layer contains not only the fiber-like basal ends of the rod and cone cells, but also the slender, unbranched, dendritic processes of the bipolar cells of the ganglion retinæ. Each bipolar cell sends one such process through Henle's layer to terminate in a little thickening near the membrana limitans externa. In the outer reticular layer, however, these dendrites of the bipolar cells send out branches which bifurcate repeatedly, becoming reduced to the finest fibrils; they form a close sub-epithelial felt-work.

Occasionally nuclei are found in the outer reticular layer. Most of these belong with bipolar cells displaced outward (Fig. 561, x). Toward the inner nuclear layer, however, there are stellate ganglion cells with neuraxons which pursue a horizontal course and then turn inward to join the optic nerve fibers. The existence of such fibers has been denied by some writers. The neuraxons of other stellate ganglion cells in this region end in relation with the bases of the visual cells (Fig. 561, +).

Toward the inner reticular layer, the inner nuclear layer contains the bodies of ganglion cells, which appear to lack a chief or large process, and are therefore called 'amakrine' cells. They send branching fibers into the inner reticular layer, where they interlace with the fine varicose branches of the bipolar cells, and with the ramifications of the dendrites from the *ganglion nervi optici*.

The ganglion cell layer consists of a single row of large multipolar cells containing Nissl's bodies. Certain of these cells because of exceptional size are known as 'giant ganglion cells,' and they occur at quite regular intervals. 'Twin cells' have been found, consisting of two cell bodies united by a short bridge; only one of the pair has a neuraxon.

The nerve fiber layer consists chiefly of the non-medullated neuraxons of the ganglion cells, arranged in plexiform bundles. Occasionally the neuraxons send collaterals back to the ganglion cell layer, where they branch about the cell bodies. The fiber layer contains also neuraxons which have come out from the brain to terminate in free branches among the cells of the inner nuclear layer.

In addition to the nervous elements, the retina contains blood vessels and a supporting framework of neuroglia cells. The largest vessels are toward the fiber layer, in which they travel to and from the central vessels in the papilla. The neuroglia framework consists chiefly of *radial* (or Müller's) *fibers*, which are elongated cells extending from the internal to the external limiting membrane. Beyond this membrane they send short processes between the rods and cones, forming 'fiber baskets.' The radial fibers are not isolated cells but are parts of a general syncytium, being connected by a network of processes which penetrate all the layers of the retina. The external limiting membrane, through which the rods and cones pass, is formed by the coalescence of the processes, and the internal limiting membrane is made up of the closely adjacent basal expansions of the radial fibers. The nuclei of the fibers are found in the inner nuclear layer. In addition to the radial fibers there are neuroglia cells with horizontal or tangential branches (Fig. 561, 00). As in the central nervous system, some of the stellate groups of fibers do not contain nuclei.[1]

[1] For further reading on the retina see, S. L. Polyak, 1941. The Retina. University of Chicago Press.

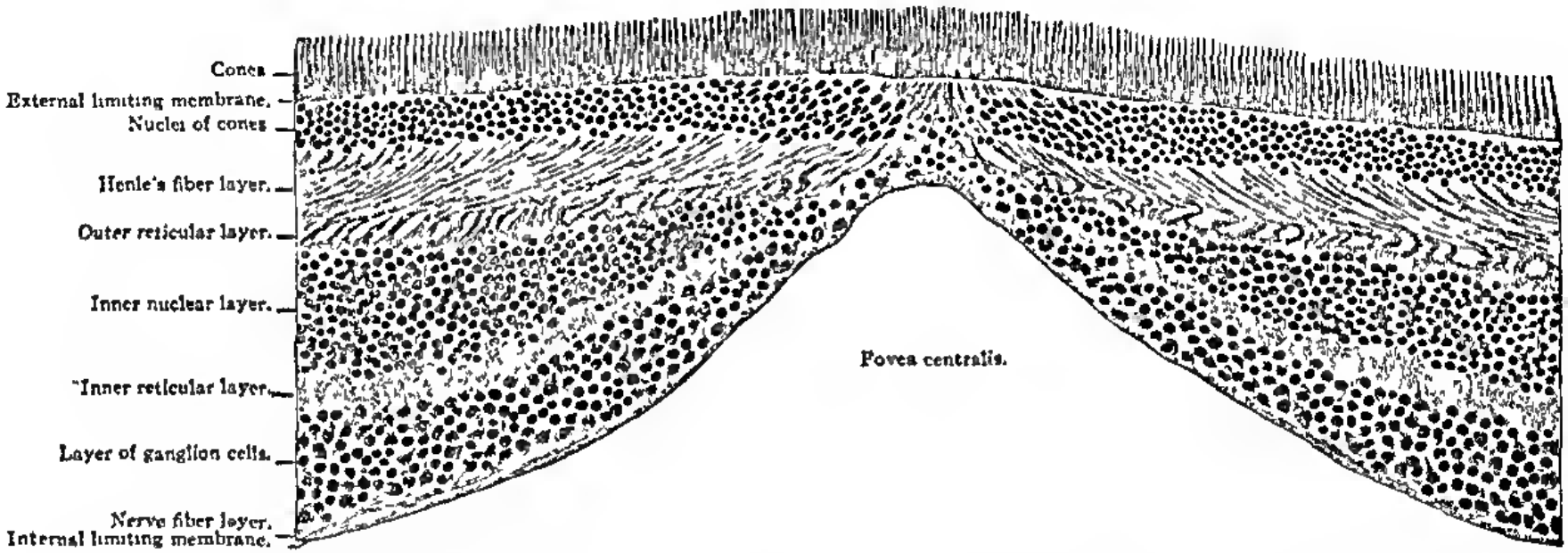


FIG. 563.—HORIZONTAL SECTION THROUGH THE MACULA AND THE FOVEA OF A MAN SIXTY YEARS OLD. × 135. (Schaper.)

The nerve fiber layer, like all the layers, is thicker on the side toward the papilla of the optic nerve than on the opposite side, in the latter situation the nerve fibers are seen in transverse section as minute dots. The section is not through the exact center of the fovea, for there only cone cells are present; no remnants of the confluence of the inner granule and ganglion cell layers are found.

Two modifications of the retina require special description, namely, the *fovea centralis*, which is the region of most acute vision, and the *pars ciliaris*, which is the rudimentary peripheral portion.

Macula Lutea and Fovea Centralis. When vision is centered upon a particular object, the eyes are so directed that the image of the object falls

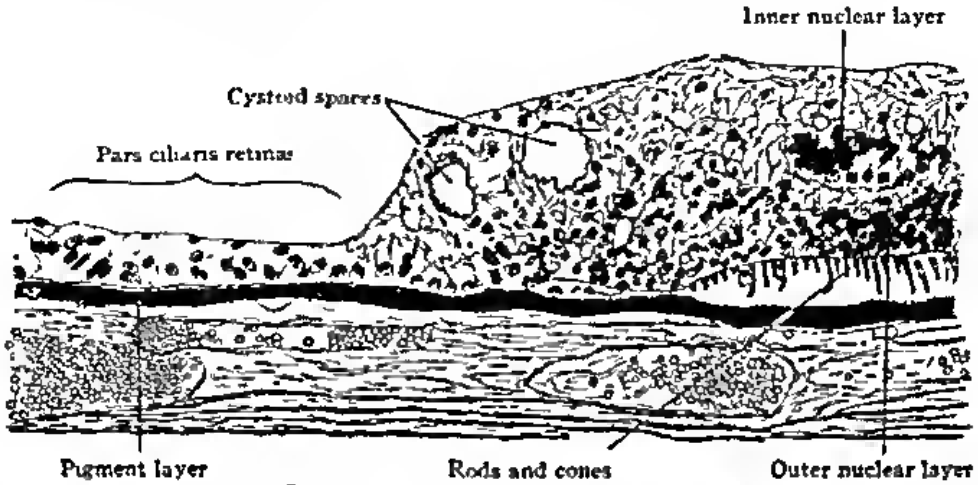


Fig. 564.—Meridional Section of Ora Serrata. The Space between the Pigment Layer and the Rods and Cones is an Artifact.

upon the *macula lutea*, or yellow spot of the retina, within which there is a depression, the *fovea centralis*. The macula sends straight slender fibers to the papilla of the optic nerve, which is close by on its median side; other coarser optic fibers diverge as they pass the macula, forming an ellipse around it. The retinal layers of the macula are arranged as shown in Fig. 563. At its border the number of rod cells diminishes, and within the macula they are entirely absent. The cones of the foveal region are more slender and longer than those elsewhere, and this more highly specialized group may be responsible for the acuity of macular vision, which is limited to approximately a 3° angle. The very center of the fovea is sometimes called the *foveola* or area centralis. The nuclei of the cone cells form an outer nuclear layer of twice the usual thickness. The basal portions of the cone cells make a broad Henle's fiber layer, and slope away from the fovea. The bipolar cells of the ganglion retinæ are so numerous that their nuclei may form nine rows. The ganglion

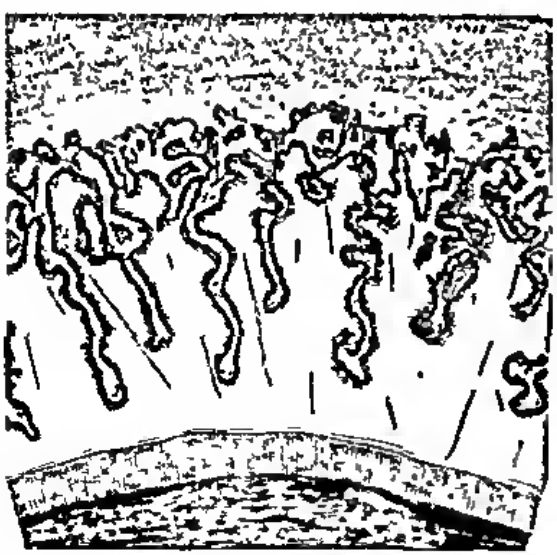


Fig. 565.—Equatorial Section of Human Ciliary Body and Lens in Region of Ciliary Processes. In Equatorial Section Only Is the Arrangement of the Ciliary Processes Markedly Similar to the Chorioidal Plexus of the Cerebral Ventricle.

cells of the optic nerve are also abundant. All of these strata become thin toward the fovea, the deepest part of which contains scarcely more than the cone cells. In some individuals the slope of the sides of the fovea is less steep than in the figure; its depth is variable. The macula and fovea are saturated with a yellow pigment soluble in alcohol.

Pars Ciliaris Retinæ. The optic nerve fibers and their ganglion cells disappear before reaching the ora serrata. The cone cells extend further

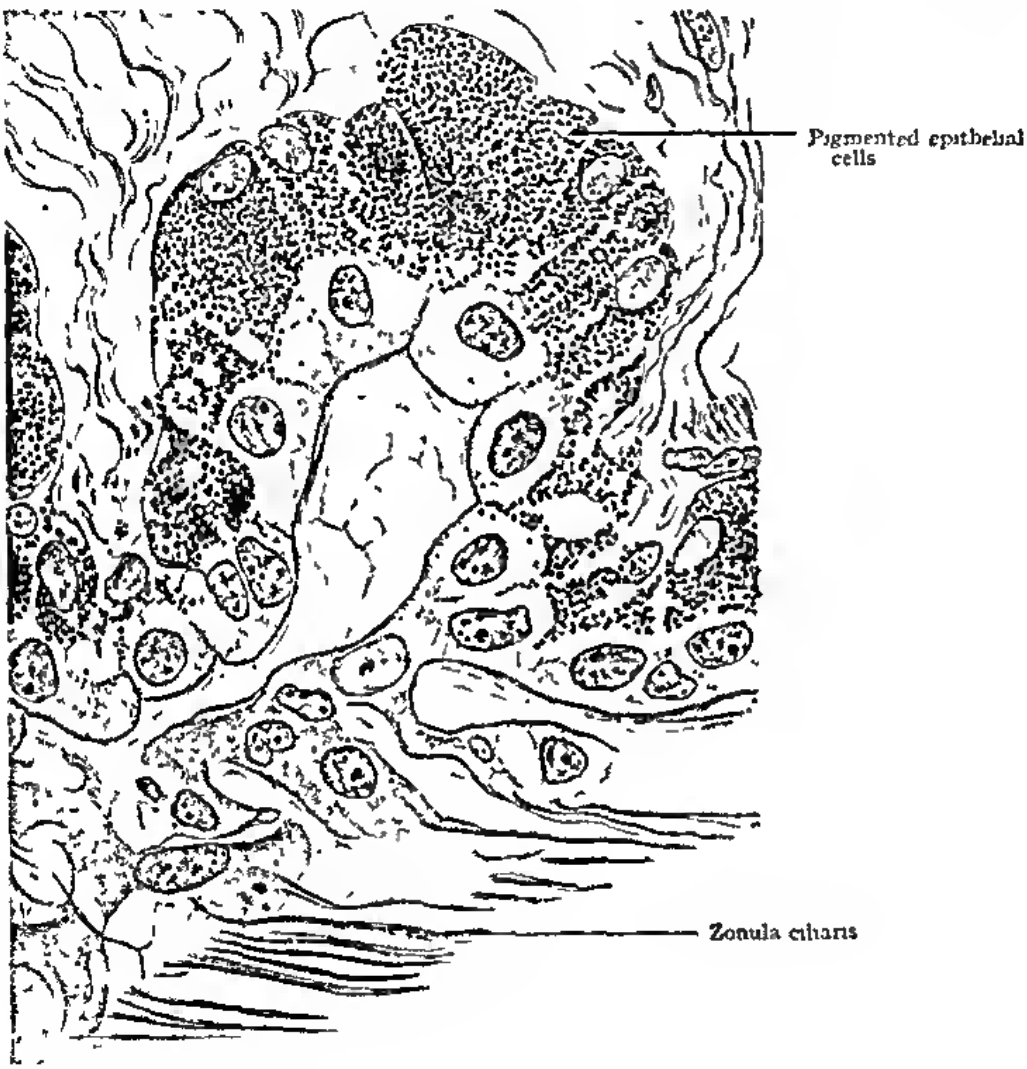


FIG. 566.—SECTION OF THE CILIARY PROCESS WITH ATTACHMENT OF ZONULA FIBERS. HUMAN. AZAN. (Drawn from a preparation by Prof. M. Heidenhain.)

toward the ora than the rods, but the last of them appear to lack outer segments. By the thinning of the reticular layer, the nuclear layers become confluent. Near the ora serrata large clear spaces, called *cystoid spaces*, may normally occur in the outer nuclear layer, and they may extend into the deeper layers. The radial sustentacular cells form a simple columnar epithelium as the other layers disappear, and they constitute the visual layer of the pars ciliaris. The pigmented epithelium is apparently unmodified as it extends from the optic to the ciliary portion. Along

the inner surface of the ciliary part of the retina, the cells of the visual layer produce closely packed horizontal fibers, which form a refractive hyaline membrane.

Zonula Ciliaris. Some of the fine homogeneous fibers arising from the pars ciliaris immediately in front of the ora serrata enter the vitreous body, but a much larger number pass between the ciliary processes to the lens. They are arranged in fine bundles embedded in a homogeneous clear matrix. They are attached to the borders of its capsule, overlapping slightly its anterior and posterior surfaces. Thus they form the zonula ciliaris (suspensory ligament) which holds the lens in place (Fig. 559). The zonula *is not a continuous layer, nor does it consist of two laminæ,*

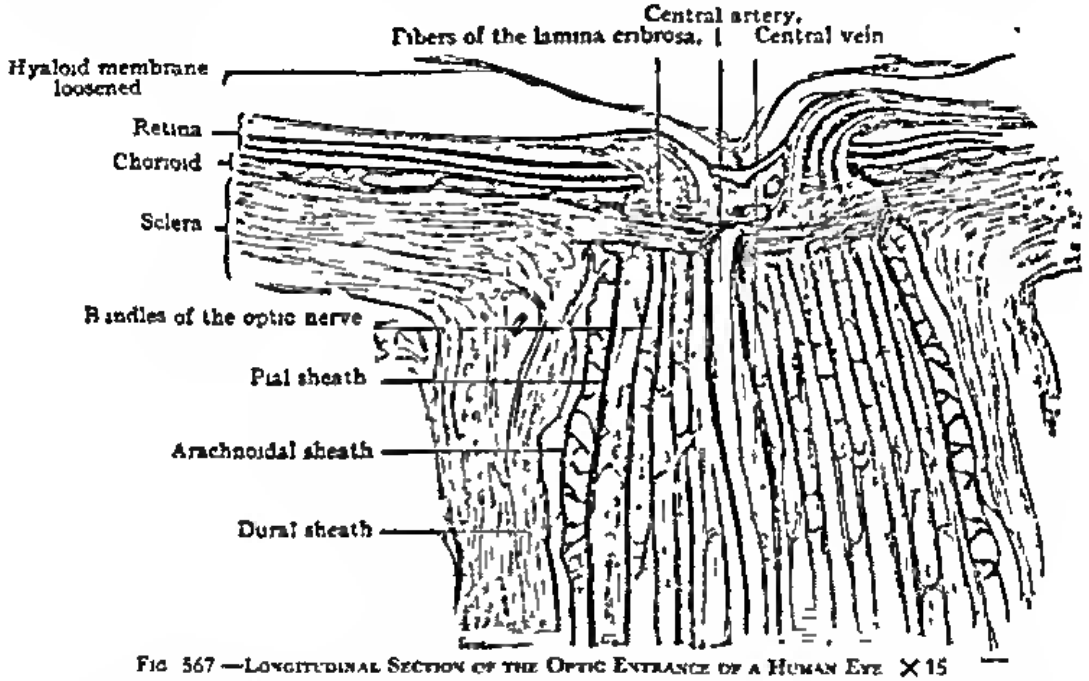


Fig. 567.—Longitudinal Section of the Optic Entrance of a Human Eye. ×15

Above the lamina cribrosa is seen the narrowing of the optic nerve, due to its loss of myelin. The central artery and vein have been for the most part cut longitudinally, but above at several points transversely

one to the anterior and the other to the posterior surface of the lens, with a space between them. It consists rather of numerous bundles, between which and the vitreous body, and among the bundles themselves, there are *zonular spaces* (canals of Petit) which communicate with the posterior chamber.

If a coloboma extends beyond the iris and includes the ciliary body, the zonula will also be absent at this spot, and the lack of tension on the lower border of the lens may cause the latter to assume an indented or reniform shape.

Optic Nerve. In its intraorbital portion the optic nerve is surrounded by prolongations of the meninges. On the outside is the dural sheath, consisting of dense connective tissue with many elastic fibers. The outer connective tissue bundles tend to be longitudinal and the inner, circular.

Internally the outer sheath is connected with the arachnoid layer by a few dense strands of tissue, and the arachnoid joins the pial sheath by many branched trabeculæ. The pia surrounds the entire nerve and sends anastomosing septa among the bundles of nerve fibers. The latter are slender and medullated, but without a neurolemma; they are supported by long-rayed *neuroglia cells*, which are found between the individual fibers, but are most numerous at the periphery of the bundles and around the entire nerve. Thus the optic nerve differs from the peripheral nerves, and resembles a cerebral commissure.

At the posterior surface of the eye-ball (or *bulbus oculi*), the dura blends with the sclera. Continuous with both is the dense elastic *lamina*

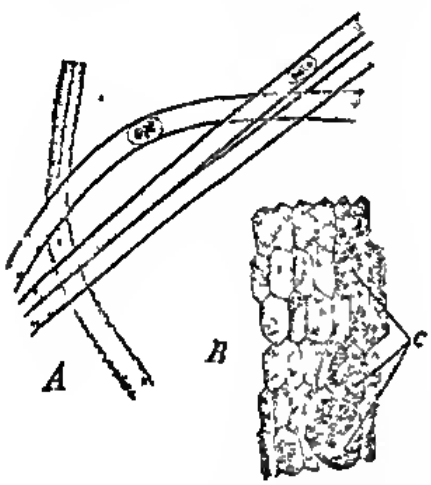


Fig. 568.—Lens Fibers of a New-born Infant

A, Isolated lens fibers, three with smooth and one with dentate borders. × 240 B, Human lens fibers cut transversely, c, section through club-shaped ends. × 560

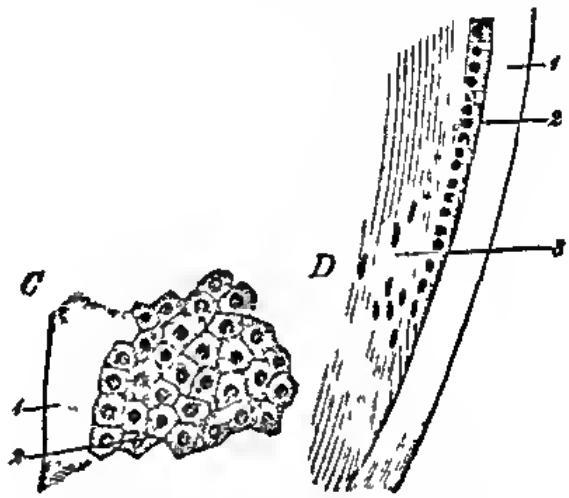


Fig. 569—Capsule and Epithelium of a Lens of Adult Man

C, Tangential section D, Meridional section across the equator of the lens, 1, capsule, 2, epithelium, 3, lens fibers × 240

cribrosa which is perforated by the optic nerve fibers. The chorioid and the pia are also in relation with this lamina (Fig. 567). As the optic nerve penetrates the lamina, its fibers lose their myelin and radiate into the nerve fiber layer of the retina. The central artery and vein of the retina enter the optic nerve in its distal half, and appear at the fundus of the eye in the center of the optic papilla. Their branches spread in the inner layers of the retina, covered by the membrana limitans interna.

Lens. The lens is a biconvex structure having an anterior and a posterior pole, and a vertical equatorial plane. It is enclosed in a thick transparent elastic *capsule*, 6.5–25 μ thick in front and 2–7 μ thick behind, which is in reality a specialized basement membrane, composed of several lamellæ.[1] Within the capsule the anterior surface of the lens is formed by the *lens epithelium*, a single layer of cells 2.5 μ thick at the pole, but becoming taller toward the equator. There they are continuous with

[1] Vogt, 1931.

the elongated *lens fibers* of the posterior layer, which collectively are called the *substantia lentis*.

Originally the fibers multiply throughout the lens, but in later stages the formation of new fibers, as indicated by the presence of mitotic figures, is limited to the region of transition between the lens epithelium and the mass of lens fibers (Figs. 556, E, and 569). When first formed the fibers are short, but they increase in length and become six-sided prisms, somewhat enlarged at one or both ends. The first fibers extend from one surface of the lens to the other. Later these become buried in by the new

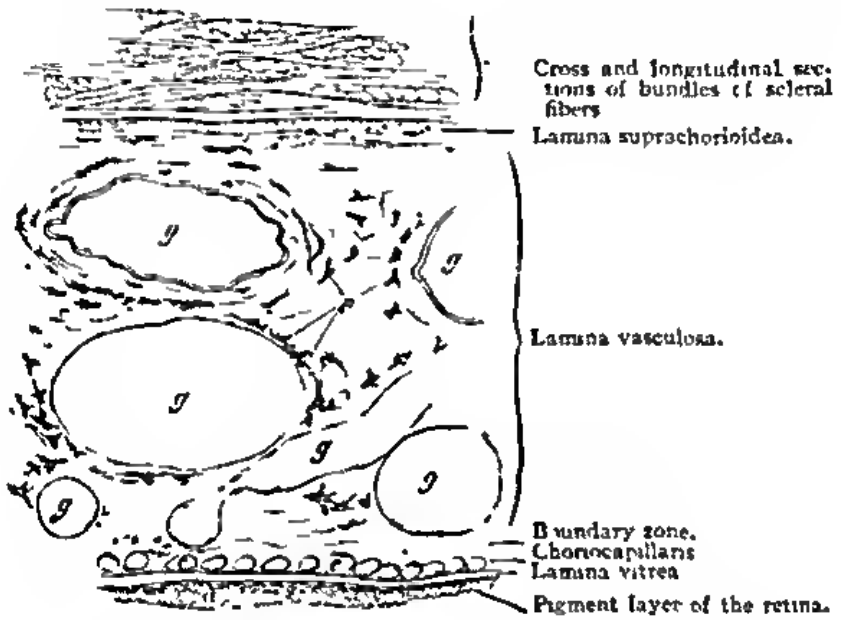


Fig. 570.—Vertical Section through a Part of the Human Sclera and the Entire Thickness of the Choroid. × 100

g, Large vessels, p, pigment cells, c, cross section of capillaries.

fibers formed at the periphery, and thus they constitute the *nucleus of the lens*. This is a dense mass of somewhat shrunken fibers, which have lost their nuclei and have acquired wavy or notched margins (Fig. 568). Their formation is somewhat analogous to the process of keratinization of the epidermis.[1] The outer fibers of the *cortical substance* are softer. They have smooth borders, and nuclei which are chiefly in the equatorial plane. Their cytoplasm is transformed into a clear fluid substance, said to be chiefly a globulin. The fibers are united to one another by a small amount of cement substance, which is more abundant at the poles, at each of which it forms a 'lens star,' usually with nine rays.

When the fibers formed at the periphery of the original nucleus elongate so as to cover it in, they do not extend from one pole to the other. Those that reach the anterior pole fall short of the posterior pole, terminating along a *horizontal suture* of cement

[1] Wolff, E., 1933.

substance; and conversely those that reach the posterior pole terminate anteriorly along a linear *vertical suture*. As the lens becomes larger, the linear sutures at either pole are replaced by tri-radiate or Y-shaped stars, one of which is inverted. Lens fibers starting near the center of one star end near the tips of the ray of the other, and vice versa. When the stars become nine-rayed the arrangement of the fibers is very intricate. Without crossing one another, and without any of them being long enough to pass from pole to pole, they cover the lens with even layers. The development of the stars is described by Rabl.[1] As a result of its structure the lens may be separated into concentric lamellæ, but Rabl considers that the meridional segments, or 'radial lamellæ,' of which the lens contains about two thousand, are its essential subdivisions.

Vitreous Body. The corpus vitreum consists of the fluid *vitreous humor*, together with looser or denser strands of fibrous *stroma* which stretch across it in all directions. Although it is difficult to recognize, certain pathological cases suggest a rather definite structural arrangement of the stroma. The cells of the vitreous body are round forms, probably leucocytes, and stellate or spindle-shaped connective tissue cells, sometimes degenerating and vacuolated, which invade the vitreous body with the blood vessels. The latter have atrophied and been resorbed, except for occasional shreds and filaments. Such opacities, which occur normally, are observed when looking at a bright light, and are frequently troublesome to those beginning to use the microscope; because of their erratic motion they are known to physiologists as *muscæ volitantes*. In old age, in eyes otherwise normal, crystals may form in the vitreous humor and float about, 'falling like a shower to the bottom of the eye when the eye is held still.' Surrounding the vitreous body there is a very resistant layer, which may be nothing more than a surface condensation.[2] It is continuous anteriorly with the hyaline membrane of the ciliary part of the retina.

The vitreous body and the cornea contain *mucoprotein*, and are the only two portions of the adult body to contain this substance. It is abundant in the embryo, and embryos are more or less transparent. The transparency of the vitreous and cornea is due to the maintenance of a fetal state, rather than to the development of transparency in opaque bodies.

Tunica Vasculosa. *Chorioid.* Between the sclera and the chorioid there is a loose tissue containing many elastic fibers and branched pigment cells, together with flat non-pigmented cells. In separating the sclera from the chorioid, this layer is divided into the *lamina fusca* of the sclera and the *lamina suprachorioidea*. Internal to the latter is the *lamina vasculosa*, which forms the greater part of the chorioid. It contains many large blood vessels embedded in a loose elastic connective tissue, some

[1] Rabl, 1900. [2] Duke-Elder, 1934.

of its cells being branched and pigmented; others without pigment are flat and arranged in layers surrounding the vessels. A thin inner layer of blood vessels, the *lamina choriocapillaris*, consists of a very close network of wide capillaries, wider and more numerous in the macular region and especially also in the ciliary bodies, where they assume the proportions of sinusoids, wide enough to allow the passage of many blood corpuscles abreast. The choriocapillaris is separated from the pigmented epithelium of the retina by a structureless elastic lamella, 2 μ thick. This lamina basalis (also known as lamina vitrea and Bruch's membrane) shows the imprint of the polygonal retinal cells on its inner surface, and is associated with fine elastic networks toward the choriocapillaris.

Between the vascular lamina and the choriocapillaris, there is a boundary layer consisting of a fine elastic network, generally without pigment. Here in ruminants and horses there are many wavy bundles of connective tissue, which give to the eyes of those animals a metallic luster Such a layer is known as the *tapetum fibrosum*. The similarly iridescent *tapetum cellulosum* of the carnivora is formed of several layers of flat cells which contain numerous fine crystals.

The *ciliary body* encircles the eye as a muscular band, attached to the inner surface of which there are from 70 to 80 meridional folds, the *ciliary processes* (Figs. 559 and 565). The equator of the eye is vertical, like that of the lens, and the meridians are antero-posterior. The processes begin low at the ora serrata and rise gradually to a height of 1 mm., terminating abruptly near the border of the lens. Each process consists of fibrillar connective tissue containing numerous elastic fibers and blood vessels, and is bounded toward the pars ciliaris retinæ by a continuation of the lamina basalis, which is thrown into intersecting folds. The ciliary processes, which are compressible, may serve to prevent the increase of intra-ocular pressure during the contraction of the ciliary muscle; and the fluid within the eye is derived from the vessels which they contain. The *ciliary muscle* is a band of smooth muscle fibers about 3 mm. broad and 0.8 mm. thick anteriorly; it arises beneath the sinus venosus of the sclera and tapers toward the ora serrata. It consists of three sets of fibers, the *meridional*, *radial*, and *circular*. The meridional fibers are next to the sclera, grouped in numerous bundles with elastic tissue intermingled. They extend to the smooth part of the chorioid, and constitute the *tensor choroideæ*. The radial fibers are directed toward the center of the eyeball. They form a middle layer of curving fibers which blend with the meridional fibers externally. The circular fibers, which vary in number in different individuals, form that part of the ciliary muscle which is nearest to the equator of the lens. The contraction of these muscles affects the shape of the lens, which is attached to the adjacent tissue by the zonula.

Iris. The iris consists of its *stroma* anteriorly, and the two layers of the pars iridica retinæ posteriorly. The stroma is partially covered by a layer of flat polygonal cells, interrupted at intervals by the *iris crypts* which extend into the underlying tissues and over which there is no mesenchymal epithelium (Fig. 571). Below this epithelium is a loose network of stellate cells, in part pigmented, resembling the reticulum of a lymph gland. This is followed by the loose connective tissue of the stroma, likewise containing networks of stellate cells, which in blue eyes are not pigmented. The very few elastic fibers are limited to the posterior layers, where they are radially arranged in relation to the pupil. The stroma contains numerous radial blood vessels with thick connective tissue coats, but (in man) without musculature or elastic fibers. In the vascular layer, toward the pupillary border of the iris, there is a band of circular smooth muscle fibers, 1 mm. deep; this is the *sphincter pupillæ.*

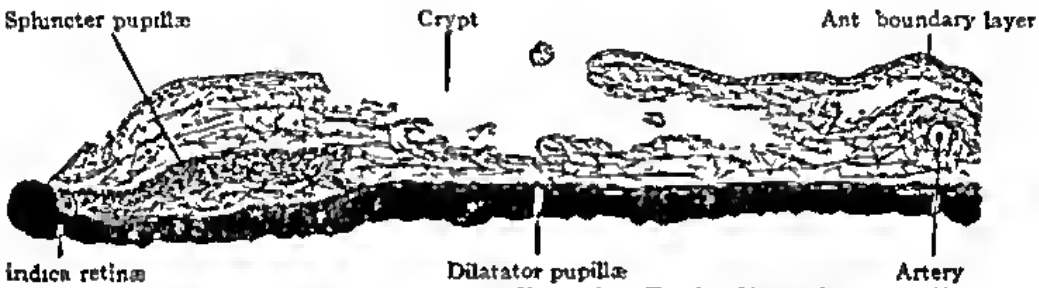


FIG. 571.—MERIDIONAL SECTION OF PUPILLARY PORTION OF HUMAN IRIS. THE IRIS VESSELS NORMALLY HAVE THICK HYALINE WALLS IN THE ADULT.

It is invested with many prolongations of the stromatic network, the polygonal meshes of which are radially elongated. The *dilatator pupillæ* is a peculiar membrane of smooth muscle fibers on the posterior surface of the vascular layer, stretching from the connective tissue between the muscle bundles of the sphincter to that between the ciliary muscles. Its fibers consist of an anterior contractile portion, and a posterior nucleated and pigmented portion. The anterior parts form a continuous layer, readily seen in radial sections as 'Henle's spindle cell layer,' which is a clear non-nucleated stripe, 2–5 μ wide. The nucleated portions of the fibers appear to blend with the pigmented retinal layer of the iris, from which they are derived. These muscles are therefore ectodermal.

The two layers of the optic cup are intimately blended in the thin stratum which forms the posterior layer of the iris. Except in albinos, this pars iridica retinæ is deeply pigmented. Posteriorly it is covered by a continuation of the hyaline membrane of the pars ciliaris.

Tunica Fibrosa. The sclera, toward the chorioid, is bounded by the pigmented *lamina fusca.* This is a loose tissue containing branched pigment cells and flattened connective tissue cells. Except for this boundary layer, the sclera consists of densely interwoven bundles of

connective tissue, chiefly meridional and longitudinal. Elastic fibers accompany the bundles, and are especially abundant at the insertions of the ocular muscles. The flat irregular cells of the connective tissue are surrounded by tissue spaces as in the cornea, and anteriorly the cornea and sclera are continuous with one another. The transition, however, is quite abrupt and the boundary is oblique, so that the rim of the cornea is bevelled at the expense of its anterior surface.

The *cornea* consists of an outer epithelium, external basal membrane, substantia propria, internal basal membrane, and mesenchymal epithelium bounding the anterior chamber. The corneal epithelium, about 0.03 mm. thick, is stratified and consists of a basal layer of clearly outlined columnar cells followed by three or four rows of cuboidal cells and

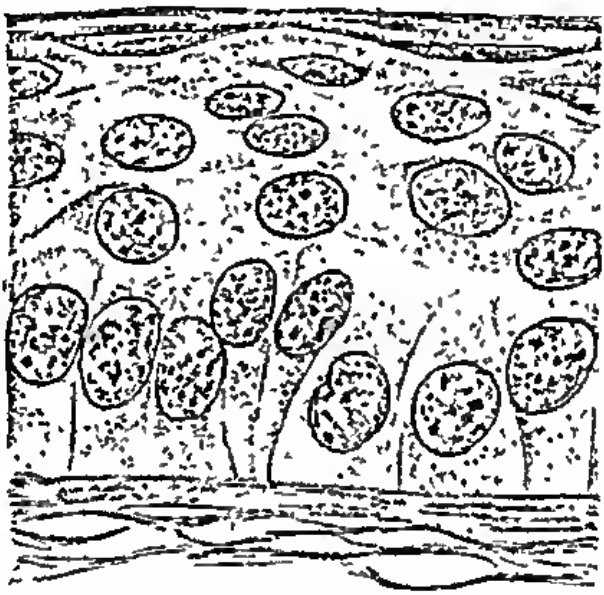


Fig. 572.—A Vertical Section of the Epithelium of the Human Cornea. Azan.

several layers of flattened superficial cells. The outer cells retain their nuclei. Peripherally the epithelium is continuous with that of the conjunctiva bulbi. The anterior basal membrane (Bowman's) is an almost homogeneous layer, sometimes as much as 0.01 mm. thick. It is pierced by minute 'pores' for the passage of nerves. Beneath, it blends with the substantia propria, of which it is a modification. Since it is not formed of elastic substance the name 'anterior elastic membrane' is not justified.

The substantia propria consists of fine straight fibrils of connective tissue, bound together in bundles of almost uniform thickness by an interfibrillar substance, perhaps fluid; these bundles are joined to one another by an interfascicular cement, so that they form a succession of superposed flat lamellæ, parallel with the corneal surface. Oblique bundles, the so-called arcuate fibers, are found especially in the anterior

layers, where they pass from one lamella to that next above or below. Numerous tense elastic fibers are found especially in the deeper layers, where they form a fine network over the posterior elastic membrane.

Within the cement substance, there is a system of branched canaliculi, dilated in places to form oval spaces. The latter are between the lamellæ, but the canaliculi extend also among the constituent fiber bundles. Within the spaces there are flat, stellate, anastomosing cells or 'corneal corpuscles,' the branches of which extend into the canals and tend to unite with those of neighboring cells at right angles. The cells and their

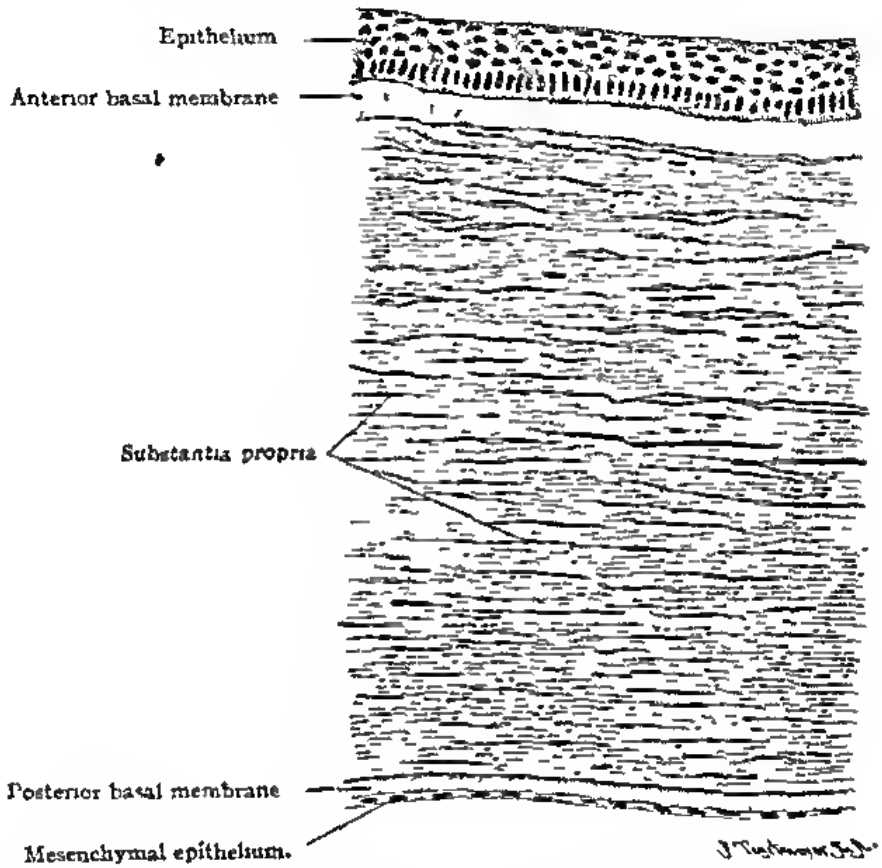


FIG. 573.—VERTICAL SECTION OF A HUMAN CORNEA. × 100

processes are more or less surrounded by serous fluid. Leucocytes enter the canals, and are normally found in the cornea; if the cornea is inflamed they become abundant. Blood vessels and lymphatic vessels are absent.

The posterior basal or elastic membrane (Descemet's membrane) is a structure clear as glass, 6 μ thick. Its posterior surface is covered by a simple layer of flat polygonal cells, which form a part of the lining of the anterior chamber. Toward the periphery of the cornea in adults, the posterior surface of the elastic membrane presents rounded elevations, and the posterior epithelium becomes continuous with the anterior epithelium of the iris (Fig. 559). In this 'angle,' the cornea receives

connective tissue prolongations from the iris, which form the *pectinate ligament of the iris*—a structure highly developed in the horse and cow, but rudimentary in man.

Vessels and Chambers. The *central vessels of the retina* supply a part of the optic nerve, and the retina; the *ciliary vessels* supply the rest of the eye. These two sets of vessels anastomose with one another only at the entrance of the optic nerve (Fig. 574).

The *ciliary arteries* include (1) the *short posterior ciliary arteries;* (2) the *long posterior ciliary arteries;* and (3) the *anterior ciliary arteries.* The three groups will be considered in turn.

1. After supplying the posterior half of the surface of the sclera, some twenty branches of the short posterior ciliary arteries penetrate the sclera around the optic nerve. They form the capillaries of the lamina choriocapillaris. Sometimes a branch of the ciliary vessels penetrates into the optic nerve and is distributed to a small area of the retina. When this distribution is to the macular region, the artery is called 'cilio-retinal.' It is found in about 17% of all cases and is of clinical importance. Anastomoses between the central artery system and the ciliary system are extremely rare. At the ora serrata they anastomose with recurrent branches of the long posterior ciliary and the anterior ciliary arteries.

2. The two long posterior ciliary arteries also penetrate the sclera near the optic nerve (Fig 574, 1). They pass, one on the nasal and the other on the temporal side of the eye, between the chorioid and sclera to the ciliary body. There each artery divides into two branches which follow the ciliary border of the iris, and connect with the corresponding branches from the artery of the opposite side, thus encircling the iris with an arterial ring. This is the *circulus iridis major* (Fig. 574, 2) from which numerous branches extend to the ciliary processes (3) and to the iris (4). Near the pupillary border of the iris, the vessels form an incomplete arteriovenous ring,[1] the circulus iridis minor.

3 The anterior ciliary arteries proceed from those supplying the recti muscles, penetrate the sclera near the cornea, and in part join the circulus iridis major, in part supply the ciliary muscle, and in part through recurrent branches, connect with the lamina choriocapillaris. Before penetrating the sclera, the anterior ciliary arteries give off posterior branches for the anterior half of the sclera, and anterior branches for the conjunctiva bulbi and the corneal border. The cornea itself is without vessels, but at its border, between the anterior lamellæ of the substantia propria, there are terminal loops.

The *veins* generally proceed toward the equator, uniting in four (less often in 5 or 6) *venæ vorticosæ.* These pass directly through the sclera and empty into one of the ophthalmic veins. Besides the venæ vorticosæ there are small veins accompanying the short posterior and the anterior ciliary arteries. The short ciliary veins receive branches from the ciliary muscle, the episcleral vessels, the conjunctiva bulbi and the periphery of the cornea. The episcleral veins also connect with the venæ vorticosæ. Within the sclera, near the cornea, there is a circular vein, receiving

[1] WOLFF, E., 1933.

small branches from the capillaries of the ciliary muscle. This *sinus venosus scleræ* (canal of Schlemm) connects with the anterior ciliary veins.

Arteria Centralis Retinæ. The central artery of the retina enters the optic nerve 15–20 mm. from the eye-ball, passes to its center and proceeds

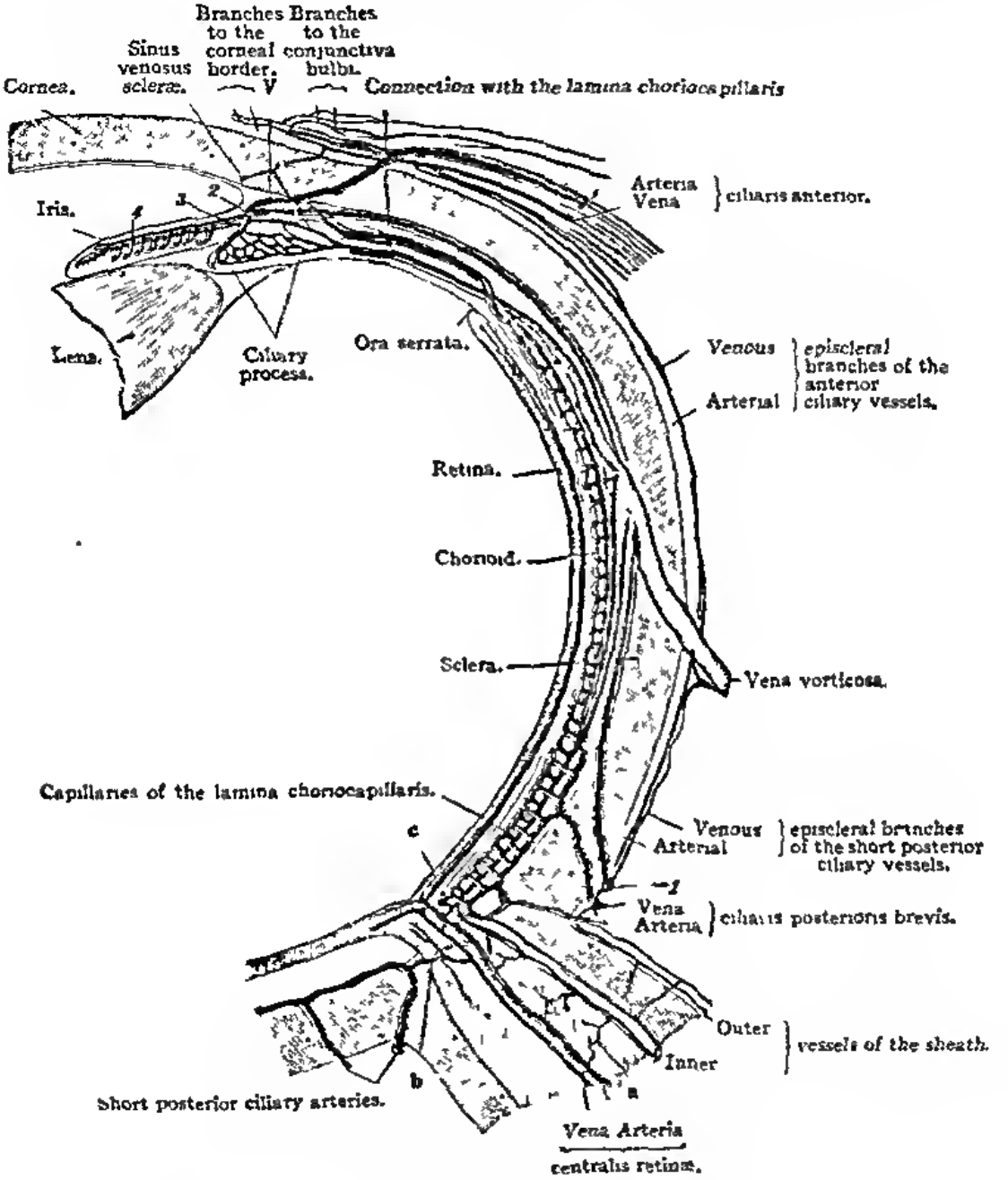


FIG. 574.—BLOOD VESSELS OF THE EYE (Leber)

The retina, optic nerve and tunica fibrosa are stippled, the tunica vasculosa is blank V, Connection of the anterior ciliary artery with the circulus iridis major (2)

to the optic papilla. There it divides into two branches directed upward and downward respectively, and these by further subdivision supply the entire pars optica retinæ. Within the optic nerve the artery sends out numerous little branches which anastomose with small vessels that have entered the sheaths from the surrounding fat; and also with branches of

the short posterior ciliary arteries (Fig. 574, *b*), but within the retina the branches of both artery and vein are end arteries, without anastomoses.

The central vein of the retina receives two main branches at the optic papilla and follows the artery along the axis of the optic nerve.

The eye contains no lymphatic vessels, but is provided with communicating tissue spaces, bounded by loose cells or mesenchymal epithelia. They include the corneal and scleral canaliculi, and the *anterior* and *posterior chambers;* the latter connect with one another through the capillary interval between the lens and iris. The posterior chamber extends into the *zonular spaces;* and there are irregular extensions of the anterior chamber, associated with the pectinate ligament of the iris, called in many animals the spaces of the angle of the iris (spaces of Fontana). The latter are not very common in man, and it is preferable to call this area the *filtration angle*. Posteriorly the tissue spaces include the hyaloid canal of the vitreous body. All these spaces contain a 'filtrate from the vessels,' and drain through the filtration angle. The very narrow *perichorioidеal space* between the chorioid and sclera; the subdural and arachnoid spaces of the optic sheaths, named the *intervaginal spaces;* and finally the *interfascial space* (of Tenon) which surrounds most of the sclera and the optic nerve contain cerebrospinal fluid. Acting as bursæ, they facilitate the movements of the eye.

Nerves. Apart from the optic nerve, the eye is supplied by the *short ciliary nerves* from the ciliary ganglion, and the *long ciliary nerves* from the nasociliary branch of the ophthalmic nerve. The ciliary nerves penetrate the sclera near the optic nerve and send branches containing ganglion cells to the vessels of the chorioid. The main stems pass forward between the chorioid and sclera to the ciliary body, where they form a circular ganglionated plexus, the *plexus gangliosus ciliaris*. Its branches extend to the ciliary body, the iris and the cornea, and are described as follows:

The nerves of the ciliary body form a delicate network on its scleral surface; they supply its muscle fibers and those of the vessels with slender motor endings; and between the ciliary muscle bundles they have branched free endings, perhaps sensory.

The medullated nerves of the iris lose their myelin and form plexuses as they pass toward the pupillary margin. A sensory plexus is found just beneath the anterior surface, and motor fibers supply the sphincter, dilator and vascular muscles. The existence of ganglion cells in the human iris is denied.

The nerves of the cornea enter it from the *plexus annularis* in the sclera just outside. The annular plexus also sends fibers into the conjunctiva, where they end in networks, and in bulbous corpuscles situated in the connective tissue close to the epithelium. Such corpuscles may be

found 1 or 2 mm. within the corneal margin. The corneal nerves become non-medullated and form plexuses between the lamellæ throughout the stroma. They extend into the epithelium and there form a very delicate plexus with free intercellular endings.

Eyelids. The eyelids or *palpebræ*[1] (Latin, *palpare*, to stroke) originate from two folds of integument which advance from above and below in front of the eye. In the human embryo, the folds appear in the seventh week (about 20 mm.) and their edges are fused about two weeks (33 mm.) later;[2] the lids commence to open in the fifth month by a cornification and splitting of epithelium beginning at the medial angle. The lids, however, are not open until the seventh or eighth month and in very rare cases may still be closed at birth. The transverse opening, the *rima palpebrarum*, between the upper and lower lid varies in length, the average in the adult being about 28 mm., the extent of which gives the appearance of a large or small eye

The skin is very thin and is provided with numerous fine lanugo hairs, small sebaceous and sweat glands; the dermal papillæ are usually few and underdeveloped except near the free borders of the lids where they are higher. Branched connective tissue cells containing a yellow to brown pigment and mast cells are present in the corium. Pigment occurs also, in the basal layers of the epidermis, especially near the medial angle of the eye. The subcutaneous tissue is very loose, rich in elastic fibers and contains little or no fat in Caucasians; but a pad of fat has been described in the center of each lid in Mongolians.

Near the anterior edge of each lid are several rows, 2 or 3, or in individuals up to 5 on the upper lid, of short, thick, curved, tapering hairs, the eyelashes or *cilia*, the oblique roots of which extend deep into the corium. Since they are shed in from 100 to 150 days they occur in various stages of development. On the upper lid, the average number of cilia is 140–150 up to 200 and on the lower 50–75 up to 100. Usually cilia are longer in children and in adult women than in men. Rich nerve plexuses surround the hair follicles. Each cilium is provided with a large sebaceous gland, the gland of Zeis, which rests upon a well-developed basement membrane. Between the cilia or opening into the ducts of the glands of Zeis are modified sweat glands, the *ciliary glands* (of Moll), with simpler coils than the ordinary sweat glands, which may show successive constrictions; 'a branching of the tubules has been observed.'

[1] Cicero, *De Natura Deorum*, II, 57 'Palpebræ sunt tegumenta oculorum . . . munitesque sunt palpebræ tamquam vallo pilorum.'

[2] Covtino, 1907.
Ask, 1908.
Keibel, 1912

The secreting epithelium is simple, but may become two or three layered in an excretory duct. The mode of elimination of secretion is apocrine, the heights of the cells ranging from columnar to cuboidal or

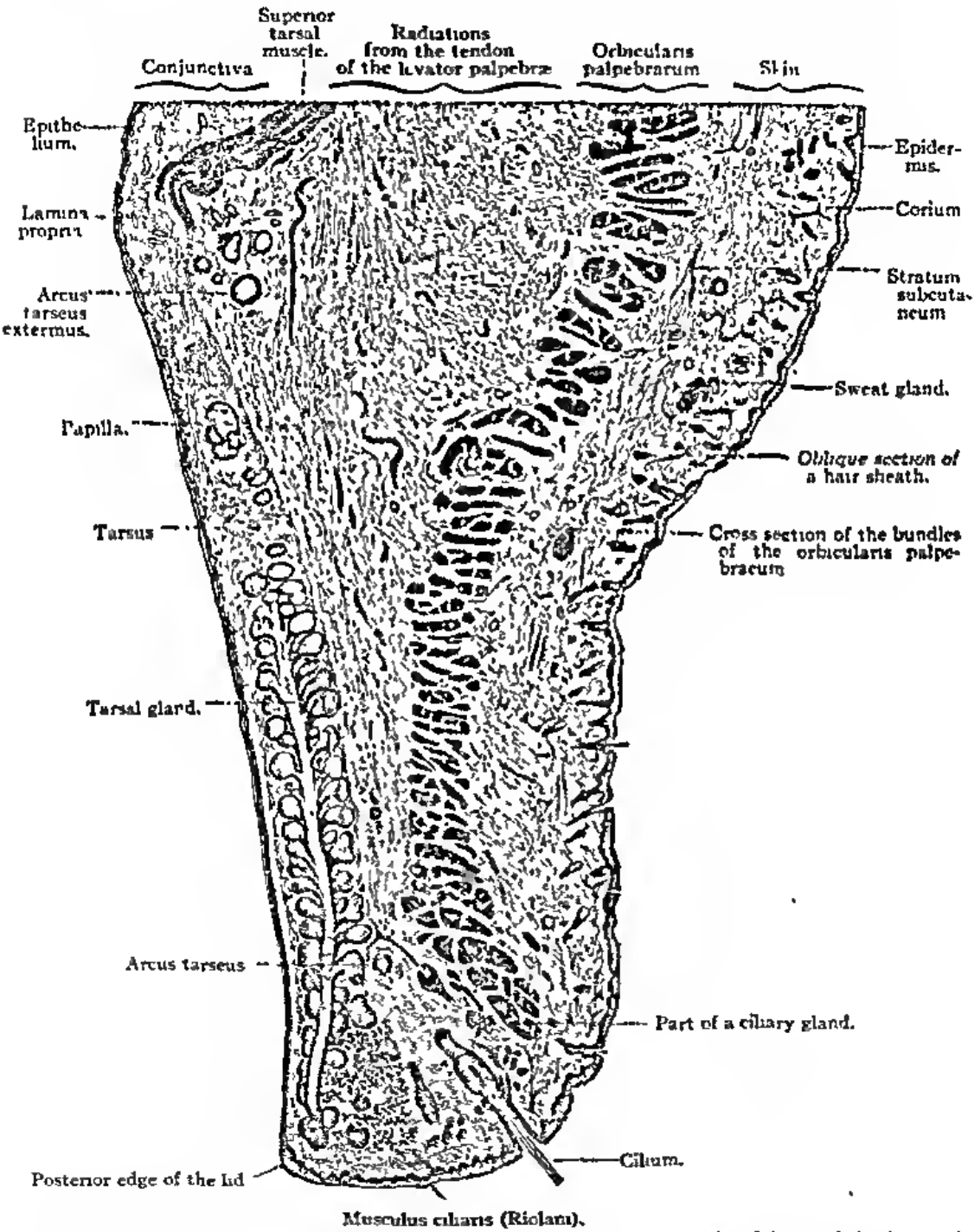


FIG. 575.—SAGITTAL SECTION OF THE UPPER LID OF A CHILD OF SIX MONTHS. The outlet of the tarsal gland was not in the plane of section. × 15

even flat depending upon differences in functional states. Basal to the secreting cells are myoepithelial cells, a well-developed basement membrane and a meshwork of fine elastic fibers. When a duct of either a

gland of Zeis, or a ciliary gland becomes blocked an inflammatory condition may arise known as 'stye.'

The central portion of the eyelids is muscular, formed by bundles of striated muscle, the *pars palpebralis, musculi orbicularis oculi,* extending lengthwise of the lid. The thickness of the muscle is subject to individual and racial differences. A subdivision of this muscle, behind the roots of the cilia, is called the *musculus ciliaris Riolani.* Posterior to the orbicularis oculi muscle are found the terminal radiations of the tendon of the *musculus levator palpebræ.* A part of these are lost in connective tissue; another part, associated with smooth muscle fibers (which are governed by sympathetic nerves) are inserted into the upper border of the *tarsus* and form the *superior tarsal muscle.*[1] This occurs in the upper lid, but correspondingly in the lower lid the radiations from the *inferior rectus muscle* contain smooth muscle fibers, making up the *inferior tarsal muscle.*

The framework for each eyelid is formed by a dense fibrous connective tissue plate called the *tarsus,* which gives form and stiffness to the lid. It is separated from the orbicularis oculi muscle by the *palpebral fascia,* a lamina of varying thickness composed of loose collagenous and elastic fibers. This fascia is affixed to the tarsal plates and continues from them to be attached along the whole margin of the orbit. The term *septum orbitale* has been applied to the fascia which forms a partition between the cutaneous and conjunctival parts of the lids.

Embedded in the substance of each tarsus, there are a series of tubuloacinous glands, the *tarsal glands* (of Meibom), 30 to 40 on the upper and 20 to 30 on the lower lid. They are elongated yellowish structures seen through the conjunctiva on turning back the lids. Each gland consists of a wide, more or less straight excretory duct, surrounded on all sides by small acini, which empty into the duct through short constricted stalks. The acini resemble sebaceous glands; the duct is lined with a simple low columnar epithelium, which becomes stratified near its opening on the posterior margin of the lid. A basement membrane, with a layer of smooth muscle outside and a network of fine nerve fibers surrounds the glands. The fatty secretion, the *sebum palpebrale* lubricates the edges of the lids. Near the upper end of the tarsus and partly enclosed in its substance, there are branched tubular *accessory lacrimal glands.* There are usually 3 to 6 glands, although as many as 30 or more have been found, occurring chiefly in the medial (nasal) half of the lid.

Behind the tarsus lies the *tunica conjunctiva palpebrarum,* the innermost layer of the eyelid, which passes above and below at the *fornix conjunctivæ* on to the eyeball as the *tunica conjunctiva bulbi.* It may be noted

[1] Müller, H, 1858.

that the entire anterior covering of the bulb of the eye is named by some the *conjunctiva bulbi*, which accordingly is divided into the *conjunctiva scleræ* and the *conjunctiva corneæ*. The conjunctivæ consist of a lamina propria and a surface epithelium: the margin between them in the conjunctiva palpebrarum is comparatively smooth, while in the conjunctiva bulbi well marked papillæ are observed near the cornea. The lamina propria is a loose connective tissue sheet comprised of

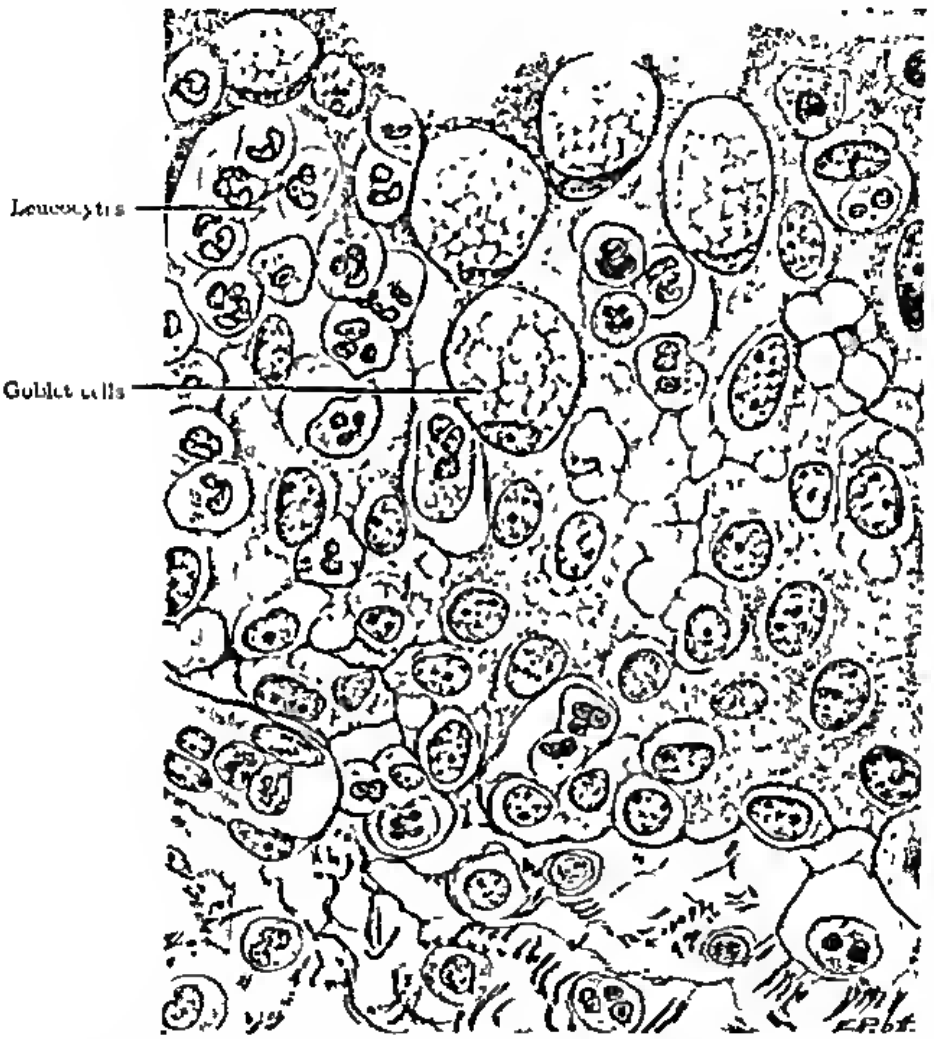


FIG. 576.—A SECTION THROUGH THE CONJUNCTIVA BULBI OF THE HUMAN EYE. NOTE THE GOBLET CELLS AND THE INFILTRATION OF LEUCOCYTES INTO THE EPITHELIUM. AZAN. (Drawn from a preparation by Prof. M. Heidenhain.)

collagenous and elastic fibers and some fat cells. It is infiltrated with plasma and lymphatic cells; the latter invade the epithelium, beneath which they may form nodules. Lymphatic nodules are said to occur regularly in ruminants; in man the larger number of nodules are seen normally in the lamina propria of the conjunctiva bulbi where there have been found as many as twenty. Excessive crying and other irritations lead to hyperæmia—the redness being most marked in the fold between the palpebral and bulbar conjunctivæ and less toward the

cornea. A migration of blood cells from the dilated vessels into the lamina propria and the epithelium, may under certain conditions be sufficient to cause thickening of the conjunctivæ.

The stratified squamous epithelium of the skin becomes stratified columnar on the conjunctivæ, with several basal layers of cuboidal and a superficial layer of short columnar cells covered by a thin hyalin cuticula. The transition from squamous to columnar cells may occur at the posterior edge of a lid or higher up on its conjunctival surface. Toward the arch, where the palpebral conjunctiva becomes continuous at the fornix with that of the bulb, the epithelium is so folded as to form pit-like indentations, the 'conjunctival crypts'[1] or the so-called 'conjunctival glands of Henle.' Sections through the walls of the crypts may show elongated epithelial islands in the lamina propria—the 'papillary bodies.' Near the limbus, the outer low columnar cells on the bulb become flatter like those on the cornea. Goblet cells are present in the conjunctival epithelium, perhaps more numerous at the fornix and on the adjacent bulb than on the palpebræ. In all races other than the white and in many mammals, the basal epithelial cells on the bulb contain pigment granules. Pigmentation is most at the corneal border, the *limbus conjunctivæ*. The yellow appearance of the exposed portion, often most pronounced near the medial border of the cornea, and known as *pinguecula* (*i.e.* somewhat fat, fattish) is not due to fat or to an epithelial pigment, but to degeneration of elastic tissue; it accompanies a thickening of the connective tissue layer.

At the medial angle of the lids there is a thin fold of connective tissue covered with stratified epithelium; this *plica semilunaris* is a rudimentary third lid. It seldom reaches any degree of development in Caucasians, but may be present to varying extent in other races. The plica semilunaris is the homologue of the nictitating membrane, which is well-developed in many vertebrates above the fishes.[2] The epithelium has been described as stratified squamous in the adult and stratified columnar in the child. Goblet cells, sparse in man, may be embedded in it.[3] Between the plica semilunaris and the lids is a small, soft, nodular body, the *caruncula lacrimalis* (Lat. diminutive, *caro*, flesh), which originates from the lower lid. It resembles skin except that a stratum corneum is lacking; and contains hairs, sebaceous and accessory lacrimal glands and in its middle part, small sweat glands. Some have described goblet cells and intra-epithelial mucous glands in it.[4]

Blood is supplied to the eyelids principally by branches from the ophthalmic artery. These form arches near the free margins of the lids,

[1] Dubreuil, 1908. [2] Stibbe, 1928. [3] Koch, 1904. [4] Stieda, 1890.

the *arcus tarseus superior* and the *arcus tarseus inferior*. A second, smaller arch is found above the tarsus in the upper lid and a less defined one below the tarsus in the lower lid. From these arches perforating twigs (*aa. conjunctivales posteriores*) pierce the tarsi and form a close capillary network in the palpebral conjunctiva, which continues into that of the bulb where it joins capillary loops near the margin of the cornea and passing inward unites with the anterior ciliary arteries. Other twigs run forward from the arches, supplying the *orbicularis oculi* muscle and the skin. Veins form a close retrotarsal network draining to the ophthalmic vein and a loose pretarsal network anastomosing with veins in the skin, which drain medially into the angular vein and laterally into the superficial temporal vein. The veins in the lids are more numerous and larger than the arteries and possess well-developed valves. The lymphatic vessels form connecting networks in front and behind the tarsus, which join a third network in the skin. They drain mostly to the anterior auricular and parotid lymph glands and some from the medial halves of the lids to the submaxillary lymph gland. Whether the lymphatic vessels of the conjunctiva bulbi end blindly toward the cornea, or connect with tissue spaces, has not been determined. Sensory nerves, to the upper lid are from the ophthalmic and to the lower from the maxillary division of the trigeminal nerve. These form rich plexuses in the conjunctivæ and in the skin, with free endings in the epithelium and modified Meissner's corpuscles and 'terminal bulbs' in the connective tissue beneath.[1] Motor nerves are branches from the oculomotor nerve to the levator palpebræ muscle and from the facial nerve to the orbicularis oculi muscle. Sympathetic nerves innervate smooth muscle and glands.

Lacrimal Apparatus. The lacrimal apparatus consists of the lacrimal or tear gland with its excretory ductules and the lacrimal passages—the lacrimal ducts, the lacrimal sac and the nasolacrimal duct conveying tears into the nasal cavity.

The gland is a yellowish-pink mass, consisting of two parts: above the larger *glandula lacrimalis superior* (*pars orbitalis*) lies deep behind the lateral margin of the upper eyelid in relation to the orbital septum and below the smaller *glandula lacrimalis inferior* (*pars palpebralis*) is found above the superior conjunctival fornix, extending down into the back of the lid. At birth the gland is small and during childhood it is only one-fourth to one-third the size seen in the adult and it becomes smaller again in old age. Usually the lacrimal gland is larger in men than in women. Each gland has several excretory ductules, seldom more than

[1] Dogiel, *1895*.

Zietzschmann, Arch, f Ophthal. Bd. 58, 1904—contains many details on the comparative histology of the eyelids.

twelve, of which two to five drain the orbital part and the others the palpebral part. The ductules open separately on the conjunctival surface along a line just in front of the superior fornix.

Near the medial angle of the eye on the free margin of each lid is a slight elevation, the *papilla lacrimalis*, at the summit of which is a small slit-like opening, the *punctum lacrimale*. From each punctum a small *ductus lacrimales* conveys the tears which have bathed the front of the eye, through a separate or a conjoint opening into the lacrimal sac (*saccus lacrimalis*), lodged in a fossa in the anterior medial wall of the bony orbit. A larger *ductus nasolacrimalis* leads from the sac, first through a canal encased by bone and then through loose tissue, into the inferior meatus of the nasal cavity. The upper opening of the duct from the sac is oval, while the lower is funnel-shape or in the form of a slit-like groove, with an imperfect value (*plica lacrimalis*). A double lower opening is rarely found in man, although it occurs constantly in some animals (*e.g.* dog).

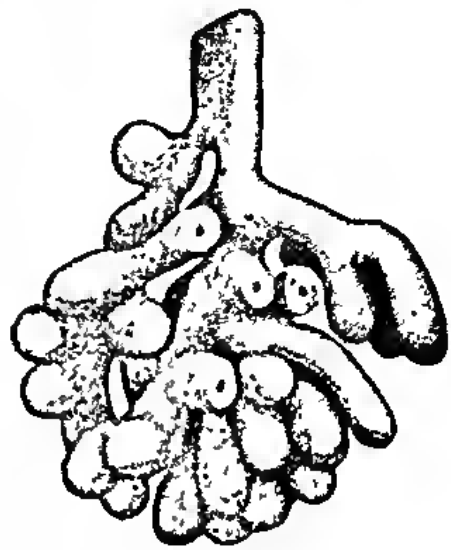


FIG. 577.—RECONSTRUCTION OF A PORTION OF A LACRIMAL GLAND FROM A HUMAN FEMALE (Maziarski.)

The lacrimal gland appears in the human embryo in the ninth week (22–26 mm.) as five or six successive knob-like ingrowths from the conjunctival epithelium. These are at first solid bodies, which soon elongate, branch and acquire lumens. Division of the gland into orbital and palpebral parts occurs about the 38 mm. stage.[1] The lacrimal passages—ducts, sac and nasolacrimal duct arise in the sixth week (9.5–12 mm.) as a ridge-like thickening of ectodermal epithelium along the line of fusion between the lateral nasal and maxillary processes of the embryonic face. The ridge is never free on the surface since it forms beneath after fusion of the processes from the deep layers of cells at the junction. As early as the 15 mm. stage the ridge becomes separated and sinks into the subjacent mesenchyma. Primordia for the lacrimal ducts are formed by budding from the upper end of the separated ridge and reach the lid margins about the same time that the lower end reaches the nasal cavity (18–24 mm.). The superior duct arises from a side bud, the inferior being from a continuation of the main stem. During its growth the inferior duct cuts off a part of the lower lid margin which

[1] CIRINCIONE, 1908.
ASK, 1910.
KEIBEL, 1912.

forms the *caruncula lacrimalis*. Canalization begins in the inferior duct late in the second month (about 35 mm.) and then in order in the superior duct, the sac and the nasolacrimal duct, the process being completed except at the terminations in the third month. The puncta open in the seventh month and the orifice into the inferior nasal meatus in the eighth month, or even after birth. Double puncta and ducts from additional buds, are very rare anomalies.

The lacrimal, is a compound tubulo-alveolar gland, with short tubules and enlarged end-pieces resembling the serous salivary glands.[1] It is surrounded by a loose connective tissue capsule from which septa pass between the alveoli and elastic tissue fibers and smooth muscle cells are found in both capsule and septa. Lymphatic tissue is present to a varying extent in the septa, it is said to be absent at birth, sparse at puberty and abundant in the adult. The secreting epithelium is simple columnar, the heights of the cells corresponding to different functional states. During rest the cytoplasm becomes filled with large, clear spherical bodies resembling vacuoles but after stimulation these are discharged leaving the cells shrunken, dark and finely granular. Mitochondria are abundant. Central bodies lie within a clear area close to the nucleus—on the side toward the free border of the cell.[2] A Golgi apparatus may be demonstrated as a network in the region of the central bodies. Fatty globules are present in the secretory cells but they are probably no more numerous than in the salivary glands or in the pancreas. In the lacrimal gland this is borne out by the composition of tears —they contain approximately 99 per cent. water the rest albumin and sodium chloride with traces of other salts, mucin and fat. Secretory canaliculi, the extent of which varies in different functional states, reach out from the lumen between the epithelial cells. The excretory ductules of the gland have wide lumens; the epithelium is composed of two or three layers, the outer cells being cuboidal or rounded, while the inner ones abutting upon the lumen are columnar. Goblet cells have been observed among them. The alveoli rest upon a reticular tissue basement membrane, between it and the glandular cells are seen stellate flattened elements called *basket cells*.[3] Some histologists interpret the basket cells as 'myo-epithelial,' while others regard them as a species of connective tissue cells.[4]

The chief blood supply to the lacrimal gland is through the lacrimal branch of the ophthalmic artery. Venules unite within the interalveolar septa to form a lacrimal vein which drains into the ophthalmic vein. The lymphatics of the lacrimal gland have not been studied completely; they are probably limited to the surface of the gland and pass from it

[1] Maziarski, 1901. [2] Dubreuil, 1908. [3] Boll, 1868. [4] Sundwall, 1916.

into the lymphatic capillaries of the conjunctivæ. The gland is innervated by the lacrimal, by the facial and by sympathetic nerves. The lacrimal, a branch of the ophthalmic division of the trigeminal nerve anastomoses with the zygomatic branch of the maxillary division and enters the gland with the blood vessels. Nerve fibers arising from cells in the geniculate ganglion of the facial nerve, after a complicated and

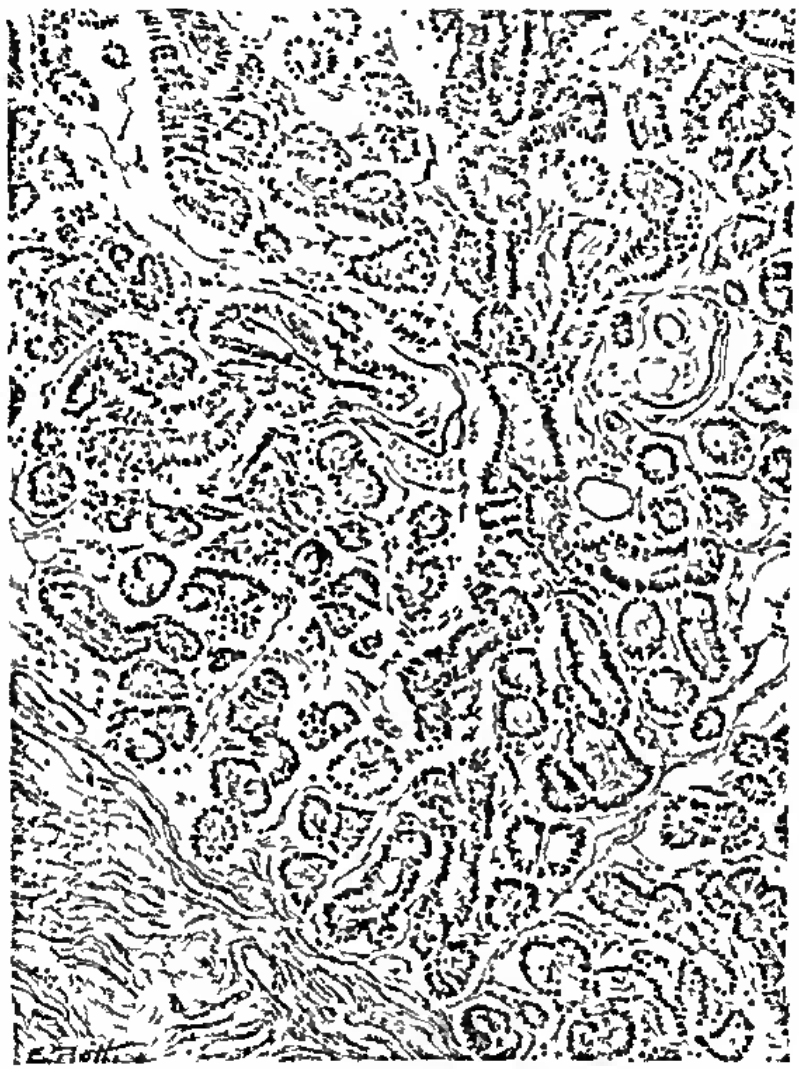


FIG. 578.—SECTION OF THE LACRIMAL GLAND FROM A YOUNG CHINESE
Alcohol fixation, hæmatoxylin and eosin

circuitous course pass to the gland by the way of the zygomatic nerve and the anastomosis with the lacrimal nerve. Sympathetic nerves derived from the carotid plexus reach the gland in company with the lacrimal artery and in the zygomatic nerve. The fibers end in the gland substance as fine non-medullated ramifications between the alveolar cells and around the excretory ductules and the blood vessels.[1]

The lacrimal ducts leading from the puncta to the lacrimal sac are lined with a stratified squamous epithelium six to twelve cells thick,

[1] DOGIEL, 1893.

which rests upon a lamina propria containing an abundance of cells and elastic fibers. Externally the ducts are surrounded by striated muscle fibers, chiefly longitudinal. In the lacrimal sac the epithelium becomes a two-layer columnar with some ciliated and goblet cells. The constancy of cilia has been questioned. Small branched serous glands occur occasionally in the lacrimal sac and extend into the lamina propria which is a loose layer rich in lymphocytes and lymphatic nodules. The epithelium of the nasolacrimal duct is similar to that lining the lacrimal sac, except there are no ciliated cells and glands are confined to the lower part of the duct. The surface is frequently folded and forms diverticuli or pits. In the lamina propria of the duct the lymphocytes unlike in the sac seldom form nodules. Beneath the lamina propria is a rich vascular submucosa, with a fibro-elastic network between the vessels; the latter are well-developed toward the nasal meatus and resemble cavernous tissue. The elastic tissue of the network decreases as the venous plexus increases. Fat cells occur singly or in groups in the submucosa.

The blood supply to the lacrimal sac and the nasolacrimal duct is derived from branches of the superior and inferior palpebral arteries and the infraorbital artery. The lower part of the duct receives a supply from the nasal branch of the sphenopalatine artery as well. The venous plexus around the lacrimal sac drains partly into the angular vein and partly into the plexus around the nasolacrimal duct. The lymphatic vessels from the lacrimal sac accompany the facial vein and pass to the submaxillary lymph glands. From the lower part of the nasolacrimal duct, the lymphatic capillaries join those in the walls of the inferior nasal meatus and drain anteriorly to the skin and the submaxillary lymph glands and posteriorly to the retropharyngeal and deep cervical lymph glands Sensory nerves of the caruncula lacrimalis and of the adjacent conjunctiva, the lacrimal ducts and sac and the upper part of the nasolacrimal duct are derived from the ophthalmic nerve through the inferior branch of the infratrochlear nerve. The lower part of the nasolacrimal duct receives a twig from the anterior superior alveolar branch of the maxillary nerve. For more details on the accessory structures of the eye, see S. E. Whitnall, 1921. The anatomy of the human orbit and accessory organs of vision, Oxford Medical Publications. Hans Lauber, 1936. Handbuch der mikroskopischen Anatomie des Menschen (von Möllendorff), Band 3, Teil 2.

EAR

Development and General Anatomy. The ear is divided into three parts: (1) the *external ear*, which includes the *auricles* projecting from the surface of the body, and the *external acoustic meatus* leading from the sur-

face to the *tympanic membrane;* (2) the *middle ear,* including the tympanic cavity or 'drum' and the chain of three bones extending across it; and (3) the *internal ear,* which is a system of epithelial ducts and surrounding tissue spaces, embedded in the temporal bone, and connected with terminal branches of the acoustic nerve.

On each side of the body, the internal ear first appears as a local thickening of the epidermal ectoderm near that portion of the medullary tube which later becomes the pons. The thickened areas are invaginated as shown in Fig. 579, A and B, and the pockets thus produced become

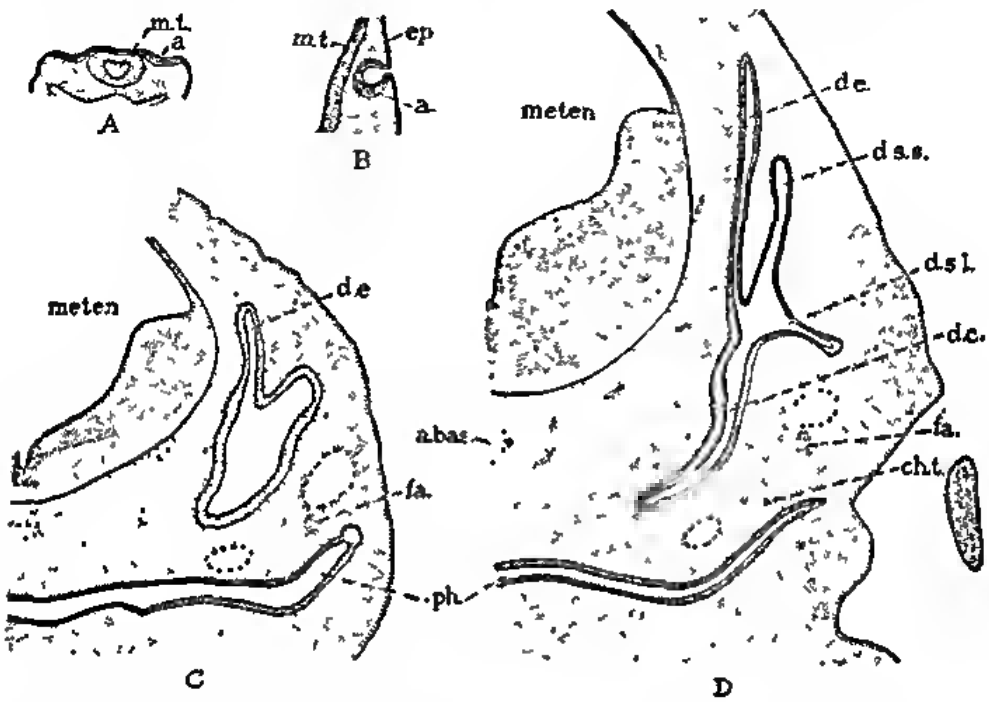


FIG. 579.—SECTIONS OF RABBIT EMBRYOS TO SHOW THE DEVELOPMENT OF THE EAR × 9

A, 9 days, 3 8 mm , B, 10 days, 3 4 mm , C, 12½ days, 7 5 mm , D, 14 days, 10 mm a , Ectodermal epithelium which forms the membranous internal ear, a. bas , basilar artery, ch. t , chorda tympani, d. c., cochlear duct; d. e., endolymphatic duct, d s. l , lateral semicircular duct, d. s. s , superior semicircular duct; ep , epidermis, fa , facial nerve, meten , metencephalon, m. t , medullary tube, ph , pharynx

separated from the epidermis in the form of *auditory vesicles* (otocysts). The place where they become detached from the epidermis is marked by a slight elevation on the medial surface of the vesicle, which soon elongates, producing the tubular *endolymphatic duct* (Fig. 579, C). The blind upper end of the duct becomes enlarged to form the *endolymphatic sac,* which, however, is only slightly developed in man; it appears in the models of the embryonic vesicle shown in side view in Fig. 580, A–C. In the adult the endolymphatic duct is a very slender tube, terminating blindly (or perhaps with secondary apertures) just beneath the dura.

In two places the medial and the lateral walls of the upper half of the vesicle approach one another, and, after fusing, the epithelial plates thus produced become thin and rupture, so that two *semicircular ducts* are formed. The space encircled by each duct may be regarded as a

hole through the vesicle. Two ducts are the *superior* and *posterior semicircular ducts* respectively. The third or *lateral semicircular duct* forms soon afterward. In Figs. 579, D and 580, B it is a horizontal shelf-like projection of the vesicle, the center of which is to become perforated so that its rim will become the duct. The portion of the vesicle which receives the terminal openings of the three semicircular ducts is called the *utriculus*. Since at one of their ends the superior and posterior ducts unite in a single stalk before entering the utriculus, there are but five openings for the three ducts. Near one end of each duct there is a dilatation or *ampulla*, where nerves terminate.

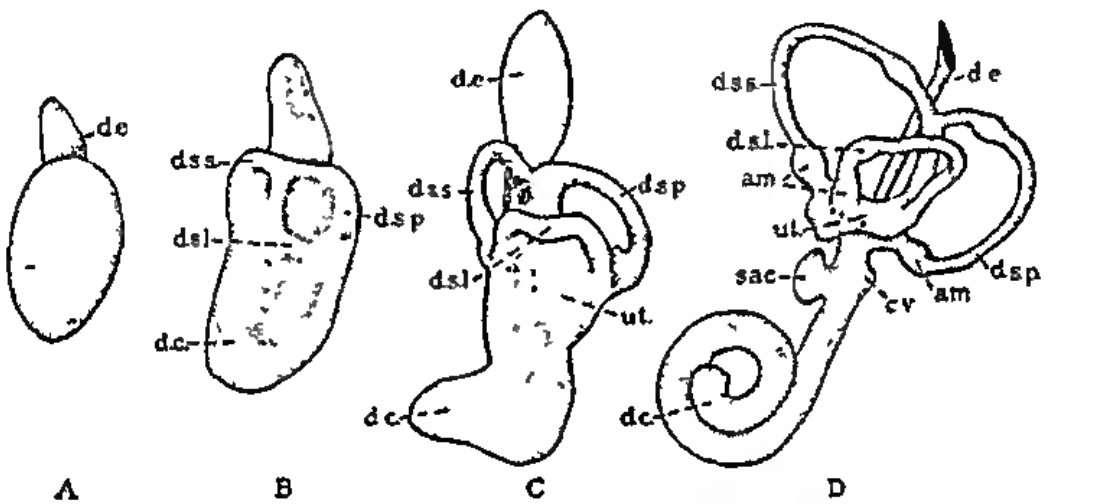


FIG. 580.—LATERAL OR EXTERNAL SURFACES OF MODELS OF THE MEMBRANOUS PORTION OF THE LEFT INTERNAL EAR FROM HUMAN EMBRYOS DIFFERENT ENLARGEMENTS (His, Jr.)

A, from an embryo of 6 9 mm., B, 10 2 mm., C, 13 5 mm., and D, 22 mm. am., ampulla; c. v., cæcum vestibulare of d. c., cochlear duct, d. e., endolymphatic duct, d. s. l., d. s. p., and d. s. s., lateral, posterior, and superior semicircular ducts, sac., sacculus, ut., utriculus

While the formation of the semicircular ducts is occurring in the upper part of the auditory vesicle, the lower portion elongates and its end becomes coiled, eventually making two and a half revolutions. The coiled tube is the *ductus cochlearis;* its distal end is the *cæcum cupulare*, and at its proximal end is the *cæcum vestibulare* (Fig. 580, *D*, *c. v.*). A dilated sac formed at its proximal or upper end, opposite the cæcum vestibulare, is known as the *sacculus;* in the adult the connection between the sacculus and the ductus cochlearis is relatively narrow, and is called the *ductus reuniens* (Fig. 587, 1). The portion of the original vesicle between the sacculus and utriculus, from which the endolymphatic duct arises, becomes a comparatively slender tube, the *ductus utriculo-sacularis* (Fig. 587, 2).

The ectodermal vesicle thus produces a complex system of connected epithelial ducts, namely the superior, posterior, and lateral semicircular ducts; the utriculus, and utriculo-saccular duct with the endolymphatic duct connected with it; the sacculus, ductus reuniens and ductus coch-

learis. They all contain a fluid called *endolymph*. The acoustic nerve sends branches between the epithelial cells in certain parts of the ducts. Round areas of neuro-epithelium, in which the nerves terminate, are called *maculæ acusticæ;* there is one in the sacculus and another in the utriculus. Elongated areas are *cristæ*, and there is one in each of the three ampullæ. The axis, or *modiolus*, about which the cochlear duct is wound, contains the nerves which send terminal fibers to the *spiral organ* of the adjoining epithelium. In this they form a line of terminations along the medial wall of the cochlear duct, following its windings from base to cupola.

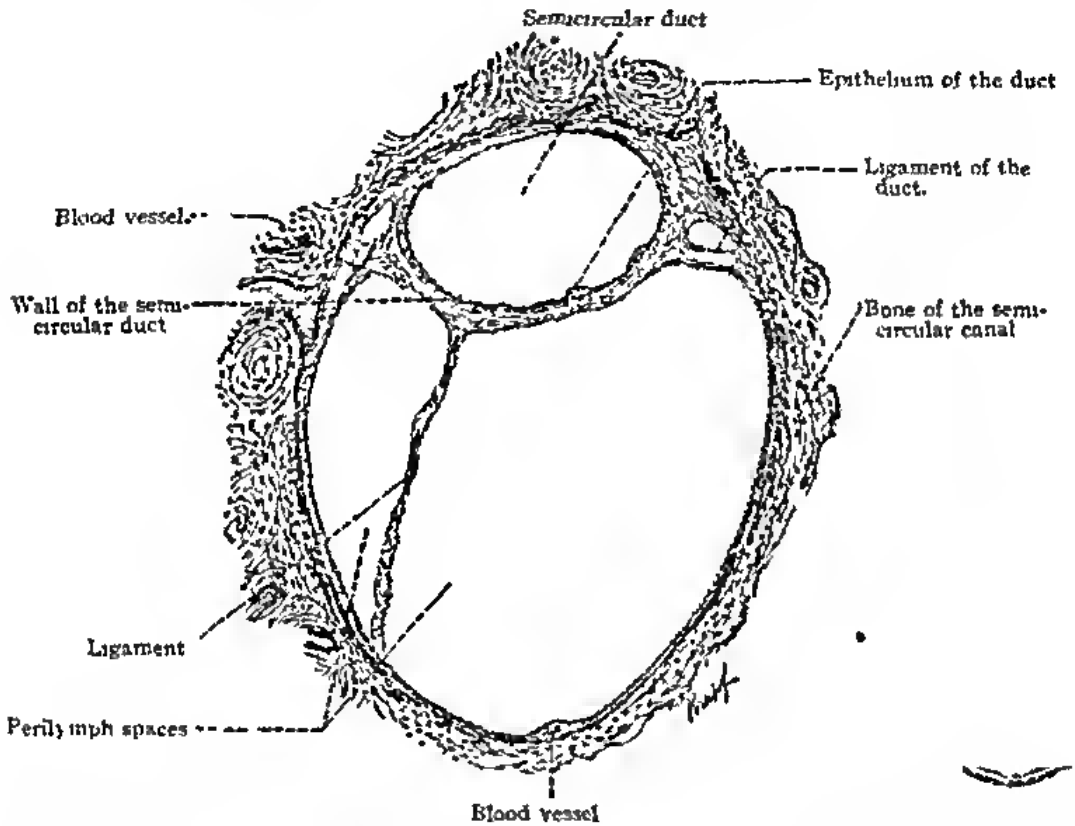


Fig. 581.—Cross Section of a Semicircular Duct and the Adjacent Perilymph Spaces together with the Semicircular Canal of Bone in which they are Lodged. From a Human Adult. × 50. (Böhm and von Davidoff.)

The mass of mesenchyma enclosing the entire system of ectodermal ducts becomes for the most part first cartilage and then bone. In the latter there are no marrow spaces; the bone is thus very dense and, since there has been little absorption by osteoclasts, the calcified cartilage matrix of the endochondral bone remains throughout life (see p. 134). Immediately surrounding the individual ducts, however, the mesenchyma becomes mucoid in appearance, and cavities lined with mesenchymal epithelium are found in it. In this way every duct is lodged in a larger *canal* in the bone, to one side of which it usually adheres. The mesenchymal cavities contain a tissue fluid called *perilymph*. Around the semicircular ducts the perilymph spaces are so large that the tissue

between them is reduced to strands as shown in Fig. 581; these are sometimes called ligaments. The spaces are irregularly arranged and communicate with one another at various points; they connect also with the perilymph cavities of the *vestibule*, which is the central part of the internal ear, from which the semicircular, cochlear and endolymphatic ducts

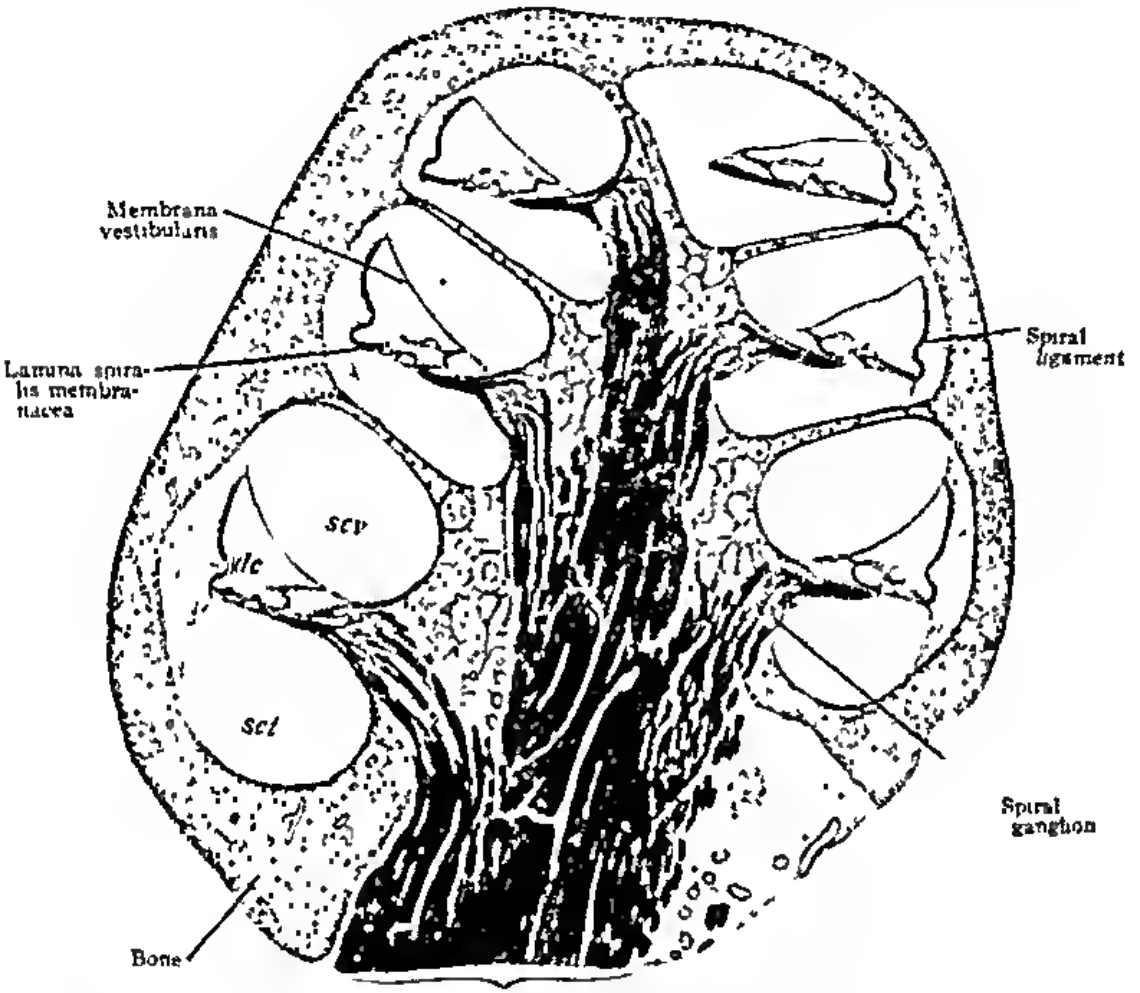


FIG. 582.—AXIAL SECTION OF THE COCHLEA OF A CAT × 25

The copula is above, the basis cochleæ below. In the modiolus are seen bundles of the cochlear branch of acoustic nerve blackened by osmic acid. Lateral branches of the nerve may be traced to the spiral ganglia where the nerve fibers arise. At the apex of the cochlea is the helicotrema affording communication between two perilymph spaces (scalæ). (Sobotta.) scv, scala vestibuli; dc, ductus cochlearis; sct, scala tympani.

proceed outward. All of these structures are partially surrounded by spaces, connecting with those of the vestibule which enclose the sacculus and utriculus. At the distal end of the endolymphatic duct, the spaces communicate with those of the cerebral arachnoid, and the perilymph mingles with cerebrospinal fluid.

Around the cochlear duct the perilymph spaces form a continuous tube. Starting from the vestibule, it ascends to the *cupola*, following the windings of the cochlear duct, to which it is closely applied. It is known as the *scala vestibuli* (*i.e.*, 'staircase of the vestibule') from which it passes out. At the apex of the cochlea it turns and becomes the descending *scala*

tympani, which ends blindly at the base of the cochlea, close against the wall of the tympanum. The two scalæ bear a constant relation to the coils of the cochlear duct. If the cochlea is so placed that its apex is upward, the scala vestibuli is always found on the upper side of the duct, and the scala tympani on the lower side, as shown in Fig. 582. In the body, the apex of the cochlea is directed forward and outward.

The temporal bone develops from the mesenchyma surrounding the ducts and their perilymph spaces, so that when the *membranous labyrinth* which they form is removed by maceration, the bone still contains a corresponding arrangement of cavities and canals. These constitute the *bony labyrinth*. Casts of it, made in soft metal, may be seen in all anatomical museums. Since the bone immediately surrounding the canal is denser than elsewhere, the labyrinth may be chiseled out from the bone. Instead of subdivisions to correspond with the utriculus, sacculus, and utriculosaccular duct, the bony labyrinth has a single space, already referred to as the *vestibule*. Into it the semicircular and cochlear *canals* open, together with the *aquæductus vestibuli* which contains the endolymphatic duct.

The middle ear and external ear arise in connection with the first pharyngeal pouch and first branchial cleft respectively (p. 301). At an early stage the ectoderm and entoderm meet one another and fuse, but later mesenchyma grows in between them. In the adult, however, the two parts are still close together, being separated by only the drum membrane, which is covered on one side with ectoderm and on the other with entoderm.

The ectodermal groove becomes surrounded by several nodular elevations of skin, which coalesce in a definite manner to make the projecting *auricle* (pinna). Its depression deepens, becoming the *external acoustic meatus*, which extends inward to the *tympanic membrane*. Much of this deepening takes place after birth, accompanying the development of the mastoid process. The entodermal portion of the spiracular cleft becomes in the adult an elongated outpocketing of the pharynx, known as the *auditory tube* (Eustachian tube). As seen in the section Fig. 583, the tube is separated from the bottom of the meatus by a very thin layer of mesenchyma, which is later included in the drum membrane.

In the mesenchyma behind the spiracular cleft, a chain of three small bones (the *malleus*, *incus*, and *stapes*) develops; it extends from the meatus to the vestibule. The bony wall of the vestibule is deficient at the small oval area where the stapes reaches it, so that the chain of bones comes directly in contact with the fibrous covering of the perilymph space. This area of contact is the *fenestra vestibuli* (*i.e.*, window of the vestibule). When the chain of bones vibrates back and forth, the motion of the stapes is transmitted through the fenestra vestibuli to the perilymph, and

waves may pass up the scala vestibuli and down the scala tympani, stimulating the nerves of hearing in the cochlear duct. The blind termination of the scala tympani rests against the lateral wall of the vestibule, where also the bone fails to develop; the round *fenestra cochleæ* is thus produced. Its fibrous membrane may yield somewhat to the perilymph waves, thus relieving tension in the cochlea.

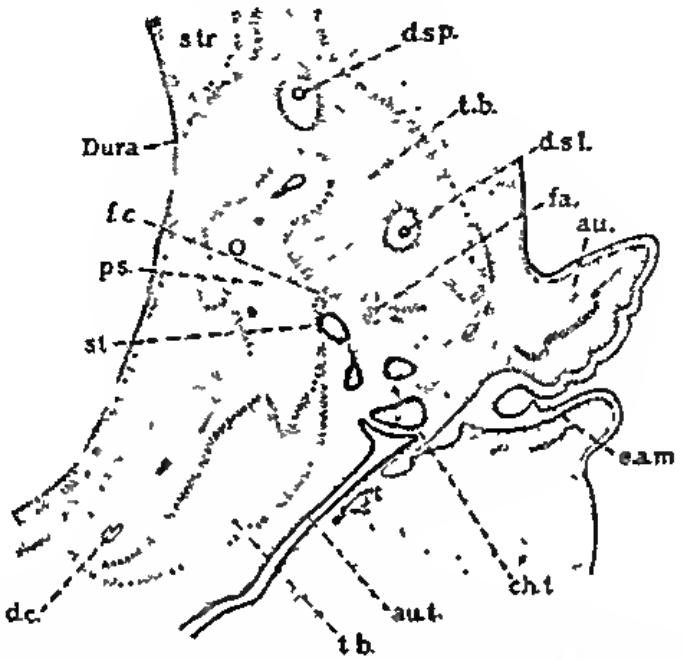


FIG. 583.—HORIZONTAL SECTION THROUGH THE EAR OF A HUMAN EMBRYO OF ABOUT 5 CM.
au., Auricle, au t., auditory tube, ch. t., chorda tympani, d c, cochlear duct, d.s l, and d.s p, lateral and posterior semicircular ducts, e.a.m., external acoustic meatus, fa, facial nerve, f.c., fenestra cochleæ; p.s, perilymphatic space, st, stapes, s. tr, transverse sinus, t b, temporal bone.

At first the fragments of the chain of bones together with neighboring nerves are embedded in a mass of mesenchyma. In a later stage the outer end of the auditory tube expands, filling all the space between the vestibule and the bottom of the meatus. Thus it forms the *tympanic cavity*. It encounters the chain of bones and the chorda tympani, and wraps itself around them so that they lie in its folds or *plicæ*. Thus all structures which extend into the tympanic cavity, or appear to cross it, are covered with a layer of entodermal epithelium derived from the auditory tube. The original contact between the ectoderm and entoderm of the spiracular cleft forms only an insignificant part of the tympanic membrane. The latter becomes greatly enlarged, extending somewhat along the upper surface of the ectodermal auditory meatus. The portion of the malleus lying near it becomes embedded in its mesenchymal layer, and its inner entodermal layer is made by the expansion of the tympanic cavity. The enlargement of the tympanic cavity continues after birth, when it invades the spaces formed within the mastoid part of the temporal bone.

In spite of these modifications, the course of the spiracular cleft is retained in the adult. The ectodermal depression and its surrounding elevations constitute the external ear; the pharyngeal outpocketing persists as the auditory tube and the tympanic cavity of the middle ear. It opens freely into the pharynx and contains air.

Sacculus, Utriculus, and Semicircular Ducts. The walls of all these structures consist of three layers. On the outside there is *connective tissue* with many elastic fibers and occasional pigment cells. This is followed by a narrow *basement membrane* said to form small nodular elevations toward the third and innermost layer, the simple flat *epithelium*. Near the maculæ and cristæ the connective tissue and the basement membrane become thicker, and the epithelial cells are columnar with a cuticular border. In the neuro-epithelium of these areas there are two sorts of cells, sustentacular and hair cells. The *sustentacular* or *fiber cells* extend clear across the epithelium and are somewhat expanded at both ends; they contain oval nuclei. *Hair cells*, which receive the stimuli, are columnar cells limited to the superficial half of the epithelium; they have large spherical nuclei near their rounded basal ends, and a clump of fine agglutinated filaments projecting from their free surface. The nerves lose their myelin as they enter the epithelium and ascend to the bases of the hair cells. There they bend laterally, forming a dense network which appears as a granular layer in ordinary preparations; the granules are optical sections and varicosities. The horizontal fibers terminate, like their occasional branches, by ascending between the hair cells, on the sides of which they form pointed free endings. They do not reach the free surface of the epithelium. This surface is covered by a continuation of the cuticula, a 'membrana limitans,' which is perforated by the hairs. Over the two maculæ there is a soft substance containing very many crystals of calcium carbonate, 1–15 μ long, which are named *otoconia*. (Large 'ear stones' of fishes are called otoliths.) Over the cristæ of the semicircular ducts there is a gelatinous substance, transparent in fresh preparations, but coagulated and rendered visible by reagents.[1]

The 'ligaments' of the ducts, the thin periosteum of the bony semicircular canals, and the perilymph spaces lined with mesenchymal epithelium are seen in Fig. 581.

Cochlea. The relation between the ductus cochleæ and the scalæ tympani and vestibuli is shown in Fig. 584. The ductus is triangular in cross section, being bounded on its peripheral surface by the thick periosteum of the bony wall of the cochlea; on its apical surface (toward the cupola) by the *membrana vestibularis* (Reissner's membrane); and on its

[1] Shambaugh, 1932.

basal or medial surface by the *lamina spiralis*. These three walls may be described in turn.

The peripheral wall of the cochlear duct is formed by the dense fibrous periosteum attached to the bone, together with a large mass of looser tissue crescentic in cross section, the *ligamentum spirale* (Fig. 584). The spiral ligament is covered by a layer of cuboidal epithelial cells belonging to the cochlear duct. Close beneath the epithelium there are blood vessels which are said to give rise to the endolymph. The thick plexus which they form is described as a band, the *stria vascularis*, which

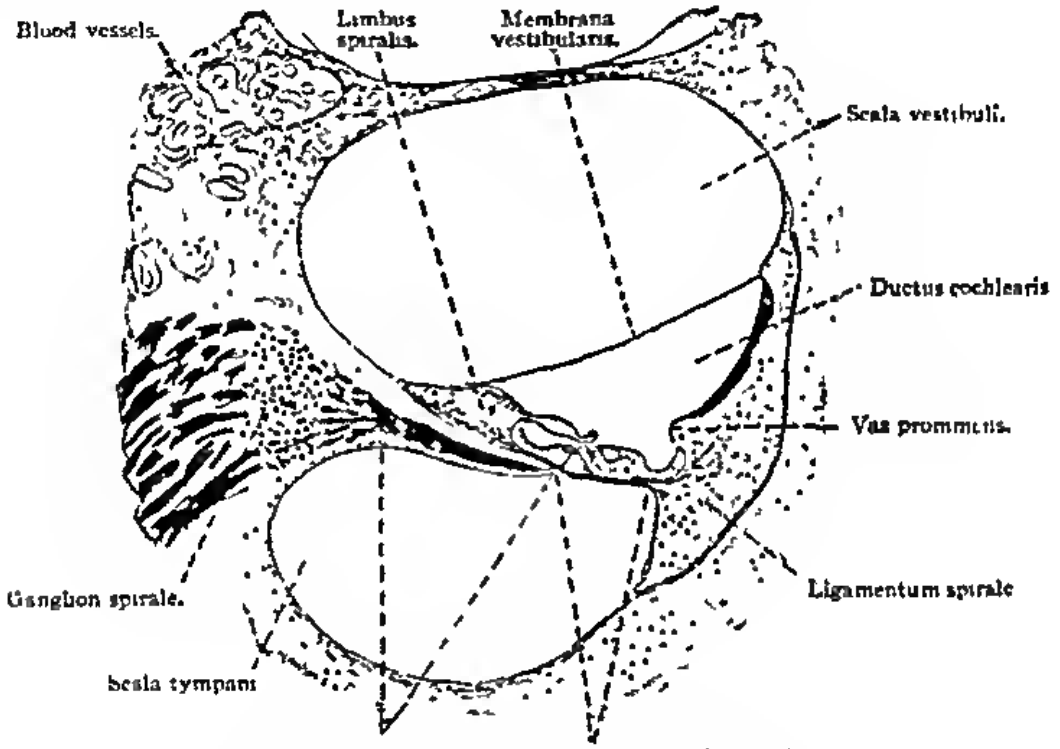


FIG. 584.—A SECTION OF THE COCHLEA. × 50

terminates more or less distinctly with the *vas prominens*. The latter occupies a low elevation of tissue which has its maximum development in the basal coil of the cochlea.

The apical wall, or *membrana vestibularis*, consists of a thin layer of connective tissue bounded on one side by the mesenchymal epithelium of the scala vestibuli, and on the other by the simple flattened ectodermal epithelium of the cochlear duct.

The basal wall or *lamina spiralis* extends outward from the modiolus to the bony wall of the cochlea. Near the modiolus it lies between the two scalæ, but peripherally it is between the cochlear duct and the scala tympani. Toward the modiolus it contains a plate of bone perforated for the passage of vessels and nerves; this part is the *lamina spiralis ossea*. The peripheral portion is the *lamina spiralis membranacea*. Both parts

are covered below by the mesenchymal epithelium of the scala tympani, and above by the epithelium of the cochlear duct, including its complex neuro-epithelium known as the *spiral organ* (of Corti).[1]

Where the membrana vestibularis meets the osseous spiral lamina, there is an elevation of tough connective tissue called the *limbus spiralis* (Fig. 584). It consists of abundant spindle-shaped cells, and blends below with the periosteum of the spiral lamina. Superficially it produces irregularly hemispherical papillæ covered with simple flat epithelium, found within the cochlear duct near the vestibular membrane. Further within the cochlear duct the papillæ give place to a single row of flat ridges or

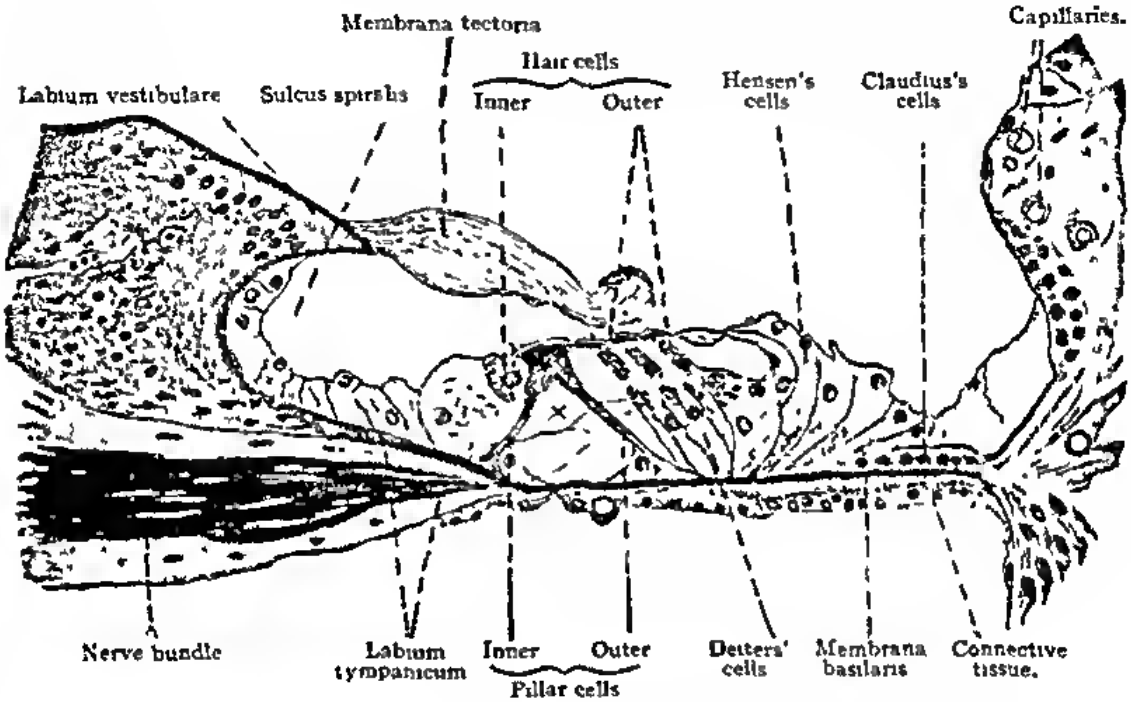


FIG. 585.—PORTION OF FIGURE 584 × 240

x, Intercellular 'tunnel' traversed by nerve fibers

plates, directed peripherally. These are 'Huschke's auditory teeth' (Fig. 586). Beneath them the limbus terminates abruptly in an overhanging *labium vestibulare*, which projects over an excavation—the *sulcus spiralis* (Fig. 585). The basal wall of the sulcus is the *labium tympanicum*, found at the peripheral edge of the osseous spiral lamina. As the epithelium of the limbus passes over the labium vestibulare into the sulcus, it becomes cuboidal. A remarkable non-nucleated structure projects from the labium vestibulare over the neuro-epithelium of the membranous spiral lamina. It is called the *membrana tectoria* and is composed of 'multitudes of delicate fibers of unequal length, embedded in a transparent matrix of a soft, collagenous semi-solid character, with marked adhesiveness.'

[1] CORTI, 1851.

It is apparently produced as a cuticular formation from the tall cells which in the fetus fill the sulcus spiralis and extend beyond the hair cells, and acquires an attachment to the labium vestibulare. Later the tall cells become shorter, or are transformed into specialized pillar cells and hair cells, thus in either case losing attachment to the membrana, which thus floats free above them; it is of nearly the same specific gravity as the endolymph,[1] and might transmit vibrations of the fluid to the hair cells, with which it is often found in contact. It should be noted, however, that the osmotic currents caused by fixing and decalcifying fluids in these closed canals are apt to cause distortions; some authors have described the membrana as attached to the hair cells, not to the limbus.

The lamina spiralis membranacea, or lamina basilaris, consists of four layers. The *mesenchymal epithelium* of the scala tympani is followed by a layer of delicate *connective tissue*, prolonged from the periosteum of the scala. Its spindle cells are at right angles with the fibers of the overlying *membrana basilaris*. This membrane, which is beneath the *epithelium* of the cochlear duct, consists of coarse straight fibers extending from the labium tympanicum of the ligamentum spirale. They cause it to appear finely striated. Peripherally (beyond the bases of the outer pillar cells) the fibers are thicker, and are called 'auditory strings'; they are shortest in the basal part of the cochlea and longest toward the apex, corresponding in length with the basal layer of the cochlear duct. These fibers have been thought to vibrate and assist in conveying sound waves to the nerves, but theories which assume that the basilar membrane is a 'vibrating mechanism' are considered untenable by Hardesty; he finds it more probable that the membrana tectoria vibrates and transmits stimuli to the neuro-epithelium.

The epithelial cells covering the basilar layer occur in rows of highly modified forms, which extend up and down the cochlear duct, constituting the *spiral organ* (organ of Corti). Next to the cuboidal epithelium of the sulcus spiralis there is a single row of *inner hair cells* (Fig. 586). These are short columnar cells which do not reach the bottom of the epithelium; each has about forty long stiff hairs on its free surface. The inner hair cells are followed peripherally by two rows of *pillar cells*, the *inner* and *outer*, which extend the whole length of the cochlear duct. As seen in cross section they are in contact above, but are separated below by a triangular intercellular space or 'tunnel,' which is filled with soft intercellular substance. Thus they rest upon the basilar membrane in Λ-form. Each pillar cell may be subdivided into a head, a slender body, and an expanded triangular base. The greater portion of each cell has been transformed into a resistant band, at the base of which, within the tunnel, there is a mass of protoplasm containing the nucleus. A protoplasmic sheath extends up from the base around the body of the cell. Dark round structures which may be found in the heads of the pillars, and at the foot

[1] Hardesty, 1915.

of the outer ones, are not nuclei, but are 'probably of horny nature.' The heads of the pillars interlock. Both pillars produce 'head-plates' directed outward, and so arranged that the plate from the inner pillar overlies that from the outer pillar. Moreover, the round head of the outer pillar is fitted into a concavity in the head of the inner pillar, as shown in the figure.

On the peripheral side of the outer pillars there are several rows (usually four) of *outer hair cells* separated from one another by *sustentacular*

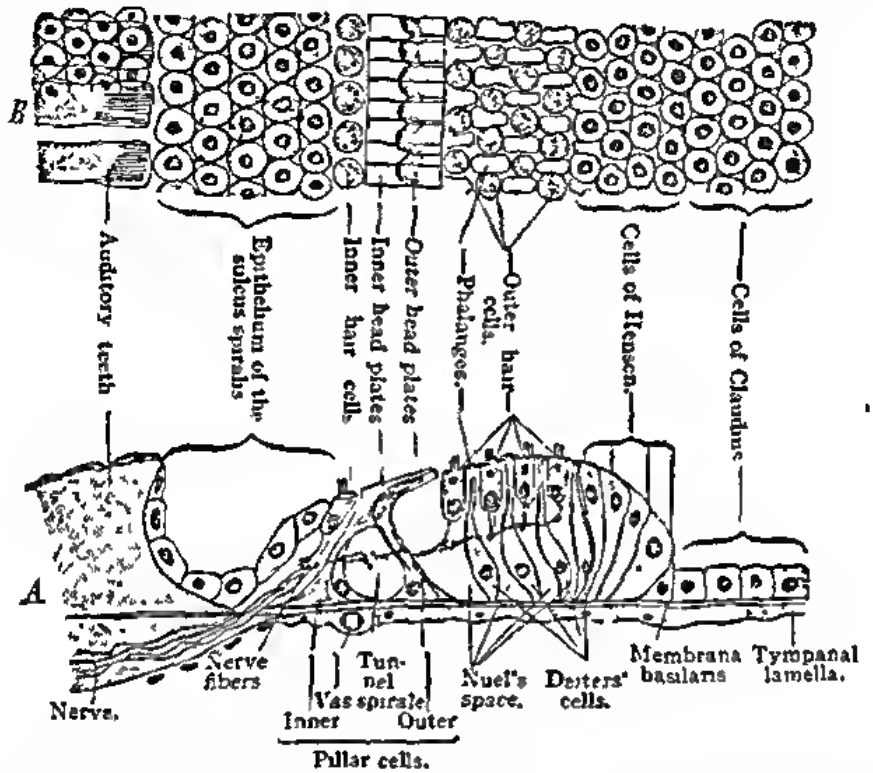


FIG. 586.—DIAGRAM OF THE STRUCTURE OF THE BASAL WALL OF THE DUCT OF THE COCHLEA.

A, View from the side B, View from the surface. In the latter the free surface is in focus It is evident that the epithelium of the sulcus spiralis, lying in another plane, as well as the cells of Claudius, can be seen distinctly only by lowering the tube The membrana tectoria is not drawn The spiral nerves are indicated by dots

cells (Deiters' cells). The outer hair cells have shorter hairs than the inner ones, and are characterized by the presence of 'Hensen's spiral bodies,' one of which occurs in the outer half of each cell. These bodies are shown as dark spots in Fig. 586. The centrosomes of the hair cells are always in their upper ends. Like the inner hair cells, the outer ones do not extend to the basilar membrane, thus leaving unoccupied the communicating intercellular spaces between the deeper portions of the sustentacular cells. These *Nuel's spaces* connect with the tunnel.

Deiters' sustentacular cells are slender bodies, each containing a stiff filament, and having at its free end a cuticular formation referred to as a 'phalanx.' The phalanges come between the outer hair cells, separating them from one another, and the inner hair cells are similarly separated by short processes—the inner phalanges, derived from the inner pillars.

(The inner phalanges are not shown in the figure.) The phalanges of Deiters' cells connect with one another, forming a trim reticular membrane. As a whole Deiters' cells resemble the pillar cells, but their transformation into stiff fibers has not proceeded so far; the cuticular border is comparable with the head-plate.

The most peripheral of the sustentacular or Deiters' cells are followed by elongated columnar cells (cells of Hensen), which gradually shorten, and are succeeded by the low 'cells of Claudius' which extend to the limit of the membrana basilaris. In both the columnar and the low forms there are single stiff filaments which are less developed than in the sustentacular cells. The centrosomes of all these cells lie near their free surfaces. Beyond the basilar membrane the epithelium is continued over the ligamentum spirale as a layer of cells with branching basal processes extending deep into the underlying tissue.

Nerves of the Labyrinth. The *acoustic nerve* is a purely sensory nerve passing between the pons and internal ear through a bony canal, the *internal acoustic meatus.* It is divided into vestibular and cochlear portions (Fig. 582). The *vestibular nerve* passes through the vestibular ganglion and sends branches to supply the utricle, the saccule and the superior, lateral and posterior ampullæ. The cochlear nerve supplies only the spiral organ of Corti. The ganglion of the cochlear nerve is lodged within the modiolus at the root of the lamina spiralis, and is known as the *spiral ganglion.* The ganglion cells remain bipolar, like those of embryonic spinal ganglia. They are surrounded by connective tissue capsules; and their neuraxons and single peripheral dendrites receive myelin sheaths not far from the cell bodies.

The peripheral fibers extend through the lamina spiralis ossea, within which they form a wide-meshed plexus, and after losing their myelin they emerge from its outer border in the labium tympanicum through the *foramina nervosa.* In continuing to the spiral organ they curve in the direction of the cochlear windings, thus producing spiral strands. Those nearest the modiolus are on the axial side of the pillar cells; the middle ones are between the pillars, in the tunnel; and the outer ones are beyond the pillar cells. From these bundles, delicate fibers pass to the hair cells, on the sides of which they terminate.

Vessels of the Labyrinth.[1] The *internal auditory artery* is a branch of the basilar artery. It arises in connection with branches which are distributed to the under side of the cerebellum and the neighboring cerebral nerves, and passes through the internal acoustic meatus to the ear. It divides into vestibular and cochlear branches (Fig. 587). The *vestibular artery* supplies the vestibular nerve and the upper lateral portion of the

[1] Shambaugh, 1923.

sacculus, utriculus and semicircular ducts. The *cochlear artery* sends a vestibulo-cochlear branch to the lower and medial portion of the sacculus, utriculus, and ducts. This branch also supplies the first third of the first turn of the cochlea. The capillaries formed by the vestibular branches are generally wide-meshed, but near the maculæ and cristæ the meshes are narrower. The terminal portion of the cochlear artery

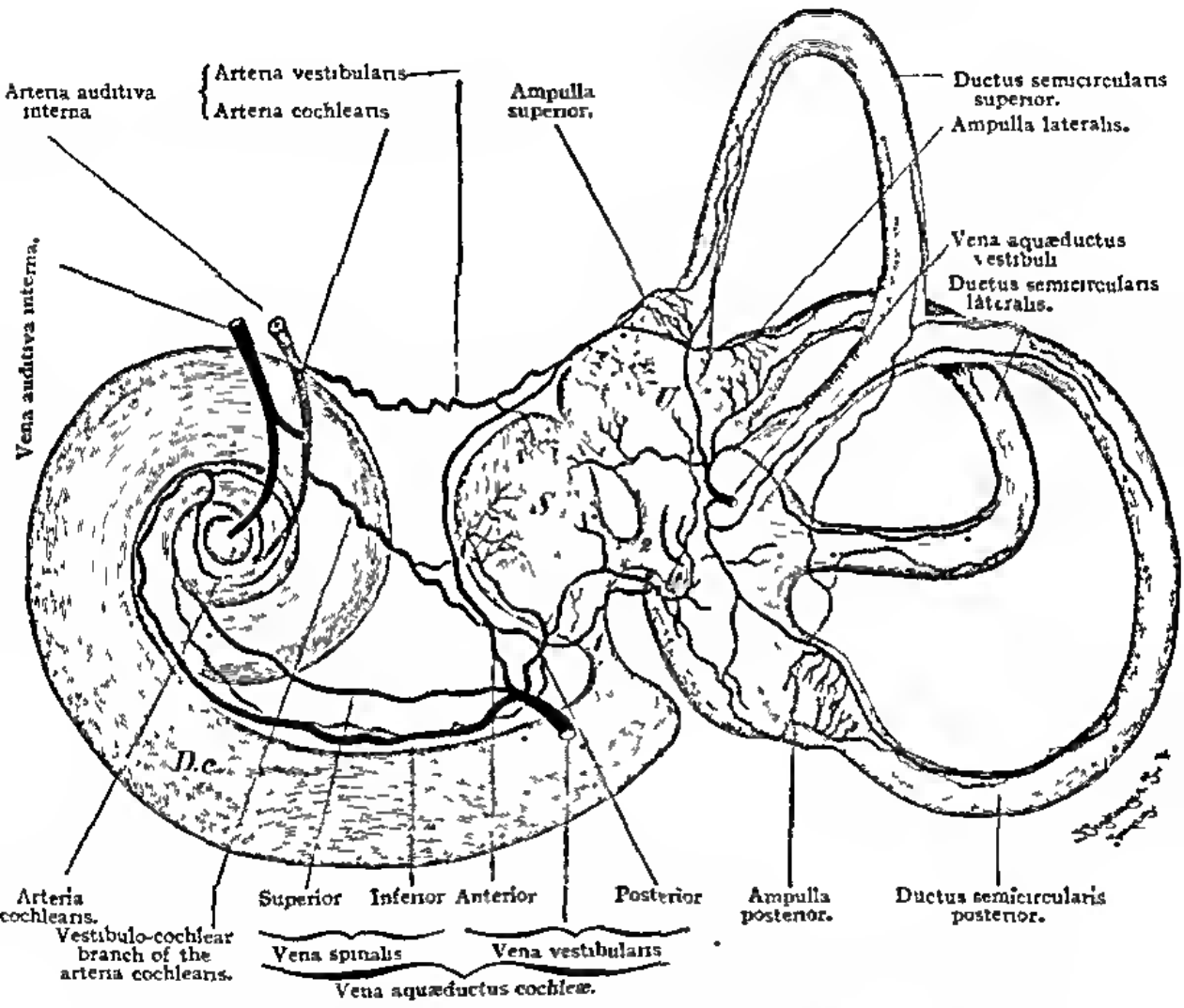


Fig. 587.—Diagram of the Blood Vessels of the Right Human Labyrinth Medial and Posterior Aspect. D. c, ductus cochlearis; S, sacculus; U, utriculus, 1, ductus reuniens; 2, ductus utriculo-sacculares. The saccus endolymphaticus is cut off

enters the modiolus and forms three or four spirally ascending branches. These give rise to about thirty radial branches distributed to three sets of capillaries (Fig. 588); 1, those to the spiral ganglion; 2, those to the lamina spiralis; and 3, those to the outer walls of the scalæ and the stria vascularis of the cochlear duct.

The *veins* of the labyrinth form three groups. 1. The *vena aquæductus vestibuli* receives blood from the semicircular ducts and a part of the utriculus. It passes toward the brain in a bony canal along with the

ductus endolymphaticus, and empties into the superior petrosal sinus. 2. The *vena aquæductus cochleæ* receives blood from parts of the utriculus, sacculus and cochlea; it passes through a bony canal to the internal jugular vein. Within the cochlea it arises, as shown in Fig. 588, from small vessels including the vas prominens (*a*) and the vas spirale (*b*). Branches derived from these veins pass toward the modiolus. (There are no vessels in the vestibular membrane of the adult, and the vessels in the wall of the scala tympani are so arranged that only veins occur in the part

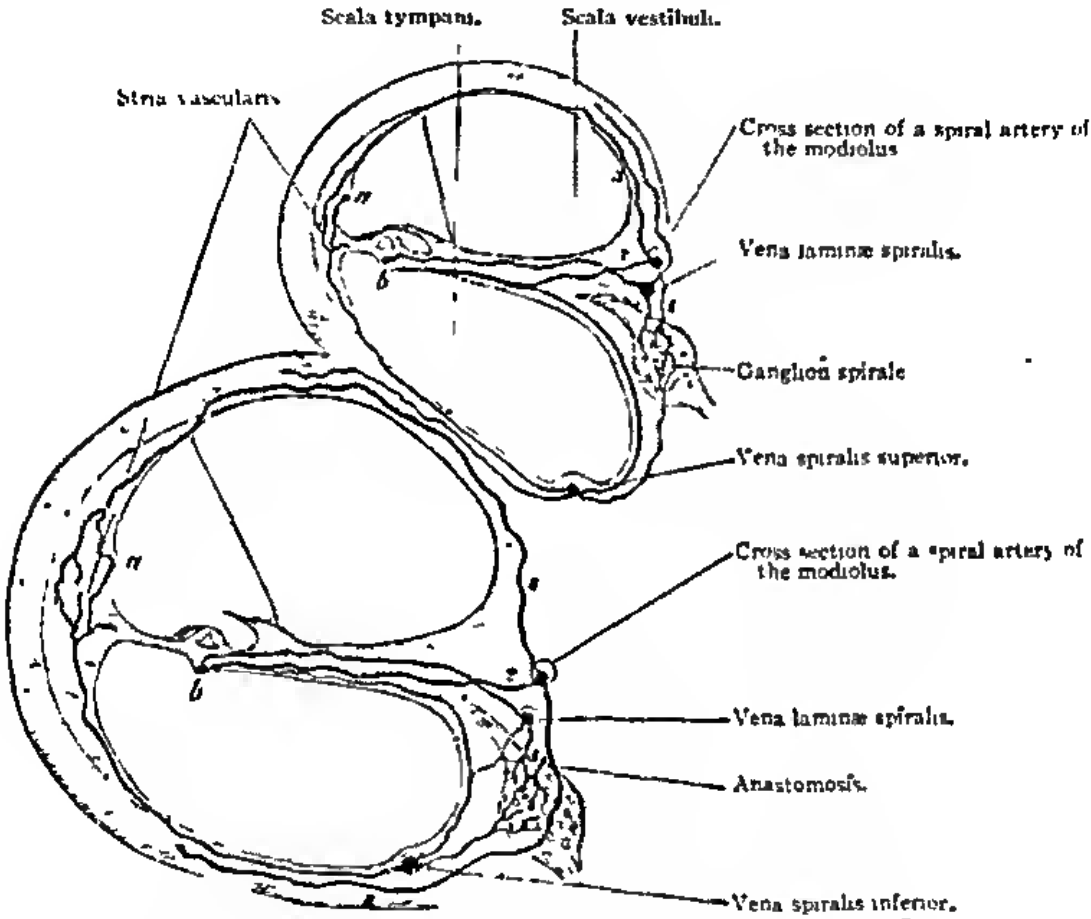


FIG. 588.—DIAGRAM OF A SECTION OF THE FIRST (BASAL) AND SECOND TURNS OF THE COCHLEA. *a*, Vas prominens, *b*, vas spirale.

toward the membranous spiral lamina; thus the latter is not affected by arterial pulsation.) Within the modiolus the veins unite in an *inferior spiral vein*, which receives blood from the basal and a part of the second turns of the cochlea, and a *superior spiral vein* which proceeds from the apical portion. These two spiral veins unite with vestibular branches to form the vena aquæductus cochleæ (Fig. 587). 3. The *internal auditory vein* arises within the modiolus from the veins of the spiral lamina; these anastomose with the spiral veins (Fig. 588). It receives branches also from the acoustic nerve and from the bones, and empties into the vena spinalis anterior.

Lymphatic spaces within the internal ear are represented by the perilymph spaces, which communicate through the aquæductus cochleæ with the arachnoid space; the connecting structure, or 'ductus perilymphaticus,' is described as a lymphatic vessel. The *saccus endolymphaticus*, which is the dilated distal end of the endolymphatic duct, is in contact with the dura, and there are said to be openings between it and the subdural space. In the internal ear perivascular and perineural spaces are found, and they probably connect with the arachnoid spaces.

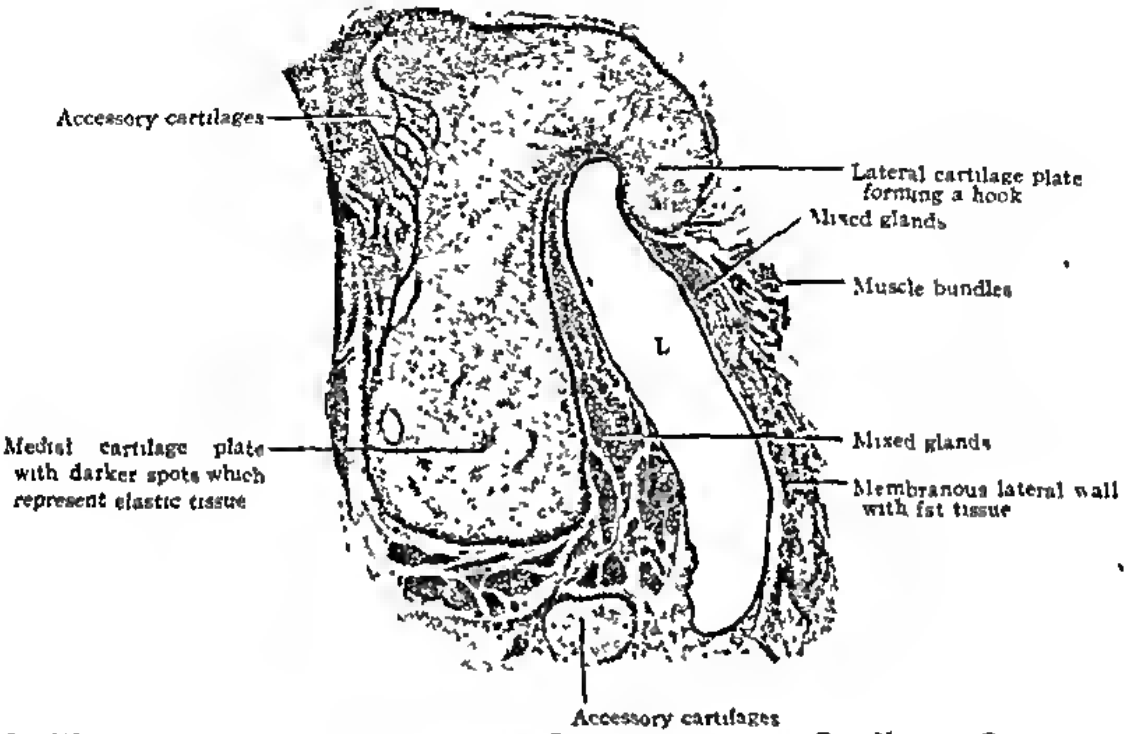


Fig. 589.—Cross-section through the Cartilaginous Portion of the Auditory Tube Near the Opening into the Pharynx. × 11 (von Ebner)
L., dilated lumen

Middle Ear. The *tympanic cavity*, which contains air, is lined with a *mucous membrane* closely connected with the surrounding periosteum. It consists of a thin layer of connective tissue, covered generally with simple cuboidal epithelium. In places the epithelial cells may be flat, or tall with nuclei in two rows. Cilia are sometimes widely distributed and are usually to be found on the floor of the cavity. In its anterior part, small alveolar mucous glands occur very sparingly. Capillaries form wide-meshed networks in the connective tissue, and lymphatic vessels are found in the periosteum.

The *auditory tube* includes an *osseous part* toward the tympanum, and a *cartilaginous part* toward the pharynx. Its mucosa consists of fibrillar connective tissue, together with a ciliated columnar epithelium which becomes stratified as it approaches the pharynx. The stroke of the cilia is toward the pharyngeal orifice. In the osseous portion, the mucosa is

without glands and very thin; it adheres closely to the surrounding bone. Along its floor there are pockets containing air, the *cellulæ pneumaticæ*. In the cartilaginous part the mucosa is thicker; near the pharynx it contains many mixed glands (Fig. 589). Lymphocytes are abundant in the surrounding connective tissue, forming nodules near the end of the tube, which blend with the pharyngeal tonsil. The cartilage, which only

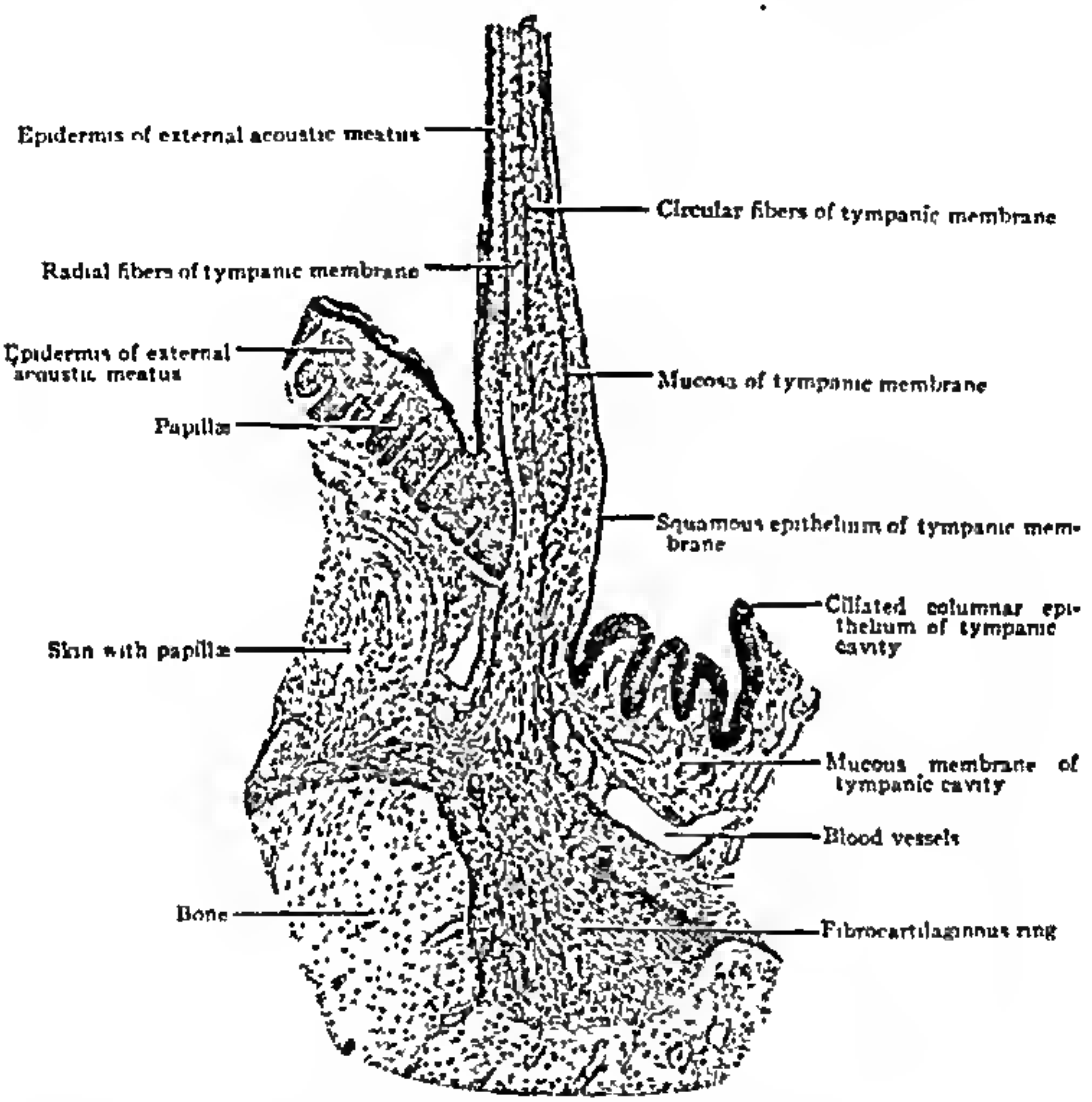


Fig. 590.—A Cross-section through the Edge of the Tympanic Membrane of a Child. × 55. (von Ebner.)

partly surrounds the auditory tube, is hyaline near its junction with the bone of the osseous portion; it may contain here and there coarse fibers which are not elastic. Toward the pharynx the matrix contains thick nets of elastic tissue and the cartilage is consequently elastic.

External Ear. Between the middle ear and the external ear is the *tympanic membrane*, which consists, from without inward, of the following *strata:* the *cutaneum*, *radiatum*, *circulare* and *mucosum*. The stratum cutaneum is a thin skin without papillæ in its corium, except along the handle or *manubrium* of the malleus. There it is a thicker layer, containing the

vessels and nerves which descend along the manubrium and spread from it radially. In addition to the venous plexus which accompanies the artery in this situation, there is a plexus of veins at the periphery of the membrane, receiving tributaries from both the stratum cutaneum and the less vascular stratum mucosum. The radiate and circular strata consist of compact bundles of fibrous and elastic tissue, arranged so as to suggest tendon. The fibers of the radial layer blend with the peri-

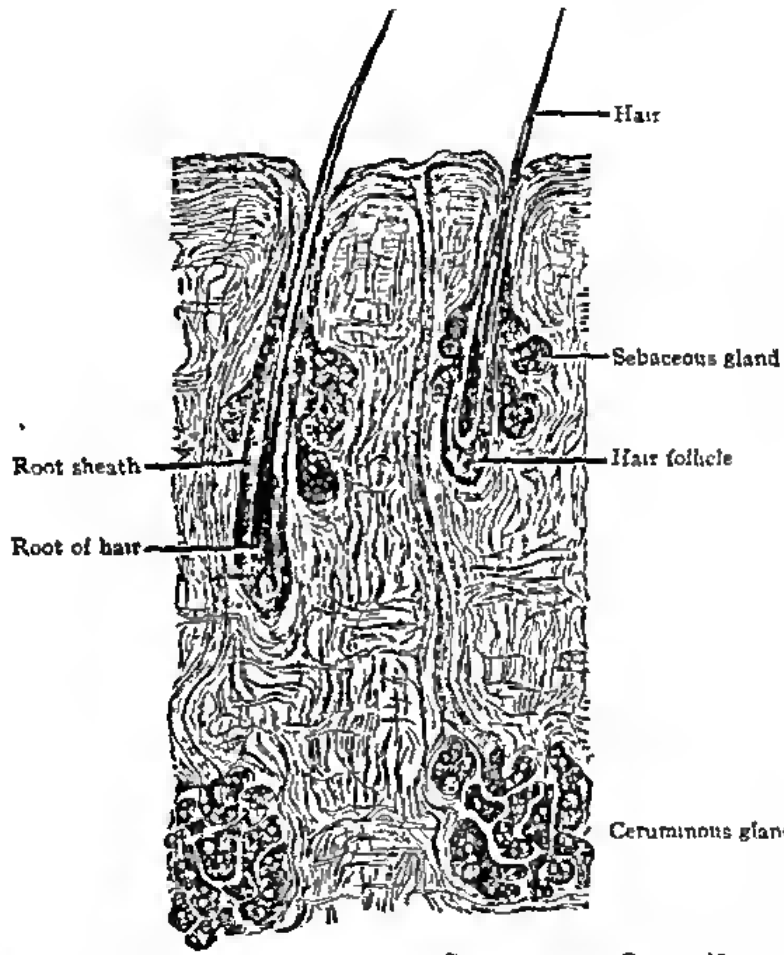


FIG. 591.—SECTION OF SKIN OF THE AUDITORY MEATUS, INCLUDING TWO CERUMINOUS GLANDS (Quain, after Gruber.)

chondrium of the hyaline cartilage covering the manubrium. Peripherally the fiber layers form a fibro-cartilaginous ring which connects with the surrounding bone. The stratum mucosum is a thin layer of connective tissue covered with a simple, non-ciliated, flat epithelium continuous with the lining of the tympanic cavity. Peripherally, in children, its cells may be taller and ciliated. As a whole the tympanic membrane is divided into *tense* and *flaccid* portions. The latter is a relatively small upper part in which the fibrous layers are deficient.

The *external acoustic meatus* is lined with skin continuous with the cutaneous layer of the tympanic membrane. In the deep or osseous portion the skin is very thin, without hairs or glands except along its upper

wall. There and in the outer or cartilaginous part, *ceruminous glands* are abundant. 'They are branched tubulo-alveolar glands' (Huber) which in many respects resemble large sweat glands. Their ducts are lined with stratified epithelium. The coils consist of a single layer of secreting cells, generally cuboidal, surrounded by smooth muscle fibers and a well-defined basement membrane. They differ from sweat glands in that their coils have a very large lumen, especially in the adult; and their gland cells, often with a distinct cuticular border, contain many pigment granules and fat droplets. Their narrow ducts in adults end on the surface

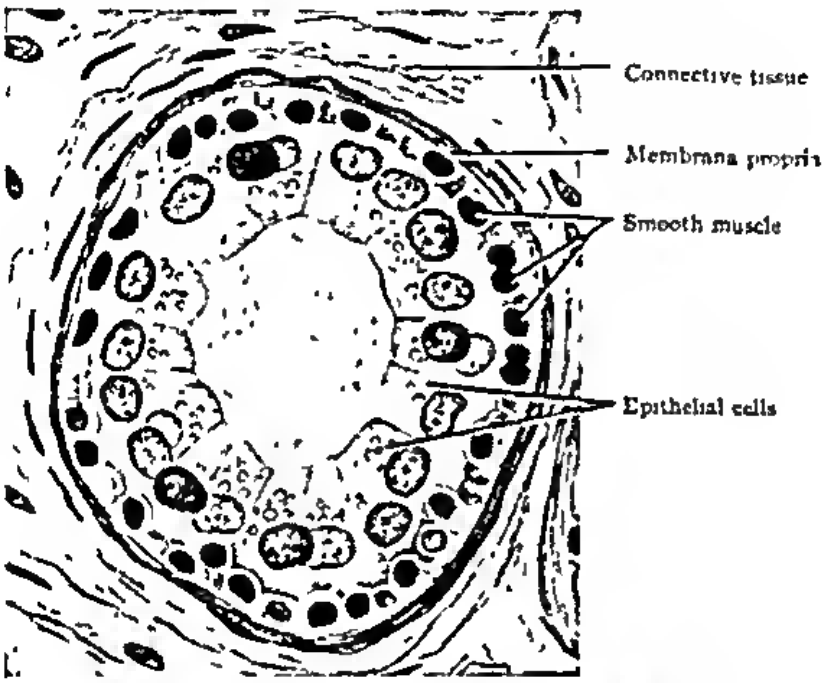


FIG. 592.—CROSS-SECTION OF A CANAL OF A CERUMINOUS GLAND. From the external ear of a human adult. × 600. *Zenker fixation, Hansen's hæmatoxylin (von Möllendorff).*

of the skin close beside the hair sheaths; in children they empty into the sheaths. It has not been shown that the ceruminous glands are more directly concerned in the production of cerumen than the sebaceous glands. The cerumen obviously is an oily rather than a watery secretion, and it contains fatty cells and pigment.

The cartilage of the external acoustic meatus and of the auricle is elastic.

NOSE

The nasal cavities are formed by the invagination of a pair of epidermal thickenings similar to those which give rise to the lens and auditory vesicle. The pockets thus produced in the embryo are called 'nasal pits' (Fig. 258, p. 302). Their external openings remain as the *nares* of the adult, but temporarily, from the third to the fifth month of embryonic life, they are closed by an epithelial proliferation. Each nasal pit acquires

an internal opening, the *choana*, in the roof of the pharynx. The choanæ are at first situated near the front of the mouth, separated from one another by a broad *nasal septum*. As the latter extends posteriorly, it is joined by the *palate processes* which grow toward it from the sides of the maxillæ. Thus the choanæ recede toward the back of the mouth, while the embryonic condition of cleft palate is being removed (Fig. 593). The lateral walls of the nasal cavities produce three curved folds one above another; they are concave below, and in them the *conchæ* (turbinate bones) develop. The nasal mucosa covers these and extends into

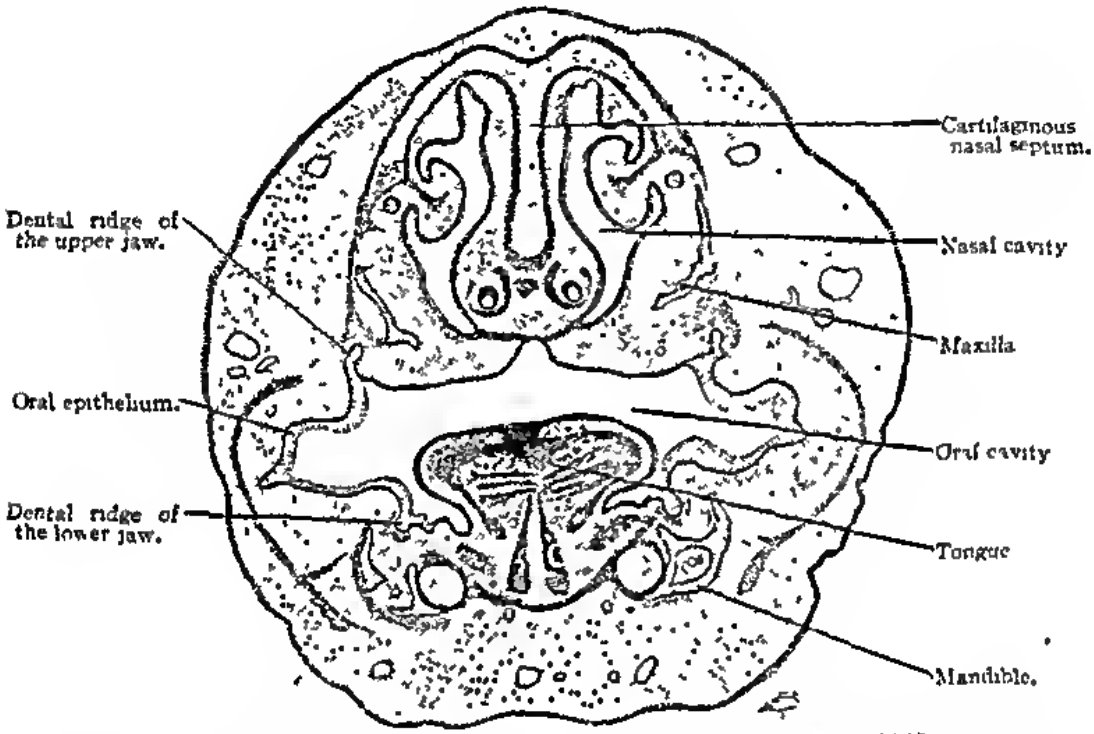


FIG 593.—FRONTAL SECTION OF THE HEAD OF A 40-MM SHEEP EMBRYO × 15.

The palate processes have united with the nasal septum. The conchæ are developing along the lateral walls of the nasal cavity. In the lower part of the septum the *vomero-nasal organs* are seen as a pair of tubes, each of which is partly surrounded by a crescentic cartilage.

excavations in the adjacent bones, forming the sphenoid, maxillary, and frontal sinuses, and the ethmoidal cells. The boundary between the epithelium of the nasal pit and that of the pharynx early disappears, and the extent of each in the adult is uncertain. Presumably the olfactory neuro-epithelium is derived from the nasal pit. In man the olfactory region is limited to the upper third of the nasal septum and nearly the whole of the superior concha.[1] This *regio olfactoria* is covered by a yellowish-brown membrane, which may be distinguished macroscopically from the reddish mucosa of the *regio respiratoria*. The latter includes the remainder of the nose. The two regions may be considered in turn.

The *vestibule*, or cavity of the projecting cartilaginous portion of the nose, is a part of the respiratory region which is lined with a continuation

[1] READ, 1908.

of the skin. Its stratified epithelium has squamous outer cells and rests upon a lamina propria with papillæ. It contains the sheaths of coarse hairs (*vibrissæ*) together with numerous sebaceous glands. The extent of the squamous epithelium is variable; frequently it is found on the middle concha, less often on the inferior concha.

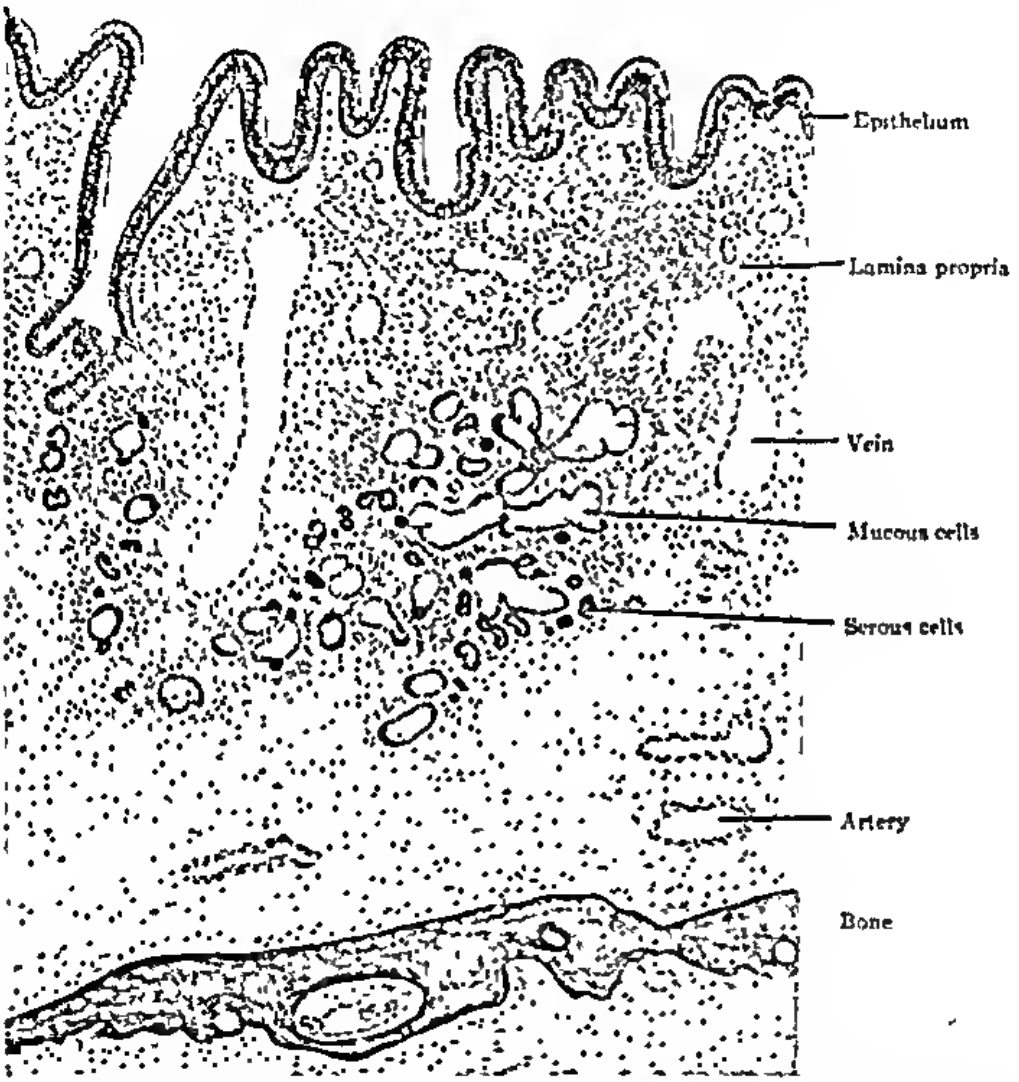


Fig. 594.—Vertical Section through the Mucosa of the Inferior Concha of Man. × 48. On the left is a funnel-shaped depression receiving an excretory duct, nearby on the right is a section of a large vein

The remainder of the nasal epithelium is variable. In the sinuses it is simple cuboidal and ranges in other parts to pseudostratified columnar, with several rows of nuclei, the surface cells being columnar, and either ciliated or mucous. Apparently the types are not fixed.[1] The simple columnar epithelium found on the sheltered sides of the conchæ may become many layered if exposed unduly to the outer air, and the more exposed surfaces may change to the stratified squamous type. The goblet cells may be few or many. The lamina propria is well developed and very vascular, being even 4 mm. thick on the interior concha (Fig. 594).

[1] Hilding, 1932

It consists of fibrillar tissue with many elastic elements, especially abundant in its deeper layers. Beneath the epithelium, it is thickened to form a homogeneous membrana propria, perforated with small holes. Lymphocytes are present in variable quantity, sometimes forming solitary nodules and often entering the epithelium in great numbers. Branched alveolo-tubular mixed glands extend into the lamina propria. Their serous portions have intercellular secretory capillaries. The glands often empty into funnel-shaped depressions, which are macroscopic on the inferior concha, and are lined with the superficial epithelium. The mucosa of the several paranasal sinuses is thin (0.02 mm.), with less elastic tissue and but few small glands. Into the lower part of the median septum on each side extends a pocket known as the vomero-nasal

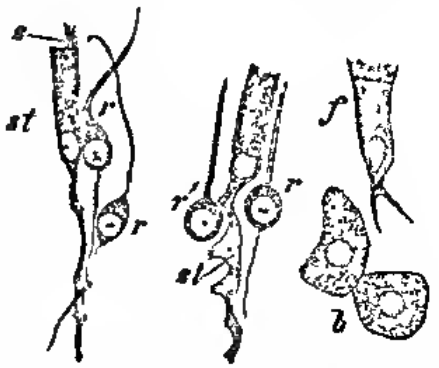


FIG. 595.—ISOLATED CELLS OF THE OLFACTORY MUCOSA OF A RABBIT. × 560

st, Sustentacular cells, s, extruded mucus resembling cilia, r, olfactory cells, from r' the lower process has been torn off, f, ciliated cell, b, cells of olfactory glands

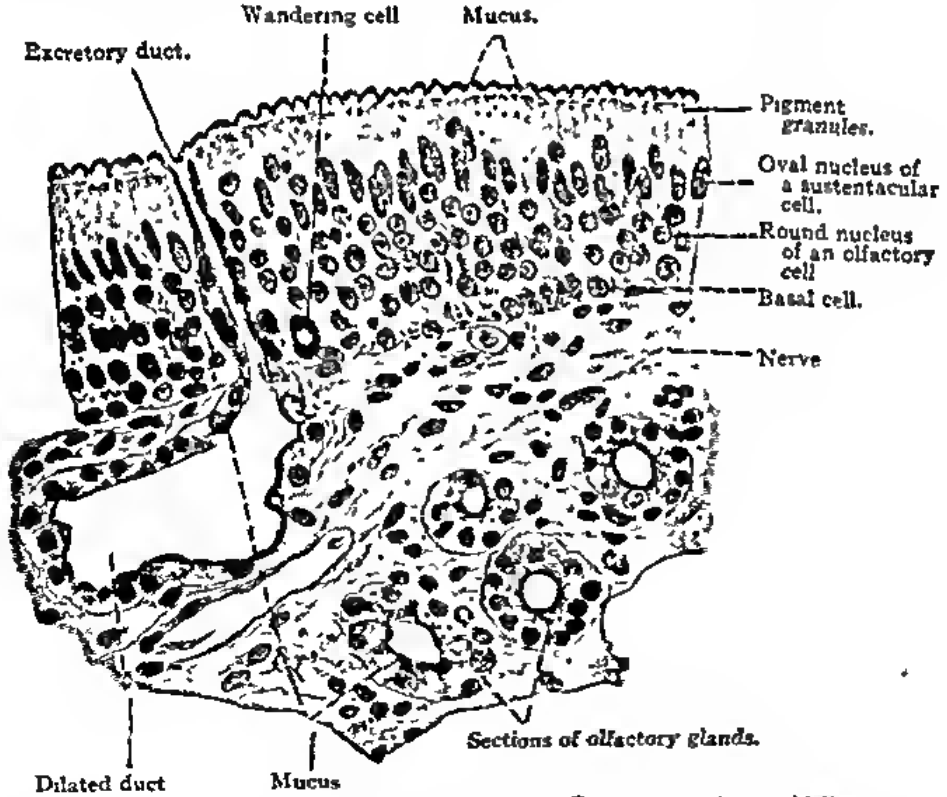


FIG. 596.—VERTICAL SECTION THROUGH THE OLFACTORY REGION OF AN ADULT. × 400

organ (Jacobson's organ). In man it is the rudimentary remnant of a sense organ, probably for the sense of taste in a fluid medium,[1] and as such is supplied by the nervus terminalis and by branches of the nervus trigeminus. It is lined with a tall columnar epithelium, and contains,

[1] BROMAN, 1920.

at least in the cat, 'sensory cells apparently identical with those of the olfactory mucosa.' In man sensory cells are said to be lacking in the adult and in embryos older than five months.

In the *regio olfactoria* the mucosa includes a lamina propria and an *olfactory epithelium*. The latter consists of *sustentacular cells* and *olfactory cells*. The superficial halves of the sustentacular cells are cylindrical, and contain yellowish pigment, together with small mucoid granules often arranged in vertical rows (Fig. 595). The more slender lower halves have dentate or notched borders, and branched basal ends which unite with those of neighboring cells, thus forming a protoplasmic network. Their nuclei, generally oval, are in one plane, and in vertical sections they form a narrow 'zone of oval nuclei' (Fig. 596). The olfactory cells generally have round nuclei containing nucleoli. They occur at different levels and so form a broad 'zone of round nuclei.' From the cytoplasm which is gathered immediately about the nucleus, each olfactory cell sends a slender cylindrical process toward the surface, where it terminates in a variety of ways. It may end in a small knob-like swelling, or in a single slender spine; sometimes the terminal knob sends out a small cluster of divergent olfactory hairs or spines. Basally the olfactory cells pass directly into the axis cylinders of the olfactory nerves (Fig. 598). Thus they are ganglion cells, their basal processes being neuraxons. Cells intermediate between the olfactory and sustentacular forms may be found, and these are doubtless imperfectly developed sensory cells. At the free surface of the olfactory epithelium there are terminal bars, and small projecting strands of mucus, sometimes suggesting cilia (Fig. 595, *s*). The mucus, which is the product of the sustentacular cells, may appear to form a continuous superficial membrane. Near the lamina propria there is a network of so-called 'basal cells.'

FIG. 597.—RECONSTRUCTION OF A MUCOUS GLAND FROM THE NASAL MUCOSA OF A 6 YR OLD CHILD (Mazzarelli.)

The lamina propria is composed of fibrous tissue and fine elastic fibers, associated with many connective tissue cells. In some animals (for example, the cat) it forms a structureless membrane next to the epithelium. It surrounds the numerous *olfactory glands* (Bowman's glands). In man these consist of excretory ducts extending through the epithelium, and of branching gland bodies beneath. They have the appearance of serous glands, but sometimes contain mucus, generally in small quanti-

ties. They are found not only in the olfactory region, but also in the adjoining part of the respiratory region.

The deeper layers of the lamina propria contain the arteries of the mucous membrane, which send branches toward the epithelium, and form a thick sub-epithelial plexus of capillaries. The veins are very numerous, especially at the inner end of the inferior concha, where the lamina propria resembles cavernous tissue. Lymphatic vessels form a

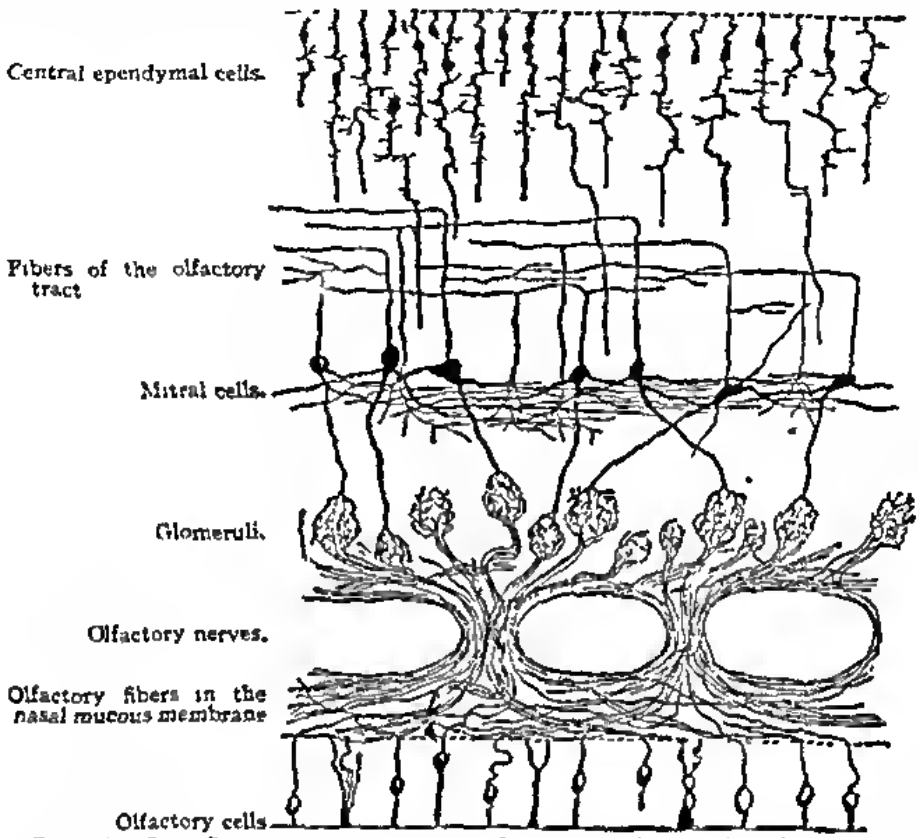


FIG. 598.—CHIEF ELEMENTS OF THE OLFACTORY BULB (Gordinier, after Van Gehuchten.)

coarse-meshed network in the deeper connective tissue. Injections of the arachnoid spaces around the olfactory bulbs follow the perineural sheaths of the olfactory nerves into the nasal mucosa, but these tissue spaces are not lymphatic vessels.

The olfactory nerves, as already stated, are formed of the basal processes of the olfactory epithelial cells, which become non-medullated nerve fibers. This is a primitive type of nervous apparatus (cf. p. 175), such as is not found elsewhere in the human body. After a tangential course beneath the epithelium, the fibers unite in bundles, and pass through the cribriform plate of the ethmoid bone to the olfactory bulb just above it, which they enter. They spread tangentially and branch, finally terminating in the *glomeruli*. The glomeruli are round or oval groups of arborizing fibers, in which the processes of the olfactory cells end in relation with the dendrites of the *mitral cells*. The latter are nerve cells with triangular

bodies, which form a characteristic layer of the olfactory bulb, and send their neuraxons through the olfactory tracts to make various connections within the hemispheres.

In addition to the olfactory nerves, the nasal mucous membrane contains medullated branches of the trigeminal nerve, distributed both to the olfactory and respiratory regions, and the nervus terminalis.

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