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by Paul Dittus

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Histologie und Cytologie des Interrenalorgans der Selachier unter normalen und experimentellen Bedingungen.

Ein Beitrag zur Kenntnis der Wirkungsweise des kortikotropen Hormons und des Verhältnisses von Kern zu Plasma.

HISTOLOGY AND CYTOLOGY OF THE SELACHIAN INTERRENAL ORGAN UNDER NORMAL AND EXPERIMENTAL CONDITIONS.

A contribution to the understanding of the function of the corticotropic hormone and of the relation of the nucleus to the plasma.

by

PAUL DITTUS .

HISTOLOGY AND CYTOLOGY OF THE SELACHIAN INTERRENAL ORGAN UNDER NORMAL AND EXPERIMENTAL CONDITIONS

A contribution to the understanding of the function of the corticotropic hormone and of the relation of the nucleus to the plasma.[1,2]

by PAUL DITTUS.

(From the Zoological Institute of the University of Tübingen and the Zoological Station at Naples.)

With 36 illustrations accompanying the text.

(Received on 23 June, 1940.)

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3 Translator's note: All page numbers refer to the page numbering in the original text.

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I. INTRODUCTION p. 41

Hand in hand with the experiments on selachians, started in 1936, which dealt with the physiological effects of the corticotropic hormone[1] of the hypophysis, further experiments were initiated on the same group

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1 corticotropic hormone: c.h. (kortikotropes Hormon: k.H.)

of animals for the purpose of clarifying the cytodynamic effects of the c.h. on the interrenal tissue. So as to have a reliable basis of comparison for the microscopic pictures obtained after the interrenal organs[2] had been affected by experiments, it was first necessary to examine closely the histology and cytology of the selachian i.o., possibly throughout the course of the entire lifetime. In these studies special consideration had to be given also to the varying conditions of the sexual cycle, since there are, of course, as has been proven by many experiments, exceedingly close relations between the i.o. (adrenal cortex) and the germ glands. In order to understand the problem of the cytological influence on the interrenal cells through the c.h. of the hypophysis, various methods were tried.

1. By total extirpation of the hypophysis in the experimental animals the c.h. was removed entirely from the body; the microscopic investigation had to reflect the action of the i.o. having no stimulation whatsoever through c.h. (Futhermore, in this method, other indirect effects of hypophysis- action were also eliminated, such as e.g. the indirect stimulation of the i.o. by the chain thyreotropic hormone → thyroid gland → i.o. or gonatropic* hormone → gonads → i.o.).

2. By injection of sufficient quantities of adrenal cortex-substance ("Cortidyn") the production of incretion by the i.o. of the

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2 interrenal organ: i.o. (Interrenalorgan: I.O.)

* Translator's note: "gonatropes" and not "gonadotropes" hormone in German text.

Zeitschrift f. wissensch. Zoologie. 154. Bd. (Journal of scientific zoology - 154th Vol.).

animals was to be made unnecessary, or was to be reduced, respectively. This process is rather to be formulated in the following way, according to more recent concepts: It is well known that the decrease in hormone production of any gland depending on the hypophysis results in increased production of the corresponding glandotropic** hormone of the hypophysis and vice-versa (Jores 1937, further literature ibid.; Dittus 1939; Zawadovsky and Vorobiew 1939). The inundation through artificially-added adrenal cortex hormone consequently had to result in restraining the production of the c.h. of the hypophysis. The decrease in the quantity of the c.h. in the blood channel causes, on its part, a decline in the i.o. activity. Thus, as under 1. the microscopic picture should show a decline in the intensity of i.o functioning. p. 42

3. Through the removal of a more or less large part of the i.o. the interrenal-tissue-part remaining in the body should be forced to assume the secretion activity of the removed part in addition to its own, or in other words: the c.h. produced by the hypophysis, which had attacked the entire i.o. until now, would concentrate henceforth on the remaining part, in such a way that a higher dose of c.h. is now affecting the individual interrenal cell than had been the case prior to the partial extirpation with regard to the entire organ. As has been stressed already above, however, the decline in hormone production in one of the glands depending on the hypophysis causes an over-production of the corresponding glandotropic hormone of the hypophysis. Since after the partial extirpation initially a shortage of interrenal active substance certainly

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** Translator's note: ".. glandotropen Hormons" in German text.

occurs, it may be assumed that the production of c.h. will exceed the normal level. Due to both these facts, a strong stimulation of the i.o. through the c.h. must take place which will supposedly also be reflected in the microscopic picture.

4. The actual experiments to test stimulation of the i.o. through pure c.h. added artificially were first carried out on normal selachians. In these experiments doses of varying strengths were given at different time-intervals. It could also be assumed here that the artificially-added stimulant would produce obvious effects in its sense organ.

As has been stated above under 2. and 3. in the Introduction, the increased production of the interrenal substance, caused by the injected c.h. in this case, represses the outflow of c.h. from its own hypophysis; thus the hypophysis assumes a regulating function. However, the doses of c.h. added were kept so high in the case of these experimental animals, that they had to be considered as lying above the regulatory range of the hypophysis.

5. So as to exclude this regulating action of the hypophysis entirely, further experiments concerning injection of c.h. were carried out on hypophysectomized selachians. Under this mode of experimental procedure it was possible to observe unmistakably the effects of c.h. At the same time the effect of the artificially-added c.h. also had to become most clearly obvious here, since an i.o. which had not been subjected to any stimulation by c.h. after hypophysectomy, was suddenly stimulated by c.h. supplied from the outside while no regulating action could manifest itself due to the absence of the hypophysis.

For various reasons the selachians are especially well suited for such experiments. First, the histological structure of the i.o. is p. 43 simple, not being arranged in zones in any way whatsoever, such as e.g. the adrenal cortex of the mammal, and this is very favourable for carrying out the planned tests. In addition to this quality suitable for these experiments - a characteristic which the anamnia actually also possess - various types of the selachians also have, in contrast with the anamnia, a quite well enclosed i.o which facilitates considerably the examination of the latter under the microscope; moreover, an additional advantage is due to the fact that among all the vertebrate, solely in the case of the selachians, parts of the adrenal system are normally not attached to the interrenal cells or even surrounded by the latter. Due to the relatively compact development of the i.o. and the lack of adrenal cells on it, there is also the possibility of appraising quantitatively, and executing technically in a satisfactory manner, the partial extirpation of the interrenal tissue, mentioned in the Introduction. It is also possible to remove entirely the hypophysis, with certainty and without any great difficulties, from these usually rather tough animals.

II. TOPOGRAPHY OF THE SELACHIAN INTERRENAL ORGAN.

For the purpose of understanding better what follows, the topography of the selachian interrenal organ and the history of its development are to be dealt with shortly here. As is known, in the case of the selachians, the i.o., the homologue of the mammalian adrenal cortex, is normally separated completely, space-wise, from the adrenal

system (suprarenal body), the homologue of the adrenal medulla of the mammals. These conditions, which differ fundamentally from the findings obtained in the case of mammals, have made it exceedingly difficult to recognize homologies. Experiments conducted by Leydig (1851, 1852, 1853) were not yet able to clarify this matter. Only Balfour (1881) recognized conditions of homologies correctly and defined them clearly. These findings have since been confirmed continually by biogenetic, histological and physiological experiments.

The topography of the selachian i.o. has been mainly described by Diamare (1896, 1899, 1903), Vincent (1897), and Giacomini (1898). The definite position and development of the organ varies with the individual representative. Moreover, some species show more or less pronounced individual differences. But for the species tested by me, three types can easily be established, which will be designated in the following text as the Scyllium-, the Raja- and the Torpedo- type.

In the scyllium- type (1 in Illustration 1), the interrenal system p. 44
is developed in the form of an azyguous dorsal strand located between the two Wolffian bodies, which is about a third or half of the length of the kidney. It begins relatively sturdy almost at the height of the caudal ends of the kidneys, and narrows more and more in its cranial stretch. Cranially to the connected strand, also dorsally between the two kidneys, some smaller interrenal islands are mostly also located, which may vary in number and size from cone (?) to cone (?). The interrenal system becomes visible only after preparation of the kidneys.

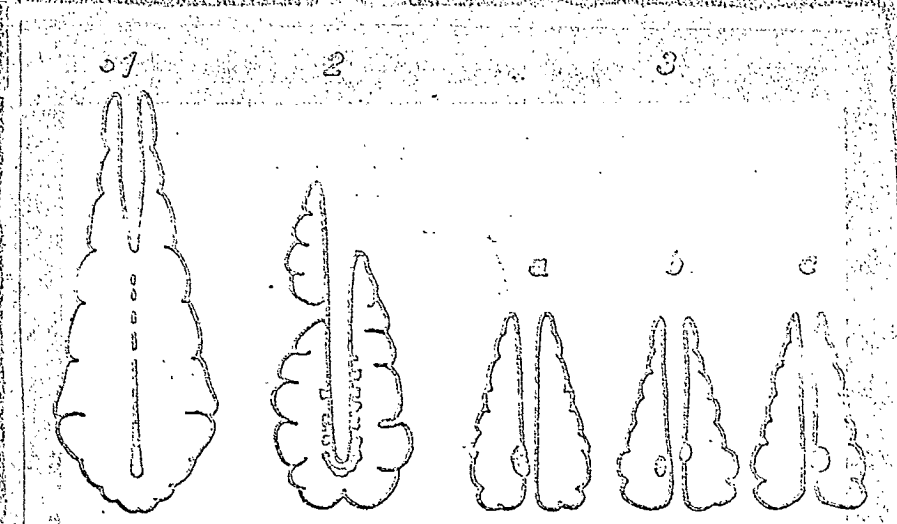

Abb. 1. Schematische Darstellung der Ausbildung des I.O. bei verschiedenen Selachiern. 1. *Scyllium*-Typus. 2. *Raja*-Typus. 3. *Torpedo*-Typus. a) Normale Ausbildung, b) und c) selten vorkommende Abweichungen (von dorsal). I.O. schwarz.

Illustration 1. Diagrammatic representation of the development of the i.o. in various selachians. 1. Scyllium- type. 2. Raja- type. 3. Torpedo- type. a) Normal development, b) and c) Deviations (from dorsal) occuring rarely. I.o. black.

The history of the development of the i.o. in sharks, which was comprehensively descrived mainly by Poll (1906; further literature ibid.) makes the position and development of the definite organ understandable; as it is of importance, also in connection with statements made below, the following is to be noted in this context: The first rudiments of the interrenal system in sharks manifest themselves as separate paired thickenings of the splanchno-pleura, in the forms of buttons or ridges, which push forward into the tissue of the root of the mesentery. They stretch from the end of the pronephros - area up to the cloaca, and they are most numerous in the region of the gonads while they are least numerous in the region shortly before the cloaca and behind the pronephros. Very soon after the appearance of the paired buds, the antimeres unite

at the median so that azygous cell disks are formed which lie closely ventrally at the aorta. The disks, lying one after another, now melt together also in a cranio-caudal direction, whereby an azygous strand is formed which is located in the root of the mesentery. Two different processes now lead finally to the definite development and position of the organ. First already very early a progressive retrogression of the interrenal parts located towards the head sets in up to the caudal end of the germ ridge. A small retrogression zone is probably also located in the most caudal part shortly before the cloaca (Hoffmann 1900, quoted according to Poll). Individual parts of the cranial zone do not undergo complete retrogression; as already mentioned above, they present themselves as interrenal cell islands located cranially towards* the connected part. Due to this fact the irregularities in the occurrence of these "Beizwischennieren" (additional intermediate kidneys) within one and the same species, and among different species, are easily understandable. The second development process consists in detaching the i.o. from the close vicinity of the mesonephros and the root of the mesentery. Both these processes lead to the above-mentioned definite development and position of the i.o. p. 45

The following species examined by me belong to the scyllium- type: Scyllium canicula, Sc. catulus, Mustelus laevis, M. vulgaris, Carcharias glaucus, Galeus canis and Chimaera monstrosa (Holocephali).

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* Translator's note: cranially towards (?). The author states: "kranial des" ? and uses similar constructions with caudal, dorsal, etc. ?

In the case of the raja- type (2 in Illustration 1) larger and smaller interrenal islands are attached on each side to the caudal halves of the kidneys, from dorsally. They are usually found near the medial edges of the kidneys; they are more or less irregularly distrubuted along the cranio-caudal direction. In the caudal region larger bodies are mostly found. In almost all cases a larger interrenal substance in the form of a "horseshoe" is developed at the caudal end. Normally, the interrenal islands located on the right kidney stretch farther towards cranial than those located on the left kidney do, which probably has a connection with the asymmetry of the Wolffian body typical of the kidney of the raja, which is also represented in Illustration 1, and has been described already by Leydig (1852), Howes (1890) and Bargmann (1937).

Unfortunately, almost nothing is known about the development of the interrenal system of the raja. However, in their case, in contrast with the sharks, the melting of the paired initial rudiments at the median would appear to occur only at the caudal end, while the remaining ones continue to exists as antimeres. Moreover, the tendency towards a more extensive melting together of the rudiments in a cranio-caudal direction would not appear to be very great, and would seem to diminish, moreover, considerably towards the head. The development and position of the organ in this type naturally varies widely among the various species and individuals. However, the type is sufficiently well characterized: 1. by the interrenal cell aggregations of various sizes, in a chain running parallel to the medial edges of the kidneys, and developed on each side, and 2. by the conglomeration of interrenal tissue appearing

in the caudal part, through which the left and the right sides are usually connected as by a bridge.

This type is found in the Raja clavata, R. asterias, R. batis and Laeviraja oxyrhinus.

The torpedo- type shows the best-circumscribed i.o. Normally it p.46 is located in the caudal third of the left kidney near the median as a uniform, oval body (3 in Illustration 1 and Illustration 2). It is in situ, especially in the case of young animals, and can also be seen from ventrally, as can also be ascertained in Illustration 2, in the cross section. In the case of older animals, most probably due to the considerable growth of the kidney, the position is rather towards dorsal, so that it is often hard to see from ventrally.

In addition to this normal type, two additional forms of development may also be observed, to which reference had already been made by Kisch (1928). Two smaller interrenal bodies[1] may be developed, the larger one

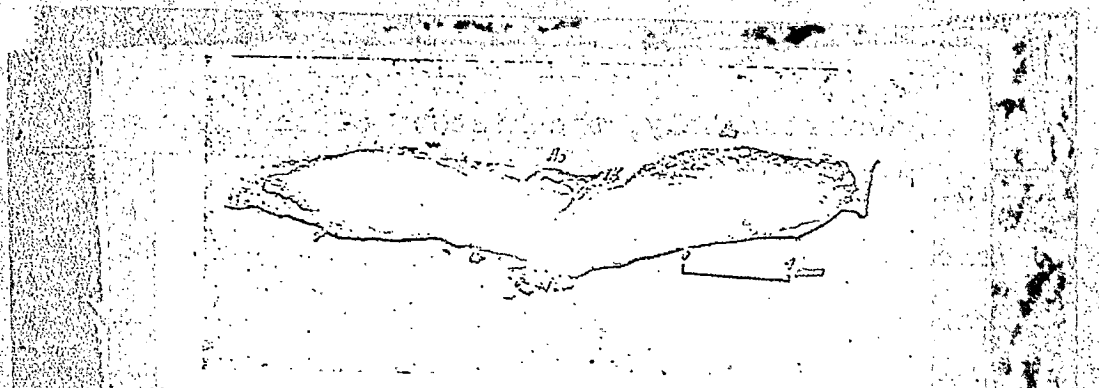

Abb. 2. *Torpedo ocellata* (Männchen kurz vor der Geburt, 20 g). Querschnitt durch die Urnieren auf der Höhe des I.O. (Mikrophotographie). Bouin, E.H. (= Eisenhämatoxylin), S.F. (= Säurefuchsin). *Ao* Aorta, *I.K.* Interrenalkörper, *M* Mesenterium, *P* Peritoneum, *Un* Urniere, *Ur* Ureter.

Illustration 2. Torpedo ocellata (male shortly before birth, 20 g). Cross section through the Wolffian bodies at the height of the i.o. (microphotography). Ao Aorta, I.K. interrenal body, M mesenterium, P peritoneum, Un Wolffian body, Ur ureter.

[1] Interrenal body - i.b. : (Interrenalkerper - I.K.)

of the two being located dorsally towards the left kidney, and the smaller one dorsally to the right kidney (the latter one always more oral and median) (3b in Illustration 1). Finally, a uniform i.b. may also be located dorsally to the right kidney (3c in Illustration 1). I could never find any scattered interrenal tissue in the animals tested by me. The following table will indicate the frequency of the three possible positions found in the animals tested by me; since in addition to the Torpedo marmorata and T. ocellata, the Trygon pastinaca and Tr. violacea also belong to this type, these two latter species are also included.

TABLE 1.

Animal species	Number of animals tested	1 i.b. dorsal to left kidney	2 i.b.	1 i.b. dorsal to right kidney
T.m.	47	43	2	2
T.o.	98	91	4	3
Trygon violacea	19	17	1	1
Trygon pastinaca	8	7	–	1

Unfortunately, the history of the development of the i.o. in torpedoes has practically not been studied as yet (except for a finding reported by Aichel, 1900, which is probably wrong); it would be interesting to examine closely the processes which lead to this type so considerably different from the developmental forms described above.

III. MATERIAL AND METHOD.

The material was collected, and the experiments were carried out at the Zoological Station at Naples in 1936 and 1938, in the course of

a two-month-stay, in each case; the material collected was treated and evaluated at the Zoological Institute in Tübingen. Since the series of experiments described in the Introduction could be carried out best on Torpedo ocellata and T. marmorata[1], and as sufficient quantities of these animals were available, mainly these two species were used for the experiments. As has been indicated in Chapter II, T.o. and T.m. usually have a uniform i.b., which is not difficult to extirpate partially because of its favourable position and development. Moreover, there is no accessory interrenal tissue in these two species so that there can be no falsification of results from this aspect. The procedure followed for carrying out the partial extirpation of the i.b. was the same as for the one described earlier regarding the total extirpation (Dittus, 1937), with the exception of the fact, however, that only a part of the i.b. was removed at the cranial end, while the other part was left to stay in its position. This method (removal of the cranial part), which I used in preliminary experiments in 1936, was also used later, since the microscopic examination of those parts of the organ which had remained in the body for some time revealed that by this mode of extirpation no damage had occurred in the organ which might have been caused by a disturbance in the blood supply. Moreover, the homogeneity of the material was thereby guaranteed. The wound surface on the i.o., created by the partial extirpation, was well dabbed with cotton wool tampons which had been moistened with sterile sea water. When the animals were opened later, it appeared that extensive bleedings never occurred when this method was used. These animals, which are somewhat lazy for about

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[1] Torpedo ocellata and T. marmorata will be abbreviated by T.o. and T.m. in the following text.

two days after the operation, later become very lively again and connot be distinguished in any way by their behaviour from normal animals, provided that not more than about half of the i.o. had been removed. They live almost as long as pseudo-operated animals do. The explanations for both these findings are given in Chapter IV, 1. The hypophysectomy on the experimental animals was carried out according to the manner described by Dittus (1939). In addition to those on the torpedoes, hypophysectomies were also carried out on Scyllium canicula, Sc. catulus, Mustelus laevis and on Raja asterias, and these species as well as the torpedoes were also subjected to injection tests with c.h. The corticotropic hormone used for the injection tests was always given intraperitoneally. It came from the Promonta company (Hamburg), and I should not like to neglect to express my gratitude to the Promonta company and Dr. Jores, at this point. About the hormone itself a detailed report has been made earlier (Dittus, 1937, 1939); it proved to be very pure after various tests. All the experimental animals were kept at an always constant supply of fresh water in the large glass basins at the Zoological Station at Naples; throughout both my stays there, the water temperature varied between 20 and 24°. As all the animals, prior to being used for any experiment, were observed for 2 days, it was possible to exclude injured animals from the experiments. p. 48

For the purpose of examining the i.o. under the microscope, so as to eliminate post-mortal changes, only the organs of freshly killed animals were used, with the exception of some species which are hard to obtain and which are usually dead when brought to the Station. This, however, will be noted where it applies. For the purpose of preserving

the i.o., the animals were killed by destroying the medulla, and the organ was fixed immediately - usually in "Susa"*. In addition, Bouin, Zenker's fluid and Helly's fluid were also used for control purposes. The duration of the fixation was 24 hours. It was embedded over methyl-benzoate-celliodin in paraffine, from the melting point 56-58°. The width of the cut normally amounted to 6-8 μ. The following materials were used for dyeing: iron-hematoxylin and "Azan"* according to Heidenhain, the hematoxylins according to Ehrlich and Delafield and the Ehrlich-Biondi - triacid dyeing, in accordance with the modification by Krause. As contrast colouring the following were used: acid fuchsin, eosin and chromotrop IIR. For the purpose of distinguishing thymo-nucleic-acid and nucleolar substance, the reaction according to Feulgen was used, with subsequent light green colouring. In order to obtain good comparisons, the colouring was always carried out in the same way.

For the purpose of representing the lipids in the i.o., the fixation was made with the admixture according to Flemming. After cutting on the freezing microtome, the sections were enclosed in glycerin, not colored, or after dyeing the nucleus with carmalum. Moreover, small pieces were embedded quickly upon cedar-wood oil in paraffin; the paraffin was separated in chloroform, and the sections treated as above. The lipids stay well preserved in this method. "Panphot"* of the Leitzworks was used for taking the micro-photographs.

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* These expressions, placed under quotation marks, may be trade names? (Translator's note).

The i.o. of all the species of the selachians mentioned in Chapter II were examined. The number of animals examined will be given in each chapter concerned. For the purpose of maintaining the uniformity of the presentation, the histology and cytology of the i.o. of normal torpedoes treated will be described first, and only at the end findings concerning the i.o. of other selachians will be presented.

IV. THE INTERRENAL ORGANS OF THE TORPEDOES UNDER NORMAL CONDITIONS.

1. Generalities.

To the earlier histological investigations on the selachian i.o., which had been conducted mainly by Diamare (1896), Vincent (1897), and Giacomini (1898), only the following publications have been added in more recent time: Fraser (1930) on Raja clavata, Fancello (1937) on Scyllium canicula and catulus, and Pitotti (1938) on T.o., T.m. and Trygon violacea. It became obvious, however, that these investigations were not sufficient for providing the foundation for the questions raised in the Introduction. Moreover, it was necessary to have an exact knowledge of the histology and cytology of the normal i.o. as such before an attempt could be made to interpret the results of the experiments. It should be noted here that in the case of this organ, which is homogeneous and parenchymatous in its structure, only the closest study of a large amount of a large amount of material will afford an opportunity to find sharp criteria for the degree of functioning of this incretionary gland, while this is much less difficult in the case of the thyroid gland with its clear histological differentiations.

Illustration not clear enough from photocopy to print.

Illustration 3. The same animal as in Illustration 2. I.o. cross. Bouin, E.H., S.F. * Bh Connective-tissue-cover, Bl Connective-tissue-lamella, S Blood sinus.

It became obvious in the course of the investigations that the species T.o. and T.m. show no differences with regard to the histological and cytological conditions of the i.o., as has also been reported by Pitotti (1938), and for this reason both these species will be dealt with together. The rough histological structure of the i.b. remains the same, essentially, throughout the entire lifetime, in the case of the torpedoes. Therefore, the general findings are to be described here. For this purpose, I refer to Illustration 3. The i.b., which normally is lying dorsally to the left kedney, is surrounded by a firm connective-tissue-cover (Bh), which is more delicate in young animals, and becomes firmer in older ones, p. 50 in accordance with the growth of the gland that has occurred. It evenly surrounds the entire organ. The parenchyma of the gland is composed of individual lobes, columns or pillars, which harbour a varying number of

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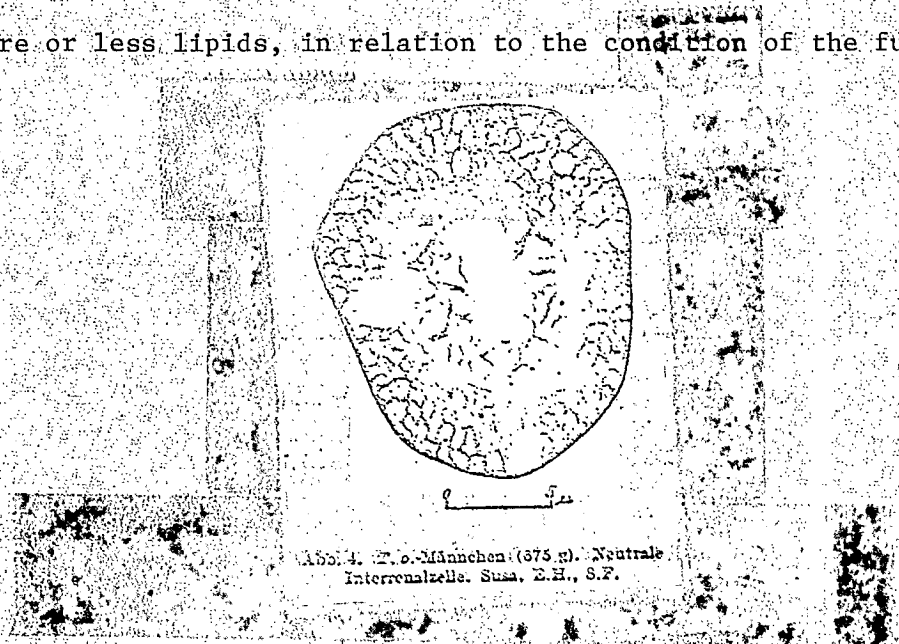
* Translator's note: E.H. (Eisenhamatoxylin) - iron hematoxylin, S.F. (Saurefuchsin) = acid fuchsine. (See Ill. 2).

interrenal cells. The individual lobes are surrounded by fine connective-tissue-lamellas (Bl). Between the lobes are many capillaries, which are widening to larger sinuses (S) at times (especially at certain functional stages). The connective tissue surrounding the lobes is exceedingly delicate on the sinus so that one might often gain the impression that the interrenal cells are separated from the vessel lumen only by the endothelium. But in reality, in addition to endothelium, connective tissue can always be identified also. The little lobes are closely connected unless vessels penetrate in between. In a well fixed material fissures never occur; if they do exist, as may be the case in postmortal preserved material, they are to be explained as artifacts.

The neutral interrenal cell itself has a polygonal shape (Illustration 4), and has a round or oval nucleus, which shows a clear chromatin frame, and always contains one to several nucleoles. The plasma is light and foamy, inasmuch as it came in contact with agents not preserving fats, as the lipids which are separated, leave small vacuoles. According to the methods for representing lipids, the interrenal cells prove to contain more or less lipids, in relation to the condition of the function



Ill. 4. T.o. male (675g). Neutral interrenal cell. Susa, E.H., S.F.*

*Translator's Note: E.H. - iron hematoxylin, S.F.- acid fuchsine (see Ill. 2)

(compare Chapter VI). The cell borders in neutral interrenal cells are always recognizable in case of good colouring. When sections of the most varying i.o. are observed, this structure is always present, in principle, although the total impression given by individual organs may vary considerably as will be shown in the following text. This phenomenon is often caused by minor deviations in the development and arrangement of the interrenal cells, the connective tissue and the vascular system, which all together may give the organ an entirely different appearance. It is to be attempted in the following chapters to describe p. 51 in detail these peculiarities concerning various conditions of the i.o., and to fit them into the general pattern, as just presented above, in such a way that the changes, which the appearance of the organ undergoes in the course of the various phases of its function, is reflected as truly to nature as possible. In the interest of a clear illustration of the processes, special emphasis was placed on appropriate pictures; microphotography was used to a large extent so as to afford an objective picture and the possibility of comparing the different conditions of functions; in the case of fine cytological details, it was, of course, impossible to omit drawings.

The final conclusions, which the microscopic pictures of the i.o. in various functional stages permit to draw, will be dealt with in detail in Chapter VIII.

2. The interrenal organs of torpedo-embryos shortly before birth.

So as to obtain material from embryos shortly before birth, a pregnant T.o. as well as a pregnant T.m. were killed, after the strong

movements of the embryos had already been recognizable from outside. The T.m.- female weighed 875g, and produced 4 male and 2 female embryos, 12-18g each, the i.o. of which were fixed immediately. The T.o.- female weighed 430g and had 3 male embryos of 20, 21, and 22g. The i.o. of one animal among these was fixed immediately. The two other animals were kept in sea water for a day and used for injection experiments; the one animal being injected with sterile sea water, and the other with c.h. Breathing frequency curves had been presented earlier for these animals (Dittus, 1937). Each of the animals (T.o. and T.m.) also had a yolk sac of about the size of a pea; they showed strong movements and gave already quite perceptible electric shocks. For these reasons it could be assumed that the time of the birth was approaching.

The i.o. examined in these animals all presented the same appearance with the exception of small individual variations. In observing the i.o., under slight enlargement, the following was noted (Illustrations 3, 22a and 23a): The strands, or little lobes of the i.o., are rich in cells and are separated from each other by obvious connective tissue lamellas. Capillaries and larger sinuses are present in abundance, and emphasize the lobated structure still more clearly. The plasma of the interrenal cells is relatively light; the nucleuses are stained with the usual nuclear stains normally, and partly also heavily. p. 52

If the i.o of these animals are observed, considerably enlarged, the multiplicity of the forms of the nucleuses are astounding. In making a careful study and subsequently classifying the pictures logically, three essentially different processes are observed which are

to be described in the following text. The accuracy of the classification and interpretation of the pictures is to be fully proved by the facts to be presented later concerning the i.o. under the influence of experiments. These pictures show that these organs, artificially stimulated, produce secretion so frequently, in such a convincing way that any misinterpretation would appear to be excluded. The neutral interrenal cell, as described in Chapter IV, 1, and represented in Illustration 4, may serve as a starting point.

The first process to be ascertained is that of mitosis (Illustration 23a). Mitoses are found very frequently; it may be concluded, therefrom, that a considerable growth is occurring through multiplication of cells, in these animals shortly before birth.

In addition to this first process, which results in a considerable multiplication of cells, very often a second process can be observed, which primarily results in an enlargement of the nuclear surface, namely the amitosis, or often also a multiple nuclear cut-off*. The frequency with which cells having two or more nucleuses were observed - it was apparent that in these animals about 10% of all cells had two or more nucleuses - would lead to the assumption that direct nuclear division is not immediately followed by a division of the plasma. So as to establish the above finding with accuracy, the material was coloured

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* Translator's note: "Kernzerschnurung" - cutting off (tying up entirely) of the nucleus? - nuclear division?

with picro-blue-black, a staining solution which makes an excellent representation of the cell borders possible.

The amitoses occur mostly without any essential changes of the shape of the nucleus by a circular ring-shaped groove which deepens until the separating membrane is formed. This form of division (Dissection according to v. Wasielewski, 1903) is represented in Illustration 5a. Due to the more lumpy development of the chromatin framework, these nuclei mostly appear to be darker than the neutral interrenal cells are. Besides this mode, which is observed most frequently, another one is also found where a nucleus first stretches, takes the shape of a dumb-bell and hour-glass, and finally divides (Severance); Illustration 23a, below, on the right, shows such a stage (bA). Finally, nuclei can also be seen which indicate an amitotic division as they are tied up unilaterally, so that the nucleuses at first look very much like kidneys, in the way described by Maximow (1908) with regard to mensenchymal cells from rabbit embryos (Illustration 5b). It was necessary to deal in greater detail with the p. 53 forms of amitoses occurring in the i.o., in order to be able to distinguish these deformations of the nucleus from those looking somewhat similar which will be described later. Illustration 5c shows a double cut-off as is often seen in the most varied forms. Regularly the nucleolar substance is about ~~even~~ evenly distrubuted in the case of the amitoses, even though the particles may differ in size, as is often the case. As we shall see later, this fact is of some importance.

As I have emphasized above, the number of the amitoses, or nuclear cut-offs, and the number of cells with tow or more nucleuses, are

relatively high. It is probable that some time after this amitomic

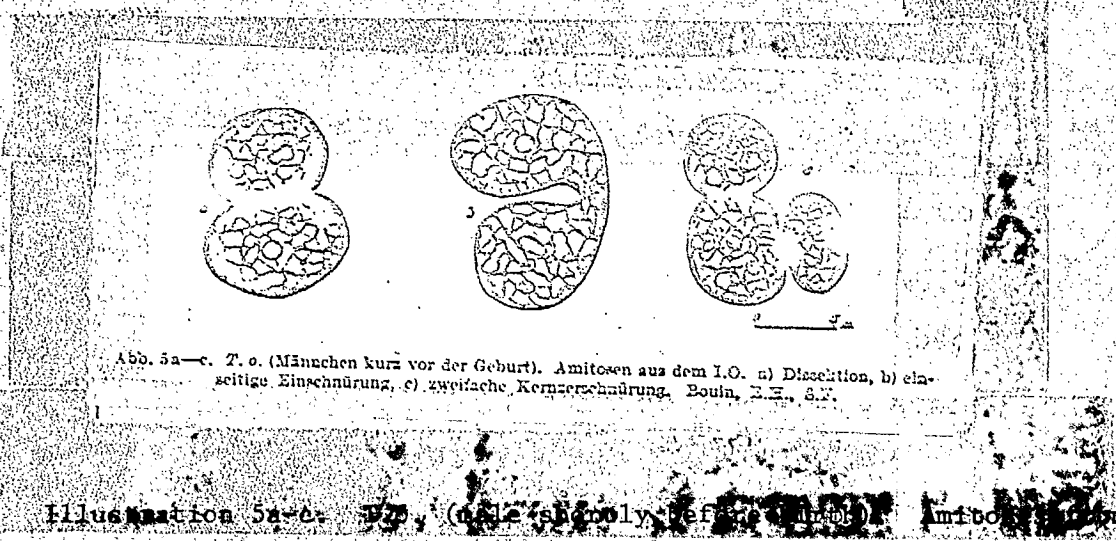

Abb. 5a—c. T. o. (Männchen kurz vor der Geburt). Amitosen aus dem I.O. a) Dissektion, b) einseitige Einschnürung, c) zweifache Kernzerschnürung. Bouin, E.H., S.F.

Illustration 5a-c. TT, (male shortly before birth). Amitoses from the i.o. a) Dissection, b) unilateral tying up, c) double nuclear cut-off. Bouin, E.H., S.F.*

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nuclear division a cell division will also follow, i.e. mononuclear cells will be formed, as this conclusion may be drawn from the decrease in the number of the cells with two or more nucleuses in later stages. Opinions with regard to the significance and the nature of amitosis are greatly divided according to the object under investigation and the researcher. (Compare G. Hertwig, 1929). It would appear to me that any explanation merely based on the rhythmical growth processes, and the amitoses of the nuclei caused thereby, would be inappropriate in this case. (Heidenhain, 1919; Jacobj, 1925). It might be more appropriate to explain this case with the concept of "reaction- amitosis" (Benninghoff, 1922), because, as will be shown again and again in the

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* Translator's Note: E.H. (iron hematoxylin), S.F. (acid fuchsine)

- see Illustration 2.

course of this investigation (compare also Chapter VIII), the enlargement of the nuclear surface and net the growth would appear to be of primary importance and effectiveness. This conception is further supported by the facts to be described now.

The third process: When studying the interrenal cells more extensively, pictures are encountered, which, after logical classification, may lead to the conclusion that processes are going on in the cells which may be described as follows: It may be observed that molded substances leave the nucleus to enter the plasma, which can be proved there - probably in a transformed state - and which may, with good reason be considered as an incretion, or the preliminary stage of an incretion of the i.o. This process may occur in various ways, and increases exceedingly in frequency in organs stimulated experimentally, and presents itself often in absolutely explosive forms. p. 54

Discussions have been held frequently about a morphologically detectable emission of nuclear substances into the plasma (compare G. Hertwig, 1929), and most scholars reject such findings, with the exception of some model samples (e.g. the spinning gland cells of lepidopterans, Maziarski, 1911, cement gland of Scalpellum, Krueger, 1926). For this reason, this subject will be dealt with only after these processes have been described. The various forms in which the above-mentioned process may occur are now to be defined.

1st form: When a nucleus enters its phase of activity, it is noted that its stainability increases and may by far surpass the normal level. It may happen that after staining with iron hematoxylin, in further differentiation, such nuclei still appear to be quite dark,

while the nuclei of the remaining tissue are already almost decolourized. In addition to an increase in the chromatin framework, a diffuse tinting of the nuclear fluid, and an increase in the nucleolar substance, which is often developed in the form of lumps, are causing this greater stainability, as a comparison of the results of the usual staining methods with the Feulgen nucleolar staining results shows. Thus, in addition to the increase of the nucleolar substance, an increase in the thymo-nucleic-acid is also noted. (Illustration 6a). In such nucleuses, one to four smaller or larger vesicles were often found, which always have a sharp boundary of basic staining and which, therefore, may not be interpreted as fixation artifacts. The contents of these nuclear vesicles normally become more or less slate-blue after staining with iron hematoxylin, up to faintly red with "Azan", and bluish with Delafield's hematoxylin. Occasionally, however, especially in the case of uriacid staining, an oxyphily also appears. It was not possible to determine any relation between oxyphily and basophily of these vesicles. According to the Feulgen-reaction the contents appear to be unstained, or at most, faintly pink, so that it may be concluded that no thymo-nucleic-acid is contained in the vesicles. Occasionally fine basophilic flakes or coagulum are found in them, also. The question as to the origin of these nuclear vesicles probably has to be answered in two ways. On the one hand, all transitions from genuine nucleoles to nucleal vesicles are found to occur in that way that each nucleole shows in its center a ball in a lighter colour, which continues to grow while the outer shell of the ball which is taking on a nucleolar-

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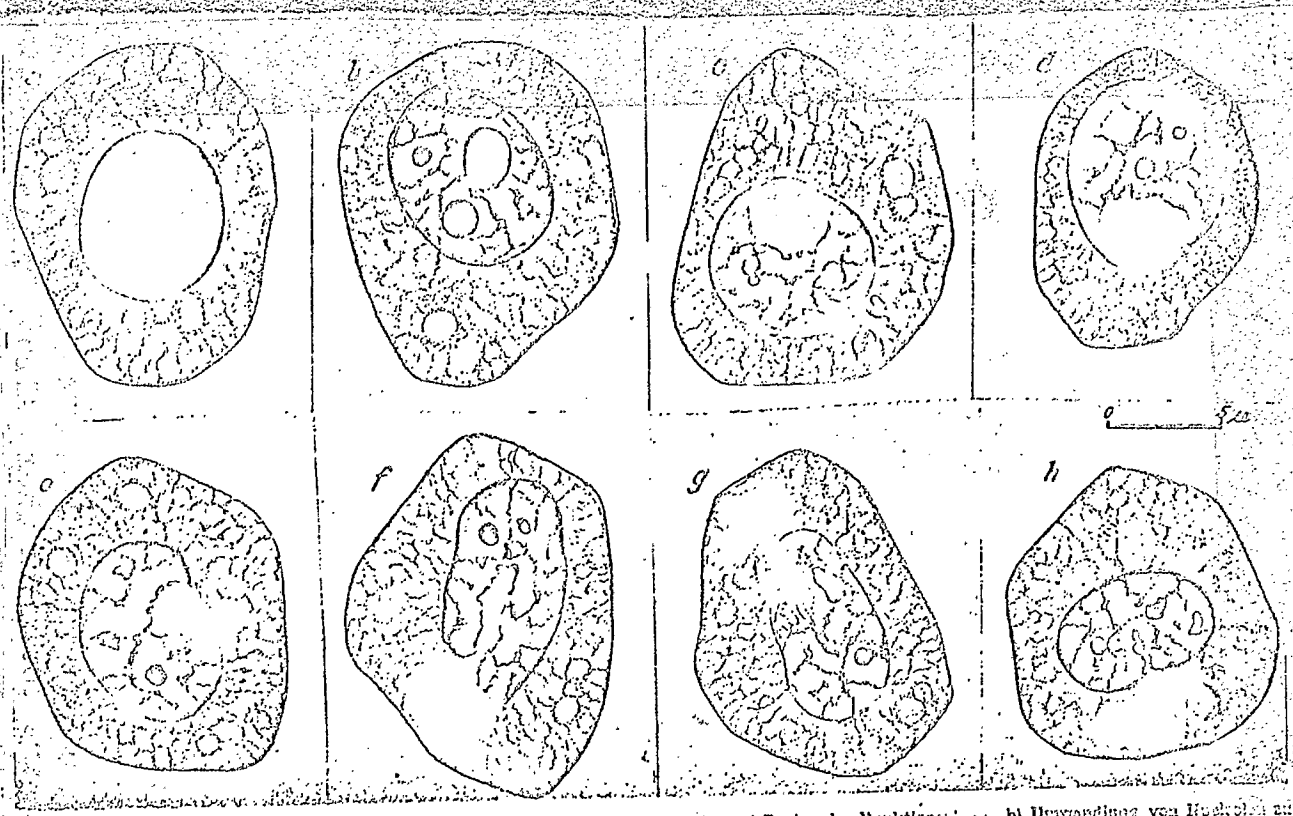

Abb. 6a—h. Tier und Technik wie in Abb. 5. Funktionsstadien von Interzellen. a) Beginn der Funktion. b) Umwandlung von Nucleolen in [illegible] Bläschen. c) Nucleäre Bläschen an der Kernmembran. d) Nucleäre Bläschen kurz vor der Entleerung ins Plasma. e) Erfolgte Entleerung der Bläschen; Kerndelle. f) Kern mit Kerneinbuchtung; Bildung von Granulationen. g) Verklebte Kerneinbuchtung. h) Kern-Narbe.

Illustration 6a-h. Animal and technique as in Illustration 5. Functional stages of interzonal cells.

a) Beginning of functional phase. b) Transformation of nucleoles into nuclear vesicles. c) Nuclear vesicle at the nuclear membrane. d) Nuclear vesicle shortly before discharging into the plasma. e) Discharging of vesicle occurred; nuclear dent. f) Nucleus with nuclear indentation. Formation of granu-

???????????? g) Agglucinated nuclear indentation. h) Nuclear "scar".

like colour becomes increasingly thinner until finally only a basic colour staining membrane is present, whereas the entire contents show the colour qualities described above. (Illustration 6b). At the same time the entire formation increases in volume. Moreover, the nucleolus becomes increasingly more liquefied due to the liquid ingredients absorbed from the nuclear room, as had been described already earlier by Berg (1932, 1934) concerning liver cells of vertebrates and Hett (1937) with regard to the epididymis cells of human beings, and granulosaluteincells of the hedge-hogs. On the other hand, often such tiny, completely unstained nucleal little vesicles are found that it can hardly be assumed that they might have developed from the mostly large nucleoles. It has to be assumed, therefore, that these vesicles can also develop directly in the nucleus. Occasionally it is noted how such vesicles melt together, and the original border membranes can often still be proved at the blending points.

When nuclei are observed in the Feulgen-preparation, which contain one or more vesicles, it is noted again and again that the thymo-nucleic-acid in the nucleus decreased, which is also revealed when these nuclei have been stained with the usual nuclear stains, in a less strong "chromatin network". This would seem to force us to assume that the "chromatin substance" transformed itself, was diffused into these vesticles and presented itself there as matter which had taken on a nucleolar-like character, a transformation process which will be demonstrated still more impressively later in connection with a somewhat different process. These vestices then wander towards the periphery of the nucleus and line the nucleus membrane inside, and may even make

it curve forward occasionally. In the case of such rounded-out nuclei it is apparent that the protruding part of the nuclear membrane is exceedingly delicate, whereas the remaining part is considerably thicker and stronger. (Illustration 6c). It is interesting that the wall of the vesticle is almost always covered by some little chromatin-or nucleolar-lumps (Feulgen), with the exception of the place where the vesicle-wall is attached to the nuclear membrane. (Illustration 6c-6h). As will be seen later, this adhesion-phenomenon very often facilitates imterpretation of some microscopic pictures. The nuclear membrane then dissolves at the surface of contact so that two lips are formed in the optical cut, which are located on each side of the vesicle. Only three times I was able to clearly observe this phase in the animals shortly before birth. This is due to the fact that 1) this stage probably lasts only a very short time, 2) only perfect profile pictures make such observations possible and 3) the phase-boundary vesicle contents cytoplasm is only noticeable in the case of tough colloidal vesicle contents, which are staining in an appropriately different way as against the plasma. Illustration 6d shows the stage just described. The pressing out of the vesicle contents into the cytoplasm by the high turgor of the nuclei always appearing to be tightly stretched leads to a relief of pressure, in the nucleus itself, the nucleus collapses somewhat, which is clearly expressed by the loss of the roundish shape. Either the nucleus shows, after discharging a smaller vesicle, a dent at first (Illustration 6a), or else it shows deep indentations having folds after larger vesicles had been discharged (Illustration 6f and 6g). These dents, or indentations in the nuclei which discharged such nucleal vesicles collapse

more and more, and finally completely close again, whereby the two agglutinated pieces of the nuclear membrane may still be ascertained for quite a long time, frequently. Such "nuclear scars" are often apparent. They become much more obvious by the stained small lumps originally sitting on the wall of the vesicle, as mentioned above. Illustrations 6g and 6h represent such nuclei. Moreover, such a stage (Kn) is also noticeable in microphotography (Illustration 22a). (Compare Berg, 1932).

The discharged vesicle contents are very hard to see in the plasma, right after having left the nucleus, since its stainability is similar to that of the plasma. After the effusion of more strongly-coloured vesicles, however, the plasma appears clearly darkly tinged immediately before the nuclear indentations described above (Illustration 6e). In cells where the emission process had occurred some longer period of time ago, which follows from the already agglutinated nuclear scars, the plasma shows fine granulations which stain darkly with iron hematoxylin (Illustration 6f to 6h). Since these granulations are always located in the cytoplasm, in close vicinity of these nuclear scars, and since they are also always present only after the elimination of the vesicles, one would seem to have to assume that the contents of the nucleolar vesicles, probably in conjunction with materials from the plasma, form these granulations. In the course of the text that follows, it will be demonstrated that it may be correct to uphold this conception.

For the sake of comprehensiveness, it may be added at this point that the pictures of the nucleuses obtained after the vesicles have been discharged cannot be confounded with the pictures of amitoses, when

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observed closely. This is obvious in the case of the amitoses through severance and dissection (circular tieing up of the nuclei). The possibility of confounding pictures of amitoses, which occur through unilateral indentation, as described in the text above (Illustration 5b), with nuclei which show nuclear indentations or nuclear scars due to discharged vesicles, may be conceivable. However, in contrast with the unilateral amitotic tying up of the nucleus, the nuclear intentations have manifold positions on the nucleus, and, in addition they are wrinkled in most cases, and curved. Moreover, as mentioned above several times, it is typical for the indentations formed by nuclear secretion that the indented membrane is covered with stainable particles. The "nuclear scars" are distinguished from the amitoses with unilateral indentation by the agglutination of the pieces of nuclear membrane, in addition to the accumulated little lumpy particles.

The entire process just described consists in the discharging of materials related to the nucleolar substance with the help of a vesicle, from the nucleus into the cytoplasm. This emission process has been described earlier in similar form by v. Volkmann (1923) concerning epiphyseal cells, by Berg (1932, 1934) on liver cells of various vertebrates and by Hett (1937) on granulosalutein cells of the hedgehog and human epididymis. This vesicle-mechanism, which has been appropriately compared by Berg with a system of locks, prevents any direct communication between the nucleus and the plasma which would have to lead to the destruction of the nucleus (Berg).

The following is still to be mentioned with regard to the frequency of the occurrence of the described stages, under observation, which may also permit to draw a onclusion concerning the chronological course of the process; very frequently nuclei with nucleoles or nuclear vesicles in transformation are seen which are still located in the centre of the nucleaus, or already at the nuclear membrane. With almost equal frequency, nuclei with scars, dents or indentations are found. Very rarely can stages shortly before or after the opening of the membrane be observed. (Illustration 6d and 6e). It may thus be concluded that the emission process takes a very short time while it takes much longer for the vesicles to develop and the nuclei to retogress.

2nd form: The second form, in which nucleolar material is discharged into the plasma, is in its first stages exactly the same as in the one described above, with the exception of the fact that an entire intact vesicle separates from the nucleus and can later be observed in the plasma. In the case of these animals shortly before birth, I could notice every now and then that such vesicles were located very close to the nucleus so that according to the picture one had to think of an emission of entire vesicles from the nucleus; however, these pictures did not seem to me to suffice for making an unambiguous definition. I encountered this phenomenon again only after the i.o. of such animals prior to birth had been stimulated experimentally, but so frequently and in such convincing manner, that it may be inferred that this form is also present in normal animals shortly before birth. It is true that this form is encountered very rarely in normal animals and for this reason this process is to be discussed in greater detail only in Chapter V,5.

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3rd Form: Closely beside the nuclei which are striking because of their great stainability, other nuclei are often located which appear light because of their lack of any obvious chromatin framework but which have, instead, numerous larger or smaller nucleoles (up to ten). (Illustration 7a). These darkly-stained nuclei and those containing the numerous nucleoles lie preferably at the capillaries or sinuses. It may be concluded from the relative positions and the observable transitional stages of both conditions of the nuclei that after an increase, originally, of the "chromatin" -substance (Feulgen), the latter, in a transformed state, is gradually used up in building up nucleoles, i.e. that the intensively stained nuclei represent a preliminary stage of the nuclei rich in nucleoles. It is striking that a large part of the nucleoles, which may have completely different shapes, are attached to the nuclear membrane from inside (Illustration 7a). In examining the preparations more closely, one often finds nucleoli shaped like dumb-bells with one part in the nucleus and the other one outside the nucleus in the cytoplasm; the nuclear membrane runs right through the middle of the connecting piece (Illustration 7b). Likewise round nucleoli are discovered, or else, also more irregularly shaped nucleolar fragments, one part of which is inside the nucleus while the other part already entered the plasma through the nuclear membrane. The question as to whether or not the nuclear membrane is dissolved, has to be definitely decided in the latter sense, according to my observations. Occasionally, when preparations are coloured in Delafield, where the nucleoles are not so intensively coloured, the nuclear membrane, which runs through the half emitted nucleolus, can be seen; still more conclusive are the pictures obtained after staining according to Feulgen as the violet-coloured nuclear membrane can be ascertained

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one hand, that the nuclear membrane becomes more penetrable at that place, and on the other hand, that the nucleolar substance perhaps becomes somewhat liquefied before the extrusion-point, and reassumes its former condition only after diffusing through, since according to various observations on living and fixed materials, the nucleoles may have a strongly gel-like condition. However, nothing can be proved by staining. After the extrusion of the nucleoles, they mostly remain lying outside on the nuclear membrane for some time, which may be concluded from the frequency of this stage (Illustrations 7c and 7d). Finally they shift more into the plasma (Illustrations 7c - 7e); their tinctorial behaviour can still not be distinguished from that of the nuclear nucleoles. Gradually they are dissolved: they then show more lightly coloured vacuoles in the centres, and corrosion manifestations at the edges (Illustration 7c). Simultaneously with this dissolution, areoles of granula, of larger or smaller sizes, are formed around them, which become intensively black with iron hemotoxylin, red with Azan, and blue when coloured with Delafield's hematoxylin. After the Feulgen- reaction they appear unstained, or at most tinted faintly pink, which may indicate their nucleolar-like nature (Illustrations 7d and 7e). Probably, in addition to the salt solution absorbed during liquefaction, in forming these granulations, materials from the cytoplasm are also added to the nucleolar substance (compare Chapters VI and VIII). These granulations now spread out in the cell plasma, and in the case of very active cells, occupy the entire plasma region, as will be shown later. But I should like to emphasize explicitly that, in the case of these animals before birth, mostly only smaller areas of plasma appear to be granulated (Illustration 7c-7e). The further behaviour of the nuclei and cells just described, and the evacuation of granulations into the blood channel will be dealt with in detail later, at an appropriate point (Chapter IV, 5).

The process of extrusion of entire nucleoles through the intact nuclear membrane has often formed a topic for discussions. Most authors reject it, because frequently particles lying outside the nucleus or which are attached to the nuclear membrane, tinging with nuclear stains, are considered as proof for the evacuation from the nucleus of morphologically demonstrable substances (compare Goldschmidt, 1904, 1909). G. Hertwig (1929) rightly indicates in his comprehensive presentation in the handbook on microscopic human anatomy that this conclusion is not justified. Heidennhain (1907) and Tischler (1921/22), to name only two representatives, most decidedly dispute any emission of "corpuscular elements" from the nucleus. G. Hertwig thus arrives at the proposition: "... it may certainly be stated that any emission of larger shaped particles from the nucleus is not frequent, if it occurs at all, and is limited to special cases (glandular nuclei, single nucleuses)". G. Hertwig considers the emission of shaped nuclear materials probable with a partly dissolved nuclear membrane (e.g.B. Maziarski (1910), Krueger (1926)) while the statement made by Koch (1928), according to which the extrusion of moulded nucleolar substance from the nucleus is supposed to occur while the nuclear membrane remains intact, "appears still somewhat doubtful" to him. Hett (1924) expressed the opinion, after having observed the pancreas of the mouse, that nucleoles do not go through the nuclear membrane, but that this phenomenon is rather a case of nucleoli translocated in the cutting process. He tries to prove this by the fact that the nucleoles "almost always" were located in the plasma on that side of the nucleus which was opposite the knife that entered.

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However, there are also those who claim with certainty that such

an extrusion of nucleoles from the nucleus does occur. I should like to forego listing all these authors here (partly quoted by Heberer, 1930, and Krueger, 1926), and to deal only with the cases of interest in this context. Harms (1921) described the emission of nucleolar substance from the nucleus, with regard to interrenal cells of amphibians and adrenal cortex cells of mammals. Dittus (1936) examined this process in greater detail on Ichthyophis glutinosus L. and, in principle, arrived at results which are equal to those just described. Also Koch (1928) observed on egg cells of spiders, and Heberer (1930, 1932) on germ cells of copepods the melting of nucleoles while leaving the nuclear membrane intact.

On the material examined by me (especially in the case of pregnant and experimentally stimulated interrenal cells) this process can be observed so clearly that its existence cannot be doubted, in my opinion. In this connection I also refer to the micro-photographs in Illustrations 13a-13f, which present several phases of this process in the i.o. of a pregnant animal. It is not possible, either, to establish any correlation, on my material, between the direction of the extrusion of the nucleoles with the incision (compare direction of the Hett, 1924) since frequently several nucleoles extruded simultaneously at various locations on the nuclei, although in most cases a certain preferred location is noticeable. As the re-examination revealed, however, there is no relation to the direction of the incision but it is obvious that the nucleoles were preferably emitted in the direction of the greater quantity of cytoplasm, so that, if one wishes to use the following expression, a certain secretion polarity existed. Besides, Groner,[1] most recently, also proved convincingly such migrations of

[1] Being printed: Z. wiss. Zool. (J. scientific zool.) 153, 310-372.

nucleoles in the sensory cells of the free sensory mounds of Phoxinus laevis, and illustrated by a micro-photogram that nucleoles may extrude simultaneously at the proximal and the distal ends of these nuclei.

In addition to these main forms in which nucleolar substance enters the plasma, other phenomena are observed in rare cases, which also aim at emitting nucleolar substance from the nucleus.

Two nuclei are shown in Illustration 8 which clearly indicate "Noses", in which larger amounts of nucleolar substance are located (Kn). As Illustration 9 shows, these Noses seem to have a tendency to become detached. It is striking how much nucleolar substance is contained in these formations. I was never able to prove later stages; even in the case of stimulated i.o., pictures which might have been related to this, were never clearly ascertainable. A similar process (Constriction of nuclear appendages loaded with nucleoles) was described by Eggert (1929) with regard to the egg of Salarias flavoumbrinus RUPP.

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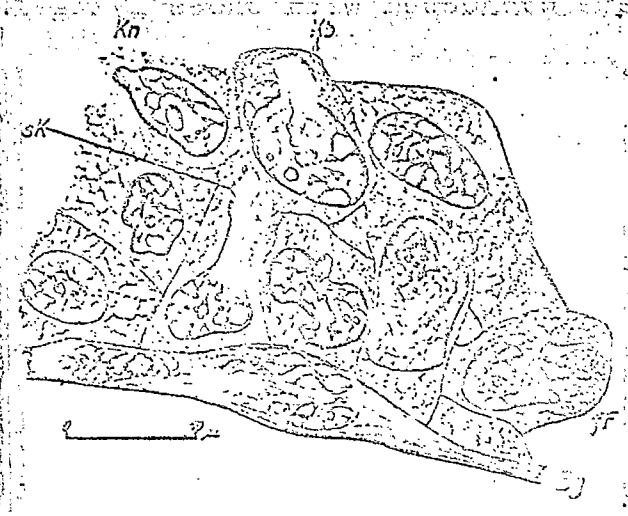

Abb. 8. Tier und Technik wie in Abb. 5. Bj Bindegewebe, Kb Kernhaut, Kn Kernmasse, sK strahlartig auswandernde Nucleolarsubstanz, f.F. fingerförmige Kernanhänge.

Illustration 8. Animal and technique as in Illustration 5. Bj Connective tissue, Kb nuclear membrane, Kn "nuclear nose", SK nucleolar substance extruding "like rays", f.F. fingerlike nuclear appendages.

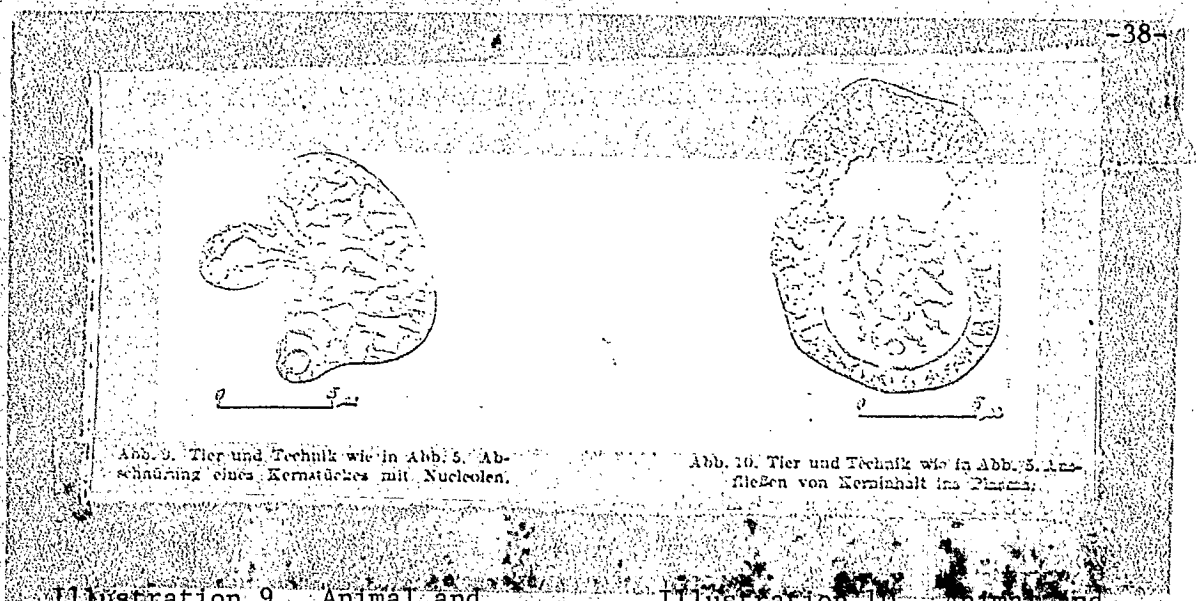

Abb. 9. Tier und Technik wie in Abb. 5. Abschnürung eines Kernstückes mit Nucleolen.

Abb. 10. Tier und Technik wie in Abb. 5. Ausfließen von Kerninhalt ins Plasma.

Illustration 9. Animal and technique as in Illustration 5. Constriction of a part of the nucleus with nucleoles.

Illustration 10. Animal and technique as in Illustration 5. Discharge of nuclear content into the plasma.

In Illustration 10 another process is illustrated, which can be observed in these animals only very rarely, though more frequently in the case of experimentally conditioned animals. At one (or more) places the nuclear membrane dissolves, and the nuclear content flows into the plasma. The same phenomenon, which ends with the degeneration of the nucleus, has also been observed in the i.o. of Ichthyophis (Dittus, 1936). In one case I also found a nucleus which emitted nucleolar substance "like rays" on one side, where the fragments with a distal location were connected with those lying in the nucleus through threads stained clearly with iron hematoxylin (Illustration 8, sK), a phenomenon which finds its parallel in stimulated interrenal organs, though in somewhat modified form (compare Illustration 28). p. 64

It is also striking that nuclei often have finger-like extensions (Illustration 8, f.F.) which might indicate that also in these places an emission of substance might occur though it is not morphologically understandable. These appendages are distinguished from the "nuclear

Noses" by their more pointed forms and by the fact that they possess less nucleolar substance.

All the processes described so far may be brought on one common denominator: in each case this is a question of emission of nucleolar substance into the plasma, which, together with material from the latter, builds up there the granulations staining basically, which represent an incretion of these cells as will be described later. All the T.o. - and T.m. embryos mentioned at the beginning of this Chapter show these processes. It is true that large amounts of granules cannot be ascertained anywhere in the organs, and this will still be described later with reference to other animals (compare perhaps with Illustrations 12, 14, 33a and 34b), but it is also obvious that the organs of these animals shortly before birth are in an active phase. This should be stressed particularly, as the signs of increased activity are not ascertainable in case of older animals (from about 35g upwards).

3ē The interrenal organs of the torpedoes shortly after birth.

In two cases it was possible to record dates accurately and to get animals shortly after birth. Once I was lucky as three T.o. animals were born in my aquarium. There were two males and one female animal; they weighed between 10 and 11g each, 3 days, and 4 days respectively, after birth, and each of them had a little yolk sac of about the size of a lentil. One animal was preserved 3 days after birth, while the two others were used for injections with c.h. (compare Chapter V, 3). ?????????fition, a male T.o., weighing 15g, also arrived, which also had a little yolk sac, and was therefore not older than a few days at the most.

What is striking about the i.o. of this animal, when slightly enlarged, is the impression that the entire organ looks much more compressed than it had in the case of this animal before birth. The little lobes are closely packed; larger sinuses cannot be proved. In some parts of the organ stronger bands of connective tissue run more or less parallel to each other through the organ (Illustration 29a). These bands of connective tissue were also found in the other two of the brothers and sister in the same litter, after injections with c.h. (compare Illustration 29b), and also in the T.m. male, so that it may be concluded that this phenomenon represents a characteristic feature of this stage. The nuclei of the little lobes which are flanked by such bands of connective tissue, are arranged with their longitudinal axes parallel to the latter. This arrangement in the i.o. is probably the result of growth processes which preceded, since the numbers of mitoses decreased considerably in these animals as compared to those before birth. In very rare cases such pictures are again found in still older animals also.

In case of considerable enlargement, very many darkly stained nuclei are noticed; and on the whole, nuclei generally stain more intensively. However, practically no where can nuclear vesicles or nuclei with nuclear indentations be observed. On the other hand, one finds all the more frequently nuclei with numerous nucleoles, migration stages of these, and cells with clearly granulated plasma areas, i.e. everywhere one sees stages of the process mentioned under the third form of incretion-development in Chapter IV, 2. The intensity of this process within the cell in the case of these animals exceeds that defined in the animals shortly before birth, without however reaching the extent which is characteristic for pregnant animals, for example (compare

Chapter IV, 5).

The peculiar fact that in the case of these animals shortly after birth the secretion mode of the nuclei with the help of vesicles practically disappeared, and that any nuclear secretion only occurs by extrusion of entire nucleoles through the intact nuclear membrane, is still to be discussed briefly here. I should like to anticipate that in all animals, from those shortly after birth up to adult ones, practically only the mode of nucleoles penetrating through the intact nuclear membrane is accomplished, and that, if nuclear vesicles occur at all in animals after birth, this occurs only in quite isolated cases. In contrast with this, nuclear secretion in animals shortly before birth, which I was able to test, occurs very frequently with the help of nuclear vesicles, in addition to the occurrence of migration of nucleoles. (Due to lack of material, I was unfortunately unable to test the i.o. of earlier embryonic stages). It would appear as if the formation p.66
and elimination of nuclear vesicles from the nucleus represented an "embryonal" secretion process, as it were, whereas the exclusive emission of entire nucleoles through the nuclear membrane would be typical for the post-embryonal time. As will be illustrated later, this finding may be confirmed on hand of the results in the case of i.o. of stimulated animals.

I.e. if animals shortly before birth are injected with the c.h. of the hypophysis, the nuclei of the stimulated i.o. respond with increased formation and elimination of nuclear vesicles (compare Chapter V, 5), whereas the i.o. of animals after birth, stimulated with c.h., show increased extrusions of nucleoles through the nuclear membrane

(compare Chapter V, 5). It may be noted, therefore, that in practice, also after stimulation, the i.o. react in the ways typical for them, and that thus, also in experimental animals, the "embryonal", and "post-embryonal" character of the nuclear secretion can be verified. These conditions will still be discussed in greater detail at an appropriate point.

The frequency of the mitoses decreased by about fifty percent, as compared to the animals before birth, according to calculations. Amitoses also decreased as compared to previously, though not to the same extent as the mitoses.

On the whole it may be said concerning the i.o. shortly after birth, that it appears to be relatively acitve, in the case of T.o. as well as T.m., which may be concluded from the amounts of granulations present in the interrenal cells. The i.o. in these animals would appear to be somewhat more active than those of the animals shortly before birth, which may probably be explained by the fact that in the case of these animals having become free through birth, an increased need for interrenal substance exists initially.

4. The interrenal organs of normal animals, weighing 35g or more, with resting gonads.

As has been pointed out already in the Introduction, and as will still be shown in Chapter IV, 5, the i.o. is in close correlation with the gonads. So as to be sure to get animals with "resting"gonads, the germ cells were always examined. In the case of young animals, not yet having reached sexual maturity, this condition is present at any rate;

in the case of the female animals, it was easily ascertainable macroscopically; in the case of the male animals, the testicles were cut for control purposes. 20 animals of the T.o. and T.m. species were examined.

The i.o. of such animals give rather monotonous pictures for animals weighing 35g up to the heaviest animals. In addition to the enlargement of the organ, the facts listed below are to be noted; Illustrations 11, 19a and 20 may serve as illustrations. The entire organ gives the impression of being very compact; the little lobes lie close to each other, and are no longer showing so decidedly roundish "lobated" formations, as was the case with animals before birth (compare Illustration 3 and 22a). Due to the arrangement of the individual little lobes, being located close to each other, they often indicate "multiangular" forms in the pictures of the intersections, which adapt themselves to the other little lobes. This may be especially due to the fact that the entire organ shelters less capillaries, and especially less sinuses than that was the case in animals before birth. This results in less "division" of the organ, and

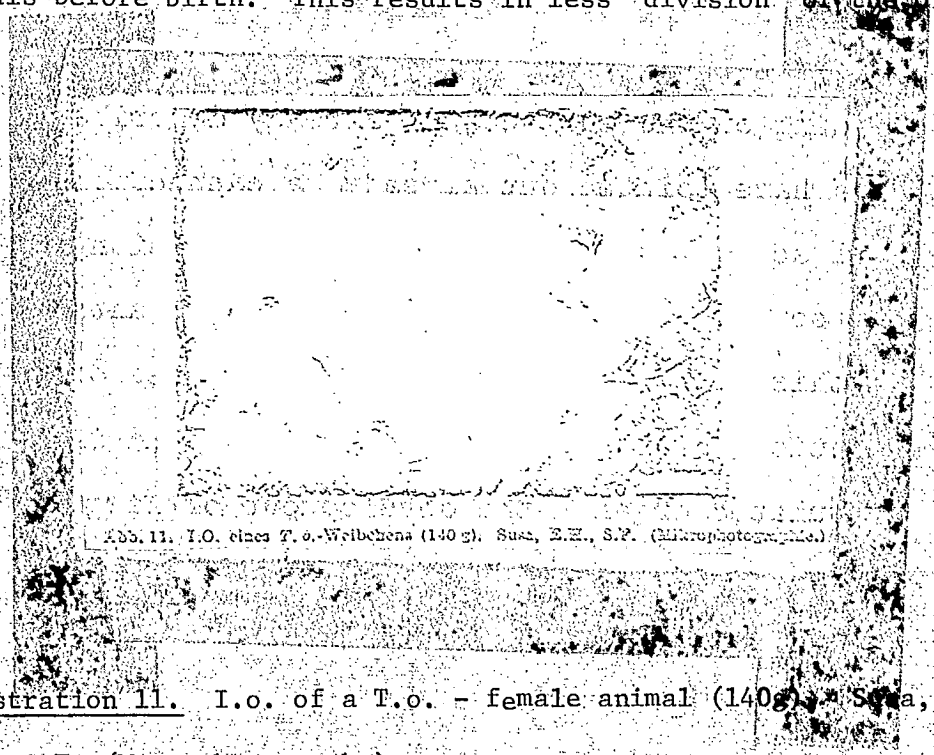

Illustration 11. I.o. of a T.o. - female animal (140g). Seiba, E.H., S.F. (Microphotography).

in preventing the lobes from protruding into the great lumina of the sinuses. Furthermore, the connective tissue lamellas which mark each little lobe off from the other ones, are developed very slightly.

The phenomenon just described is more pronounced in an older animal than in a still younger one (weight ranging approximately from 35 to 100g). Moreover, in the case of older animals, elongated lobes are often found immediately underneath the connective tissue capsule, parallel to its direction and tightly pressed against it. The longitudinal axes of the nuclei and cells also extend parallel to the outer boundaries of the organ. These narrow lobes are often marked off towards the inside by capillaries and smaller sinuses found lengthwise in crosscuts. Thus, especially in the case of an older animal, a sort of border zone is p. 68 formed, which stands out somewhat against the more irregular structure of the parts of the organ located farther inside. Frequently, the cells in this border zone also show a more pronounced granulation of the cytoplasm, so that this zone may appear very clearly at times. This increased activity in these cells, expressed in the granulations, is probably due to the more abundant capillary network, extending on the surface, as already described above, since, as I have pointed out already, a connection between the cell activity and the position in relation to a blood vessel can be established. (I should like to anticipate already at this point, however, that in the case of a very strongly functioning organ - during pregnancy and in an experimentally stimulated organ - the development of larger blood sinuses occurs mainly inside the organ).

The interrenal cell of this animal removed from any sexual activity shows a round to oval nucleus, which contains a normal chromatin framework and one to four nucleoles. The cytoplasm is slightly granulated and shows small light lipoid vacuoles in the case of a non-fat-preserving treatment (Illustration 4).

Occasionally cells with darker nuclei, or also more intensively granulated cells, mostly in the immediate vicinity of vessels, are observable. Furthermore, extrusions of nucleoles from the nucleus can always be noticed, even though not frequently. All the stages illustrated in Illustrations 7a - 7e are present, although not with such marked intensity. Thus, the microscopic pictures permit us to conclude that a slight incretion is constantly being developed in the organ. The displacement of nucleolar substance occurs only by extrusion of entire nucleoles; nucleolar vesicles are practically not at all observable.

Mitoses occur only very rarely. Stages of amitosis can only be seen in very small numbers, and the same applies to cells with two or more nuclei. Due to the fact that cells with two nuclei decrease in number, or almost disappear in older animals, as compared to the animals around their birth dates, it may be concluded that some time after the amitotic nuclear division, a complete division of the cytoplasm occurs. This fact is still to be dealt with in greater detail in connection with the discussion of the significance of amitosis in the selachian i.o. (see Chapter VIII).

Thus, this development of the i.o. is characteristic for normal T.o. - and T.m. animals not veing in the activity phases of the gonads. Slight variations may, of course,

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Thus, this development of the i.o. is characteristic for normal T.o.- and T.m.- animals not being in the activity phases of the gonads. Slight variations may, of course,

be noted from case to case, especially with regard to the number of capillaries and sinuses, and the frequency of deeply stained nuclei and the intensity of granulations of p.69 the cytoplasm; they are so small, however, that they remain within the framework of the picture presented above.

To summarize, it may be stated that the organ of this animal shows a very slight activity which remains steady, and which - as will be described in the next Chapter - is interrupted only by the phase of more intensive activity during the development of the sex products.

5. The interrenal organs of the torpedoes during the development of the germinal products and pregnancy.

The fact that a close correlation exists between the germ glands and the adrenal cortex, or i.o. respectively, has been proven by many studies, especially on mammals. But statements concerning increased activity of the i.o. during the maturing of the germinal products and pregnancy of the selachian were only made by Fancello (1937, Scyllium) and Pitotti (1938, Torpedo and Trygon).

At first I shall describe my own findings, which had mainly been ascertained in female animals, as the conditions of the gonads are easily recognizable macroscopically in their case. The first group, of which five animals were examined, contained female animals with ovarial eggs, 0.5cm in diameter at most. Of the 2nd group with ovarial eggs up to 3 cm in diameter, six animals were examined. Unfortunately I was unable to obtain female animals with small embryos in their uteri, during my stay at the Station. I was only able to again test the i.o. of animals having carried their young almost to full time. This refers to

the two female animals, mentioned above in Chapter IV,2, and the T.o.-female weigh-ing 430 g produced three embryos, 20-22g each, and the T.m.-female weighing 875g produced six embryos of 12-18g each (Group 3). Shortly after the act of delivery, only the mother animal, mentioned in Chapter IV,3 was available to me, which had produced three embryos of 10-11g each. Its i.o. was fixed 5 days after birth.

In examining animals in Group 1, i.e. animals with ovarial eggs up to 0.5 cm, it becomes obvious even under slight magnification, that well-circumscribed little lobes are developed, which are surrounded more fully by connective tissue than this is the case in normal animals. Between these compact cell groups, an extensive capillary network appears, which is partly widened into smaller and larger sinuses. Moreover, the nuclei of the interrenal cells all show a strikingly strong stainability. In case of strong magnification, it appears that the entire cell space, which partly also stains, is filled with intensively stainable particles. Moreover, larger fragments are partly seen, which may be classified as nucleolar substance, as proved by the Feulgen-reaction (Illustration 12a). A large part of these fragments lies already at the nuclear membrane. In addition, one finds all stages of passage of nucleolar substance through the nuclear membrane. The plasma itself already shows a more or less intensive granulation, which is much more intensive than it can ever be found in animals outside the sex phase. This very typical condition, indicating an increased activity of the i.o., could be ascertained more or less clearly in all specimens of this group.

p.70

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Abb. 12a—d. I.O. von *T. o.* und *T. m.* während der Ausbildung der Keimprodukte und der Schwangerschaft. a) *T. o.*-Weibchen (620 g) Eier bis 0,5 cm Durchmesser. b) *T. m.*-Weibchen (2000 g) Eier bis 3 cm Durchmesser. c) *T. m.*-Weibchen (910 g) Eier bis 3 cm Durchmesser. d) *T. m.*-Weibchen (875 g) kurz vor der Geburt von sechs Tieren mit je 12—18 g. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 12 a - d. I.o. of T.o. and T.m. during the development of germinal products and pregnancy. a) T.o.-female (620 g), eggs up to 0.5 cm in diameter. b) T.m.-female (2000 g), eggs up to 3 cm in diameter. c) T.m.-female (910 g), eggs up to 3 cm in diameter. d) T.m.-female (875 g) shortly before birth of six animals of 12 - 18 g each. Susa, E.H., S.F. (Microphotography).

In the 2nd Group, i.e. in the case of animals p.71 with eggs up to 3 cm in diameter, the development of compact little lobes, the increased occurrence of capillaries and sinuses in the i.o., were still much more clearly observable. (Illustration 12b). This phenomenon may often assume extreme

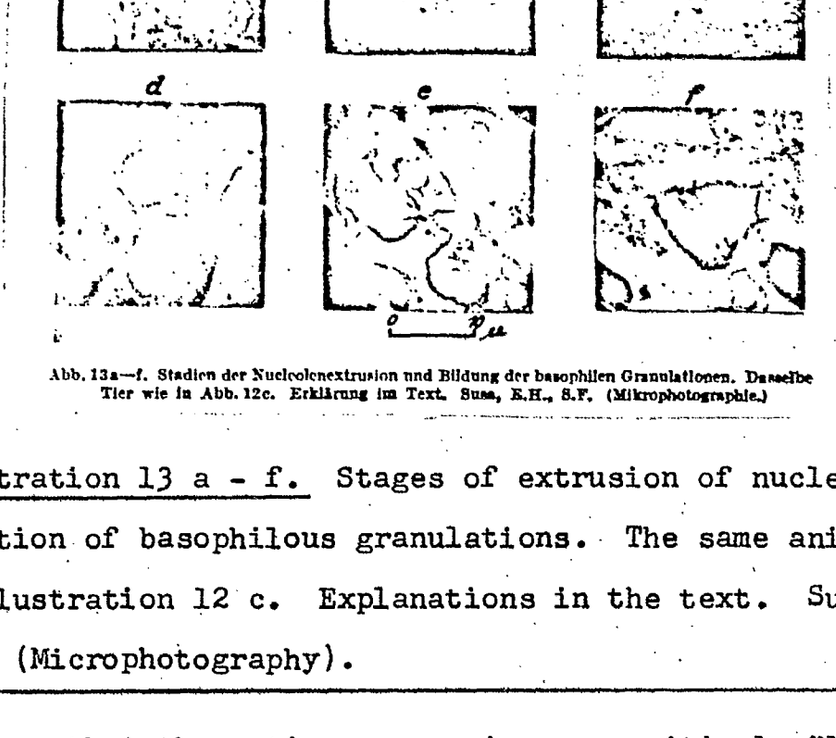

Abb. 13a—f. Stadien der Nucleolenextrusion und Bildung der basophilen Granulationen. Dasselbe Tier wie in Abb. 12c. Erklärung im Text. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 13 a - f. Stages of extrusion of nucleoles and formation of basophilous granulations. The same animal as in Illustration 12 c. Explanations in the text. Susa, E.H., S.F. (Microphotography).

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forms so that the entire organ gives a positively "loosened-up" impression (Illustration 12 c). The general stainability of the nuclei increased further. It is mainly based on the development of large nucleolar fragments, frequently lying on the nuclear periphery. The number of migrations of nucleoles increased further/as compared to the stages of Group 1, and it is obvious that partly rather large nucleolar fragments, which may often be more or less irregularly shaped, emigrate.

In view of the fact that the description of this phenomenon is usually viewed with great skepticism, as has

been pointed out before, we should like to refer to the microphotographies in Illustrations 13a-f, which record various stages of extrusion of nucleoles in the i.o. of such an animal. In Illustrations 13a and 13b, a nucleolus is shown as it "melts" through the nuclear membrane, whereas in 13c this process is almost terminated; in Illustration 13d the nucleolus is already located in the plasma, and in 13e and 13f it is obvious how the nucleolar substance is just dissolving in the plasma and is transformed into granulations. Unfortunately finer details of the dissolution of the nucleoles could not be shown in the photographic presentation. p.72

Due to the occurrence of granulations, the plasma of the interrenal cell gets a dark tone in case of staining with iron hematoxylin. (Illustration 12b and 12c).

Naturally, individual differences are obvious among the animals within this group, which can also be seen in Illustrations 12b and 12c. However, as compared to the animals in Group 1, all the animals have the following in common: a stronger development of the lobated structure, a firmer connective tissue surrounding the latter, more ample blood supply, increased stainability of the nuclei, an increase of the extrusions of nucleoles and an increment in the amount of granules in the plasma.

At this point I should like to refer briefly to the behaviour of the connective tissue in the i.o. of the torpedo. In case of the animals just described which show increased activity of the i.o. in connection with the formation of an egg, it is noted that the "little lobes" contain far fewer cells than are contained in those of animals without any increased

i.o. activity (compare Illustration 12 with Illustration 11). This fact is due to the inward proliferation of connective tissue lamellas whereby the lobes originally rich in cells are subdivided into smaller elements. In the case of a very active i.o. it can even be observed that fine fibers of connective tissue may grow into these little lobes so that individual cells are often surrounded by delicate lamellas. As this phenomenon only occurs in connection with an increased activity, as we have already seen, its interpretation is difficult.

Various reports have been made on the varying behaviour of the connective tissue within the three zones of the mammalian adrenal cortex. (Arnold: 1866; v. Brunn: 1872; Räuber: 1881, Gottschau: 1883, Flint: 1899, Comolli: 1908; Celestino da Costa: 1913; Graham: 1916; Plenk: 1927; Peniachetti: 1932; Zalesky: 1934).

In more recent times Bachmann (1937) noted in human adrenal glands that in the Zona glomerulosa and fasciculata entire cell groups are surrounded by argyrophile connective tissue, whereas it is obvious in the Zona reticularis that a single reticularis cell is surrounded in such a way. As compared to the conditions in the Corpus luteum, Bachmann concludes that the fact that individual reticularis cells p.73 are surrounded by argyrophilic connective tissue proves that the Zona reticularis is the stratum of cell disintegration, whereas the Zona fasciculata is the mainly functioning layer; this would provide a new proof for Gottschau's hypothesis. It has not been established whether the argentophilic connective tissue has any nutritive importance (Schiefferdecker, 1911) or any signficance for the lymph stream (Castaldi: 1919,

Volterra: 1925); the most likely interpretation may be to assume that the lattice fibers have a supporting function (Cesa-Bianchi: 1908, Levi: 1916; Luna: 1921). It may well be regarded as certain that the Zona reticularis is the zone of cell disintegration; but it is questionable whether or not it has a lesser importance as a "functional layer". On the basis of my present findings it would appear to me that the fact that the reticularis cells contain granules, which blacken with iron hematoxylin staining (e.g. Kolmer, 1918), again supports the interpretation that the discharging of a secretion (granulations) is mainly occurring in the Zona reticularis, as has been pointed out before (Dittus, 1936, p. 493). It may very well be possible that the formation of lattice fibers is connected with the discharge of granulations into the blood channel; already earlier it has been observed in the i.o. of Ichthyophis that there was always a proliferation of connective tissue inside the i.o. as well as the formation of connective tissue capsules around cell complexes, the cells of which were filled with basophilic granulations. After the granulations had been emitted into the blood channel, the connective tissue retrograded again (Dittus, 1936).

After this digression, the description of the i.o. in the pregnant animal is to be continued. As mentioned earlier, the phases immediately following Group 2 (animals with small embryos) were not available to me. (For your guidance it may be added here that according to statements by Lo Bianco (1909) the period of pregnancy is supposed to last 10 months for T.m. and 7 for T.o.) The i.o. of each animal, shortly before birth (Group 3) showed slightly different conditions, and for this reason they are to be dealt with separately.

The i.o. of the T.m.-female weighing 875 g, which contained six embryos, is represented in Illustration 12d. It showed the most pronounced granulation in the plasma, which I was ever able to observe in animals in the sexual phase; it is so strong that it can only be compared to that of an extremely artificially stimulated animal (compare Illustrations 33b and 34b). These granulations, staining black intensively with iron hematoxylin give the entire i.o. a dark appearance in the picture of the intersection. The capillary net is still very strongly developed, but larger sinuses are lacking.

The cell nuclei show the greatest changes. Some of them are still stained intensively; they contain larger irregularly shaped nucleolar fragments which lie preferably at the periphery of the nuclei (Illustration 14). Extrusions of nucleoles are again frequently encountered in the case of such nuclei. The p.74 plasma of the cells containing such nuclei is completely filled with basophilic little grains of various sizes. We should like to add here, that due to the fact that the plasma is full of little grains, it is often hard to determine the cell membrane which often gives the impression of being partly dissolved. Due to the transformation of the "chromatin substance" into nucleolar-like products, as mentioned often above, and the discharge of these products from the nucleus into the plasma, such nuclei are increasingly deprived of substance. They possess light nuclear fluid centres, while the stainable substance, which mainly represents nucleolar substance, lies at the nuclear membrane. Illustration 14 shows the various transition stages of the nuclei just described. At the same time it is noticeable that these nuclei appear considerably larger and more blistery than the nuclei still stained more intensively, and this is

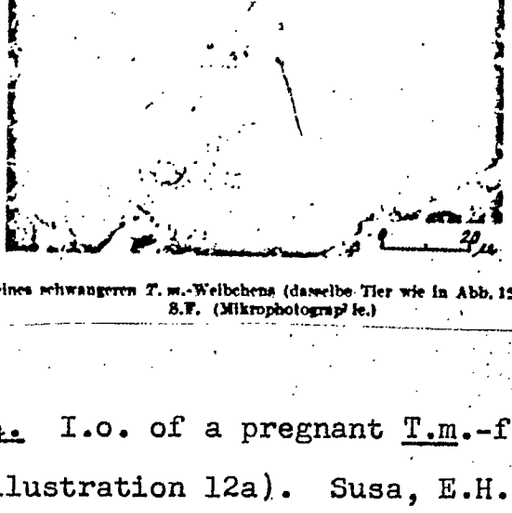

Abb. 14. I.O. eines schwangeren *T. m.*-Weibchens (dasselbe Tier wie in Abb. 12a). Susa, E.H., S.F. (Mikrophotographie.)

Illustration 14. I.o. of a pregnant T.m.-female (the same animal as in Illustration 12a). Susa, E.H., S.F. (micro-photography).

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also clearly obvious later in Illustrations 34b and 34b. (sic). Gradually the original oval shape becomes more round. This impressive picture can be observed in about 50% of all the nuclei of the i.o. of this animal (compare Illustration 12d and 14). One gains the impression that these nuclei again absorb large amounts of dissolved substances from the plasma, through diffusion, for purposes of their restitution. In addition to these nuclei with vesicles, with stainable substance on their nuclear membranes, one always encounters those where this stainable substance migrates towards the interior of the nuclei. At more or less distance from the nuclear membrane these particles are then seen in a "wreath-like" arrangement in the optical section. This characteristic p.75 stage is demonstrated in the microphotography in Illustration 15a (KK). Finally, nuclei are seen where a light nuclear fluid area follows the clearly outlined nuclear membrane. The entire stainable substance (KB) is concentrated in the centre of the

Abb. 15a und 15b. Dasselbe Tier wie in Abb. 14. Verschiedene Kernstadien. *b.K.* blasige Kerne mit hauptsächlich peripher gelagerter färbbarer Substanz. *K.K.* Kerne mit Abwanderung der färbbaren Substanz ins Kernzentrum. *K.B.* Kerne mit »Binnenkörper«. Weitere Erklärung im Text. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 15a and 15b. The same animal as in Illustration 14. Various nuclear stages. b.K. blistery nuclei with stainable substance located mainly at the periphery. K.K. Nuclei with migration of stainable substance into the centre of the nucleus. K.B. Nuclei with "interior bodies". Further explanations are given in the text. Susa, E.H. S.F. (microphotography).

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nucleus of this kind, and the picture may almost be compared with a "karyosome nucleus" (Illustration 15b). It would seem as if the stainable substance, located originally at the periphery of the nucleus, were "deposited" in the centre of the nucleus by a flow of fluid, diffusing everywhere through the nuclear membrane into the nucleus. The absorption of

p.76

dissolved materials from the plasma, as postulated above, would appear probable due to the increased volume and the observed increase of the turgor of these nuclei, but also because of a third phenomenon. While those substances at the nuclear peripheries which stain with basic colouring materials prove to be mainly nucleolar substances in the Feulgen- preparation, those which are "karyosome nucleus"-like show that the proportion between nucleolar substance and thymo-nucleic-acid shifted in favour of the latter. It may thus be assumed that with the help of the absorbed dissolved substances, the restitution of "chromatin substance" is already setting in again. One may hardly go wrong in considering this process in its entirety as a nuclear restitution process, especially since stages are frequently observed which indicate a secondary loosening up of the interior body. It is, of course, difficult to distinguish any commencing agglomeration of the stainable substance from any secondary loosening of it; but if a nucleus with a somewhat dissolved, dispersed interior body shows considerably more thymo-nucleic-acid ("chromatin substance") than nucleolar substance in the Feulgen-preparation, the conclusion that an interior body which is loosening rather than getting concentrated would appear to be justified. Moreover, the cytoplasm belonging to this nucleus hardly shows granulations any more, which constitutes a second criterion for this condition, as will be discussed later. It may thus be assumed, that most of these nuclei recover, even though it can be observed occasionally in the preparations that such nuclei with interior bodies degenerate. The entire process does not, of course, always assume these pronounced forms; probably most of the blistery nuclei with stainable substance mostly located at the

edges are transformed again into normal nuclei without the formation of such "central bodies". A similar though not so striking process of nuclear restitution has been described earlier in connection with the i.o. of Ichthyophis glutinosus (Dittus, 1936).

The development of such nuclei with "interior bodies" may also be proved in the experimentally stimulated i.o. - in the way as described above - and it is even feasible under certain conditions to step up this process so much that it will assume a pathological character and lead to the absolute disintegration of these nuclei and cells, as will be described later in Chapter V, 6.

Conditions in the cytoplasm also provide some evidence for the accuracy of the described perception. It was observable that the plasma of the interrenal cells of these animals, containing intensively staining nuclei, where extrusions of nucleoles had occurred and could still be seen, was completely filled with granulations. In the case of cells with blistery nuclei, or where migration of the stainable substance into the centre can already be seen in the nuclei, areas free from granules are already noticeable within the cells, which are due to the evacuation of basic granulations. In the case of cells located on a vessel, it is noticeable that the granulations become finely dispersed towards the vessel, probably in order to pass through the wall in a colloidal condition. (Illustration 16). In the vessels of the i.o. in this animal (as also in other strongly secreting ones of the i.o.) granulations can be proved which are completely similar to those within the interrenal cells - from the point of view of their behaviour in the case of staining - so that it must be assumed that the granulations

p.77

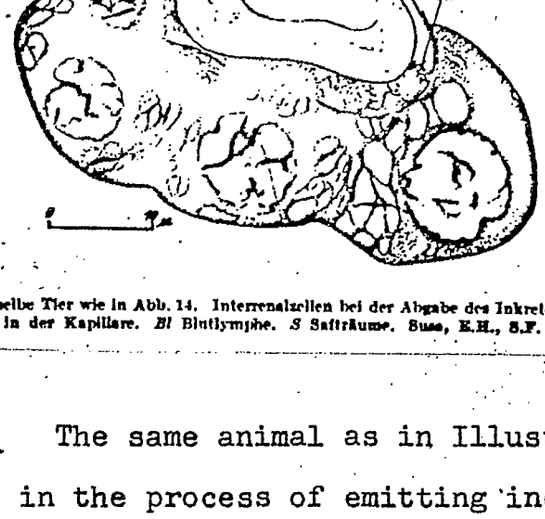

Abb. 16. Dasselbe Tier wie in Abb. 14. Interrenalzellen bei der Abgabe des Inkretes. *SK* Inkret in der Kapillare. *Bl* Blutlymphe. *S* Safträume. Susa, E.H., S.F.

Illustration 16. The same animal as in Illustration 14. Interrenal cells in the process of emitting incretion. SK - Incretion in the capillary. Bl - blood lymph. S fluid spaces. Susa, E.H., S.F.

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a somewhat
passing in/liquefied condition through the cell- and capillary wall, are precipitated in the vessel - by the preserving media applied - entirely or almost completely in their former composition (Illustration 16). I am aware that such findings are usually not believed, and with good reason in most cases. It is, however, obvious that such granulations, staining blue to black with iron hematoxylin, red with Azan, and not at all with Feulgen, exist only in the i.o. capillaries, whereas I did
at all
not find them/in the capillaries of the kidney, e.g. Also, I was able to produce this evidence only in the case of animals with greatest i.o. activity, such as shown by this animal, for instance (as well as by some other experimentally stimulated ones), in a very obvious way. The transgressions of these granulations into the blood were also established in the i.o. of Bombinator by Harms (1921), and in the case of Ichthyophis by Dittus (1936). There is no doubt in my mind, that these granulations represent incretions of the interrenal cells.

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As has been indicated earlier, the cells, where part of the granulations have been evacuated, show light fluid areas which have no structure except for the plasma trabeculae passing through; they must have been filled with a less dense solution (Illustration 16). On the basis of lipids-testing to be discussed later, it cannot be assumed that these areas may have contained lipids or fats. They presumably belong to the materials found in the fluid spaces are substances which are absorbed again by the cell being restored. Part of this solution is absorbed by the nucleus, since the cells which show these "fluid spaces", always have a blister-like nucleus which gives all the appearances of extrusion of nucleoles having occurred earlier. The plasma in cells with a "karyosome-nucleus"-like structure is relatively devoid of granules; in the plasma of cells in which the nuclei again assume their normal forms, granules are hardly found any more. It would thus appear that changes occurring in the cytoplasm run parallel with the phenomena in the nuclei.

The i.o. of the pregnant T.o.-female (430 g, three embryos with 20-22g each) differs widely in appearance from the one just described. It does not have such a pronounced granulation of the interrenal cells; furthermore, in general only nuclei shaped like small vesicles with stainable substance located at the edges are noted, while nuclei with interior bodies are encountered only very rarely. In principle, the picture of the entire organ is more like the one shown in Illustration 12b, with the exception, however, that the nuclei do not appear to be so intensely stainable, and look more blistery. The entire organ shows a much weaker activity, as compared to that of the pregnant T.m.-female.

As an explanation for these differences the following is to be noted: As has been pointed out earlier, it would appear that both animals were in about the same stage before birth. Since otherwise the histological pictures of the i.o. are completely alike for T.m. and T.o., in all animals tested, as has also been reported by Pitotti (1938), the possibility of explaining it as a difference of species is excluded. The only difference between these two animals thus would appear to consist in the number of offspring. While the T.o.-female weighing 430 g, with weaker i.o. activity, had carried three young animals, the T.m.-female weighing 875g carried six. The great difference in the histological development of the i.o. in the case of these two animals might be due to this fact. (The relative weight of the young animals as compared to the weight of the mother animal is however about the same in each case.) p.79

The T.o.-female weighing 480 g (group 4), which gave birth to three young animals, weighing 10-11g each, showed a relatively compact i.o. 5 days after birth. The obvious formation of little lobes had almost disappeared again. It is true that the nuclei are still somewhat lighter and more blistery than this is the case for normal animals, and still more granulations are contained in the plasma than usual, but the restitution of the i.o. is unmistakable, as well as its return to the stage characteristic for the animal outside its sexual activity.

Also of interest is the question of frequency of mitosis and amitosis in the female animals during the sexual acitivity stages. Mitoses were not found in the i.o. of animals of any of the groups. The finding ascertained regularly in pregnant animals (e.g. Kolmer, 1918, on pregnant guinea pigs) to the

effect that mitosis occurs more frequently in the adrenal cortex does not seem to apply here. This will be discussed at greater length in Chapter VIII. Amitoses are very frequently observed in the i.o. of animals at the beginning of their sexual phases (eggs attaining full growth). About 10% of all cells are binuclear - a finding also ascertained by Pitotti (1938). In heavily secreting organs, such as, for instance, the i.o. of the T.m.-female of group 3, pictures of amitoses cannot be seen any more; the tendency towards nuclear secretion probably suppresses this process.

On the whole, it may be stated that throughout the development of the eggs and the subsequent gestation period, i.o. activity increases which becomes obvious in the early stages at first by intensive stainability of the interrenal-cell-nuclei, transformation of "chromatin" into nucleolar substance, migration of nucleoles into the plasma, and considerable granulation of the cytoplasm, while granulations are constantly being evacuated into the blood channel. Towards the very end of the pregnancy, the frequency of the extrusions of nucleoles from the nuclei would appear to decrease, while the quantity of granulations, which is larger or smaller in accordance with the brood care performance, are emitted completely into the blood channel. After birth the i.o. is again restored to the "normal form" as described in Chapter IV, 1.

In the case of the three male animals, which were in the state of complete spermatogenesis, their i.o. showed increased activities, which were, however far below those of the females. The i.o. of these animals (Illustration 17) show more definite lobated structure, a stronger connective tissue- and capillary network, and more intensive colouring of the nuclei and plasmas than this

p.80

is the case in normal animals (Illustration 11). The same behaviour of the i.o. during the maturing of the spermatozoa has been described earlier for Ichthyophis glutinosus (Dittus, 1936).

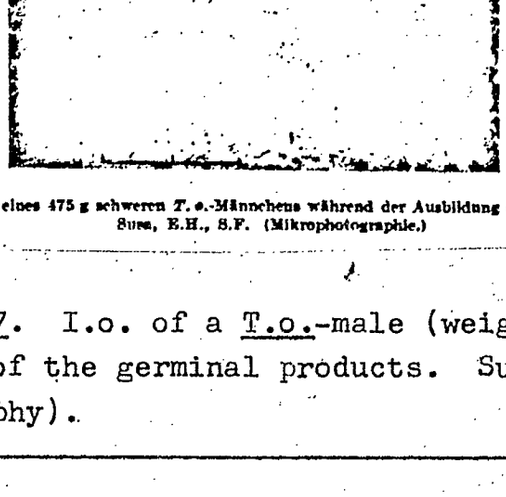

Abb. 17. I.O. eines 475 g schweren *T. o.*-Männchens während der Ausbildung der Keimprodukte. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 17. I.o. of a T.o.-male (weighing 475 g) during the formation of the germinal products. Susa, E.H., S.F. (microphotography).

Pitotti (1938) reported on i.o. changes during pregnancy for Torpedo. Here also, in the case of animals with ovarian eggs, the network of blood vessels is strengthened, and amitotic divisions occur frequently in this stage. Furthermore, Pitotti states that animals during the growth of eggs up to animals at the beginning of pregnancy, possess blistery cell-nuclei with clearly visible chromatin networks and abundant nuclear fluids as well as more than normally increased and granulated plasmas, but that in the further course of pregnancy, the nuclei become smaller again, the chromatin networks become denser and the nuclear fluids are reduced to a minimum. This latter statement does not agree with the findings ascertained by me. Unfortunately these pictures cannot be evaluated more precisely because of

insuffient illustrations. Moreover, Pitotti did not recognize the genesis of the granulations. Fancello (1937), who studied the changes occurring in the i.o. of Scyllium during the development of the egg, ascertained that the little lobes unite to form more distinct units, which are also surrounded by strong connective tissues. He also ascertained that the nuclei appeared to become larger and more blistery and that "numerous stainable granules which preferably placed themselves at the nuclear peripheries" were found in them. But he did not describe either the connection between these "stainable granules" and the granulations of the cytoplasm.

V. The INTERRENAL ORGANS OF THE TORPEDOES UNDER EXPERIMENTAL CONDITIONS. p.81

1. Generalities.

The results which had been obtained from the i.o. of normal animals, were used as the basis for evaluating the pictures resulting from experimentally influenced i.o, in the course of the second part of these investigations. In this second, experimental part the cytological and histological changes occurring in the i.o. after elimination, or varying influence, of the c.h. are to be examined.

Strictly speaking, the existence of the c.h. has not been clearly proved until recently, and we therefore find in the 2nd Volume of the Methodik der Hormonforschung (methodology of hormone research) by Bomskov (1939) the c.h. under the section on "So-called "hormones" of the hypophyseal anterior lobe, the existence of which has not been proven."

Gfaersberg (1939) does not consider the existence of the c.h. as strictly proved. The relations between the

adrenal cortex (i.o,) and the hypophysis have been known for quite some time; an enumeration of these facts is found in the descriptions by Anselmino and coworkers (1934) and Bomskov (1939). The atrophy occurring after hypophysectomy in the adrenal cortex, which could be counterbalanced by hypophysis-anterior lobe-extracts, led to the investigations which aimed at the separation of the material acting specifically upon the adrenal cortex. At first Evans and his assistants (1933) produced adrenally-active extracts which were free from gonadotropic and growth hormones. Collip, Anderson and Thompson (1933) claim that their cortex-effective substance is free from all anterior-lobe-hormones known so far, i.e. also free from thyreotropic hormone. They called the new substance which is acting upon cortex "adrenotropic" hormone. Anselmino, Hoffmann and Herold (1934) published a method for the presentation of the principle of action on the cortex of the hypophyseal anterior lobe, in a pure condition; according to their statements all the other hormones of the hypophysis could be separated. They called the new hormone "corticotropic" hormone. It should be noted that the thyroid gland, or the thyreotropic hormone respectively, also strongly is acting indirectly upon the adrenal cortex (Herring, quoted after Hammet, Hammet (1920), Loeser (1933). Although according to the statements by Collip and his coworkers, and Anselmino and coworkers, the absence of the thyreotropic hormone in their substance is stressed specifically, the following sentence by Bomskov (1939) is newertheless justified: "Since the thyreotropic hormone has a strong effect upon the adrenal gland, (via the thyroid gland), it would appear obvious to identify the corticotropic hormone by this factor. As long as no decisive proof to the contrary is furnished, (studies on animals which actually do not have any thyroid glands), the existence

of the corticotropic hormone remains questionable."

Experiments carried out by McQueen-Williams (1934) also spoke in favour of this concept. He found that the otherwise regularly observable enlargement of the adrenal gland did not occur after implantation of ox-hypophyses in the guinea-pig, when the thyroid gland had been removed. Emery and Winter (1934) were also unable to note any effect upon the adrenal cortex after implantation of hypophyses or after injection with an extract of hypophyseal anterior lobes in the case of rats p.82 without thyroid glands.

Atwell (1937) was able to demonstrate on tadpoles which had been hypophysectomized and thyroidectomized that after supplying them with "adrenotropic" extracts by Collip, Anderson and Thompson (1933), of the i.o. that a hypertrophy occurred as well as an increase of the lipoid substances, as compared to the atrophic i.o. in each animal which had been hypophysectomized, deprived of its thyroid, and had not been injected. These results, which were, it is true, gained from only limited material (four tadpoles operated and injected; and seven operated and not injected), speak in favour of the existence of c.h., which seemed to be more than probable already beforehand. Furthermore, Jores and Boecker (1937) were able to prove that totally thyreoprivic guinea-pigs (five animals) showed an equally pronounced enlargement of the adrenal glands after supply with pure c.h. as did the animals which still had their thyroid glands. Moreover it was obvious in the histological picture, due to the fact that the hyper-activity of the thyroid gland after injection of its c.h. failed to occur, that it certainly did not contain any thyreotropic active substance. In another context, Dittus (1939) stated that in axolotls, on the one hand hypophysectomized and

thyreoprivic, and on the other hand only hypophysectomized ones, show equally strong reactions after injection with melanophoric hormone-free c.h. (melanophoric reaction typical for c.h. - Dittus, 1937, 1939).[1] Neither was I able to eliminate the atrophy of the i.o. in the case of the hypophysectomized torpedoes through thyreotropic hormone, whereas even small doses of c.h. caused an extremely strong functioning of the i.o. (compare Chapter V, 6). Unfortunately I was unable - due to lack of time - to extend my experiments also to thyreoprivic selachians.

Moreover, the absence of thyreotropic hormone in the positive c.h. used by me was proven in two additional ways. Interrenoprivic torpedoes - after injections of 0.05-0.15 ccm/g of the c.h. used my be (Promonta, preparation VP 31/32 and VP 32) - did not show any increase of their breathing frequency which is typical for normal animals after having been supplied with c.h., whereas also interrenoprivic torpedoes after supply with small quantities of thyreotropic hormone (0.5- 1 mg thyreotropic hormone per 100 g animal weight) increased their breathing frequency by 20 - 50%. It must thus be concluded that the c.h. did not contain any adulteration by thyreotropic hormone. Furthermore, interrenoprivic torpedoes, which had been injected with c.h., never showed any increased activities of the thyroid glands in the histological pictures, whereas they set in immediately after supply with small doses of thyreotropic hormone (Dittus, 1939). There would appear to be no doubt left that the hormone solution used by me really does represent a specifically "interrenotropic", or "corticotropic" active substance.

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[1] Will be published in detail in the near future.

2. The interrenal organs of hypophysectomized torpedoes.

Through removal of the hypophysis (see Chapter III for technique), the place where the c.h. is produced, was eliminated from the body of each experimental animal; the stimulation of the i.o. through the c.h. was thus impossible. As is to be anticipated here, the i.o. of each of these animals showed a manifest atrophy. The i.o. of 15 experimental animals were examined which were preserved 2 - 24 days after hypophysectomy. p.83

It became obvious after the investigations that the histological and cytological changes in the i.o., typical for hypophysectomy, became apparant only after 4 - 5 days. But they were so typically manifest after 8 - 9 days that e.g. the i.o. which were taken out only 3 weeks after hypophysectomies did not show any further increases of these symptoms, according to my investigations. Possibly the total weight of i.o. which had not been subjected to any stimulation by c.h. for a long time, might have decreased still further as compared to i.o. in the case of short-term experiments (8 - 9 days post operationem).

At first we should like to describe the histology and cytology of the i.o. which was deprived of stimulation by the hypophysis for more than 8 days. In this connection we should like to refer to Illustration 34a. The little lobes which had been tightly stretched in the i.o. of normal, and particularly pregnant animals and clearly surrounded by connective tissues and were thus impressive as well-circumscribed elements of structure and form (compare Illustration 11), collapsed considerably and are densely pressed against each other so as to make it difficult to still recognize the formerly clearly visible "lobated" structure of the i.o. Due to the tight

and crowded arrangement which may be ascribed to the lack of turgor within the interrenal cells, they are deformed, often looking like little tubes or strings. Moreover, the entire connective tissue apparatus of any such organ manifests considerable retrogression. The boundary lines of the little lobes which used to be clearly visible, and strongly developed in pregnant animals, are fine and hardly discernible now. The network of vessels has also retrograded very considerably; sinuses are no longer found at all. All these facts contribute to obliterate the "lobated" structure of the organ. In observing the individual cells (Illustration 33a) it is striking that the nuclei are very light in appearance, although chromatin networks are developed fully though relatively poor in substance. (Compare - as against Illustration 4). Furthermore, the absence of larger quantities of nucleoles is quite typical - as this is characteristic for the i.o. of normal animals. The plasma is not granulated and gives the appearance of fine foam, due to the dissolved lipids.

The results of experiments with material fixed according to Flemming, and cut on the freezing microtome, indicated that considerably less lipids are embedded in the interrenal cells than this is true in the case of normal animals (compare Chapter VI).

In the case of these animals I never found any signs of any kind of increased activities, such as, e.g., some more intensively stained nuclei, migrations of nucleolar substances extruding from the nuclei, or granulations in the plasmas. Mitoses or amitoses could never be ascertained either. p.84

To summarize, it may be stated that the organs of animals which had been hypophysectomized longer than about 8 days before,

showed clear atrophies in the i.o., which became apparent in the following ways: obliteration of the lobated structure of the organ, disappearance of the cell plasmas, decreased blood supply, decreased stainability of nuclei, absence of any kind of granulation in the plasma, and disappearance of the lipids. These phenomena are exactly opposite to those found in the case of increased i.o. activity (such as, for instance, during pregnancy).

As I have stressed before, these changes become apparent as early as 4 - 5 days after hypophysectomy. In the case of these animals it is noted that the above-mentioned phenomena gradually become more pronounced, while e.g. animals 2 days after hypophysectomies are not yet distinguishable in any way from normal animals, from the point of view of their i.o. structure. On the other hand, the symptoms have fully developed around the 8th or 9th day after hypophysectomy, so that later no further intensification of these phenomena can be observed, as has been pointed out before.

The microscopic pictures give the impression as if about 4 days after hypophysectomy the activity of the i.o. were suddenly "waning". (Smith (1927) also observed a "sudden retrogression" of mammalian adrenal cortex about 4 - 5 days after hypophysectomy). Whether or not this latent period is due to c.h., which was secreted in prior to the hypophysectomy and is still circulating in the body, or else, to a certain continuing increased activity of the i.o. itself, is hard to decide, as I cannot make any statements about any amounts of c.h. still existing in the vascular system, or its use, or its decomposition. Hypophysectomized experimental animals also showed that T.o. and T.m. reacted in the same way.

3. The interrenal organs of normal torpedoes which had been given additional adrenal cortex hormones.

Since - as has been mentioned above several times - a surplus of adrenal cortex hormone in the organism represses the production of c.h. in the hypophysis, it should be possible to decrease, or eliminate, the production of c.h. of the hypophysis through sufficient doess of artificially supplied adrenal-cortex-substance. For this purpose p.85 six normal animals of varying weights were supplied with additional adrenal cortex hormones (Cortidyn)[1]. By supplying the experimental animal with this hormone artificially, its interrenal system was forced to be inactive, so to speak.

This consideration which was first of a theoretical nature provedto be right in the histological pictures of animals treated in this way, which will be discussed in detail below.

In an earlier study (Dittus, 1937) it was possible to ascertain on interrenoprivic torpedoes how much Cortidyn was required approximatively so as to eliminate the symptoms arising from the removal of the interrenal system. In order to cancel out the contractions of the melanophores in torpedoes after extirpation of the i.o., it was necessary to inject approximatively 0.002 to 0.003 ccm Cortidyn per day per gram animal weight. So as to remove the adynamia occurring also, it was necessary, however, to supply at least the double to triple amount of adrenal cortex hormone.

In accordance with these guidelines, the experimental animals were, in addition, injected with amounts somewhat larger than the doeses appropriate for the body of the animal concerned. Consequently, doses of 0.01 ccm per gram and per day were

[1] The adrenal cortex hormone "Cortidyn" was kindly supplied by the Promonta Company in Hamburg.

injected. So as to save hormone, four animals, weighing 30 - 80 g, were used, and they were supplied with hormone for 8 days. The histological examinations of these i.o. revealed distinctly decreased activities. They appeared to look just like those of hypophysectomized animals even though the phenomena described in their case did not seem to be quite so obvious. It is, therefore, not necessary to give another description, and I refer to the preceding Chapter.

4. The interrenal organs of partly interrenoprivic torpedoes.

In contrast with the experimental conditions just described, where the aim was to suppress the function of the i.o., the experiments which will be described in the following Chapters, will aim at bringing about the contrary effect, namely a stimulation of the i.o.

The first schedule of experiments is based on the idea that in removing part of the i.o., the remaining interrenal tissue, left in the body, will be forced to assume the secretion activity of the removed portion, in its entirety, or in part, in addition to its own secretion activity, or expressed better, in line with the more recently acquired knowledge of the correlation of hypophysis and interrenal tissue: the c.h. secreted steadily by the hypophysis can no longer act upon the entire i.o., but only upon a portion of it, i.e. that the concentration of the effective c.h. is - after removal of half the i.o. - twice as large upon the remaining half left in the body than it had been before the operation. As I have already pointed out in the Introduction, it should also be noted that the interrenal portion remaining in the body is probably unable to take over the additional function immediately and fully to make up for the extirpated part. Thus a shortage of interrenal- active substance will

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occur resulting, in turn, in an overproduction of the c.h. stimulating the secretion of the interrenal tissue, in accordance with the often-quoted correlation between the hypophysis and interrenal tissue. This fact also - especially a short time after the ~~partial~~ extirpation - will cause also an additional increase in activity of the interrenal portion remaining in the body.

18 animals were used for these experiments, and they were killed after 2 - 18 days. The portion of the i.o. which had been removed in each case was fixed immediately and prepared for control purposes to be checked against the part of the organ to be taken out at the end of the experiment. The initially justified objection that the operation might have an adverse effect upon the remaining portion, e.g. due to disturbances in the blood supply, or necrohormones formed on the wound surface, etc., has to be rejected, according to my experiences. It was obvious that no damage could be detected on the entire remaining i.o. portion. Only a very small line of degenerated cells was immediately below the scab of the wound, but it never became larger than three to four cell-diameters. Otherwise the tissue below the wound appeared to look exactly like those parts of the organ which were farthest removed from the wound surface.

Before starting to describe the results, I should like to mention some biological reactions which would seem to be of interest in this context.

The life span for completely normal rays kept in the aquaria of the Zoological Station in Naples, amounted to a maximum of 17 days for T.o., and 28 days for T.m. (Compare Dittus, 1937). These figures agree with the findings obtained

by the Station in Naples. If the i.o. is extirpated, which has a very vital function, of course, the species of T.o. will die after 2 - 4 days and in the case of the T.m. species after 5 - 7 days (Kisch, 1928; Dittus, 1937). In case this operation is performed on a mock basis and the i.o. is left in its original place, the T.o. will survive 8 - 14 days and the T.m. 9 - 21 days. The life spans of mock operated animals are somewhat shorter as compared to normal animals (Kisch, 1928; Dittus, 1937). p.87

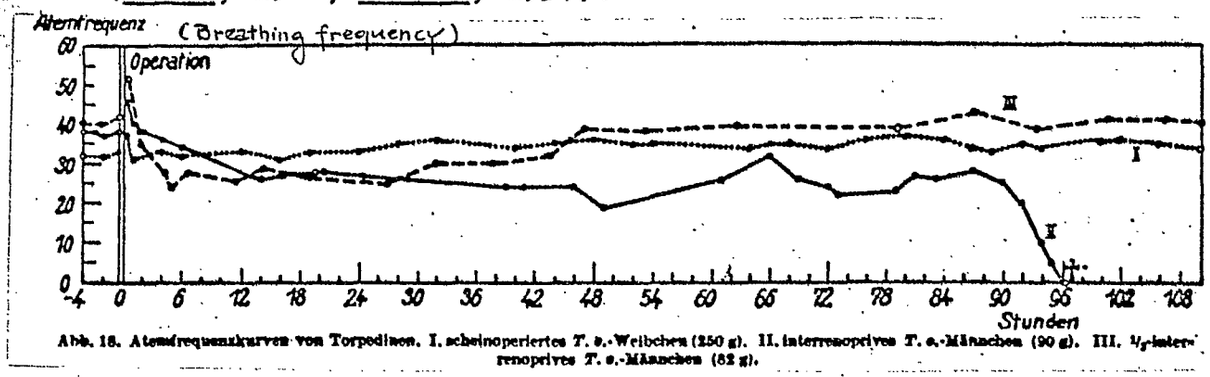

Abb. 18. Atemfrequenzkurven von Torpedinen. I. scheinoperiertes T. o.-Weibchen (250 g). II. interrenoprives T. o.-Männchen (90 g). III. ½-interrenoprives T. o.-Männchen (82 g).

Illustration 18. Breathing frequency Curves for Torpedoes. I. Mock Operated T.o. - Female (250 g.). II. Interrenoprivic T.o. - Male (90 g.). III. 1/2 Interrenoprivic T.o. - Male (82 g.).

Animals with 50% of their i.o. extirpated by me survived for 7 - 10 days in the case of T.o., and 8 - 18 days in case of T.m. These life spans are somewhat lower than those for pseudo-operated animals, but they indicate clearly that the animals can survive with only half of their i.o. without any difficulty, i.e. that this operation is "biologically" feasible. The somewhat shorter lifetime of the 1/2 - interrenoprivic as compared to the mock operated animals might be due to the fact that the partial removal of the i.o. (the same way as in the case of total extirpation) has a detrimental effect on one kidney of the experimental animal while this is not the case for mock operated animals.

Another fact is also noteworthy: while the breathing frequency of the mock operated animal remains practically the same before and after the operation (with the exception of a slight increase shortly after the operation) (Illustration 18, Curve I), and the breathing frequency of the totally interrenoprivic animal decreases after the operation until breathing stops completely while the heart is still beating (Illustration 18, Curve II), the 1/2-interrenoprivic animal shows a different picture. The breathing frequency curves of the latter animals (except for an increase of short duration after the operation due to the fact that the animals have been irritated) clearly decreases initially to a level below that recorded prior to the operation but reaches again the level prior to the operation after about 2 - 3 days and maintains this breathing frequency value until shortly before death (Illustration 18, Curve III). Six animals for which the breathing frequencies have been recorded showed practically the same pictures. [1] Since according to the investigations by Kisch (1928) and Dittus, (1937, 1939) the breathing frequency of the ray is directly influenced by the interrenal organ, the Curve presented above indicates an important fact applying to the partly interrenoprivic animals. It had been pointed out before that shortly after extirpation of part of the i.o., a shortage of interrenal active substance would probably occur initially which will be compensated for gradually afterwards by increased functioning of the part remaining in the organism. If this concept is correct, in view of the

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[1] The method for measuring breathing frequency has been described earlier (Dittus, 1937, 1939).

earlier findings on the i.o. and breathing frequency, initially a decrease and later again an increase of the breathing frequency would be noted, and this is what actually did occur in the case of the 1/2-interrenoprivic animals. The proof for the above-mentioned conception is provided by the breathing frequency curves.

Experiments for clarifying the histological changes of the interrenal tissue after partial removal of the interrenal organ were carried out in two different directions. At first the animals in the one series (14 animals) composed of normal ones outside their sexual activities, were deprived of 50% of their i.o./ in the same way, in each case. The animals which had been treated in this way/were killed in varying intervals (2 - 18 days). In this series it was planned to examine the behaviour of the remaining i.o. at varying periods after the operations. In the second series of experiments (4 animals) about 75% of the i.o. was removed in each case and all the animals were killed on the fifth or sixth day afterwards. In this way it was hoped to study the effect on the remaining part after/more considerable loss of interrenal tissue.

As has been pointed out in Chapter IV, 4, Torpedoes from 35g in weight on which were outside their sexual activities, did not show any essential differences with regard to the development of their i.o. Only the absolute quantity of interrenal tissue varies according to the individual weight groups. But it appeared that the weight of the adrenal cortex of adult mammals is always in fixed proportion, i.e. well balanced, with the body weight concerned (Freeman, 1934; Loeser, 1933; Jores and Beck, 1936). The adrenal gland is the only organ with incretion for which this weight correlation has been established. It may be assumed

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that this fact also applies to the i.o. of the Torpedoe. The weight of the animal to be tested is thus of no importance in connection with the question to be dealt with. The possibility, therefore, exists to compare the results recorded for Torpedoes of varying weights provided that exactly 50% of the i.o. was removed in the case of each experimental animal.

Illustration 19a shows a picture of the part of ... the i.o. removed by operation from a 1/2-interrenoprivic; T.o.-male; it is in no way distinguishable from the condition of the normal animal shown in Illustration 11. The other half remained for two days in the body of the experimental animal, and had to produce more during this period in accordance with the statement made in the introduction to this chapter. The microscopic picture of this stimulated interrenal tissue is represented in Illustration 19b. As compared to the normal condition (Illustration 9a) it clearly shows a more pronounced development of the lobated structure and a richer capillary network which is expressed in Illustration 19b through the frequency of dark nuclei with red corpuscles. Moreover the interrenal cell-nuclei showed considerably greater stainability, just as the plasma , which appears very darkly stained by the embedded basophilic granulations. An increase in nucleolar substance occurred in the interrenal cell nucleus; it is mostly located in the nucleus at the periphery on the nuclear membrane. One notices very frequently all the stages described above with regard to the extrusion of nucleolar substance from the nucleus into the plasma while the nuclear membrance remains intact. This process has been described in detail so often before that it is not necessary to repeat it. It is characteristic that numerous

amitoses occur again, i.e. always in those cells which do not have maximum granulation. This fact will be discussed in greater detail in Chapter VIII.

To summarize it may be stated that the portion of the i.o. which remained in the animal for two days, in the microscopic picture, shows considerably increased activity as compared to that noted in the portion that had been removed; and the remaining part shows exactly the same characteristics which had been noted in animals in their sexual phases (Chapter IV, 5), namely clearly developed lobes, stronger connective tissue, richer capillary network, greater stainibility of the nuclei, increased nucleolar substance and extrusion of the latter into the plasma, development of basilophilic granulations, absence of mitoses and increased occurrence of amitoses. p.90

The halves of the interrenal organs which had been left in the organisms for 5 - 6 days, show the same phenomena as just mentioned, only in slightly increased form, with the exception of amitoses which decreased considerably in number. Obviously each nucleus is occupied in producing nucleolar-like granulations so that the possibility of amitosis is eliminated. It is typical that very often blistery nuclei occur in the halves of the interrenal organs 5 - 6 days after the operation, and these nuclei contain very little stainable substance then. p.91 As has been pointed out earlier, these nuclei have completed their secretion phases and are absorbing again dissolved materials from the plasma. Another factor demonstrating or of this is the fact that the plasma of these cells is much less granulated due to the evacuation of the granulations into the blood channel, as compared to the plasmas of cells with nuclei still showing

extrusions of nucleoles. In addition to these nuclei, those in the next stage are also observable in which the entire chromatic substance is concentrated in the centre of the blistery nucleus and in which case a light fluid space developes on the periphery. Illustration 20a. shows an intersection

p.90

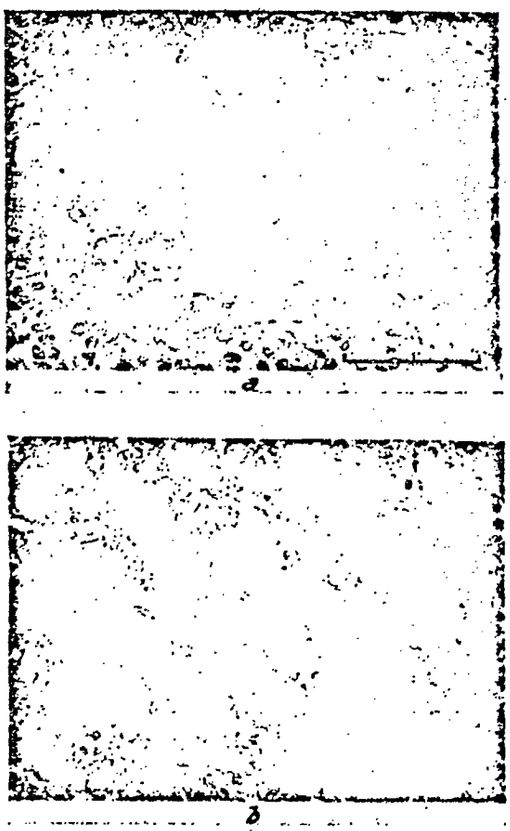

Abb. 19a und 19b. T. o.-Männchen (620 g). a) Interrenalgewebe der exstirpierten I.O.-Hälfte. b) Interrenalgewebe aus der 2 Tage im Tier belassenen Resthälfte. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 19a and 19b. T.o. - Male (620 g.).
a) Interrenal tissue of the extirpated i.o. - half.
b) Interrenal tissue from the portion remaining in the animal for two days. Susa, E.H., S.F. (Microphotography).

cont'd p.91

of the "normal" half of the i.o. which had been taken out, at the operation, from a 600 g. - T.o. - Female, as compared with the portion remaining in the organism for 5 days (Illustration 20b) under considerable enlargement. The striking difference is again

obvious and can be seen in the plasma granulation and the condition of the nuclei as compared to the normal development. Illustration 20b clearly shows "blistery" nuclei and the nuclei with the intensively stainable "interior body" (KB). As has been proved before these forms of nuclei indicate that a strong secretion of the nuclei had already taken place and that they are being restored again. Exactly the same pictures are found for these animals (6 days after the operation) as had been seen in Illustrations 12d, 14, 15a and 15b with regard to the pregnant T.m. -female weighing 875 g.

In case of later stages (7 - 10 days after the operation) the intensity of the secretion would appear to decrease somewhat again. The blistery nuclei have been regenerated in most cases, and the entire picture resembles that represented in Illustration 12b or 12c. It may be concluded from this, in the same way as from the breathing frequency curve shown in Illustration 18, that through the sudden lack of half of the i.o. and the resulting shortage of interrenal active substance, an increased secretion of c.h. occurs that goes beyond the level observed before the operation. Subsequently a certain balance is regained as the one half of the i.o. takes over entirely the function of the former complete organ, or in other words, one gains the impression that due to the initial slow start of the increased function of half of the interrenal organ, the hypophysis initially overcompensates by discharging c.h. so that increased activity of the interrenal tissue results to a greater degree than actually necessary for the organism later.

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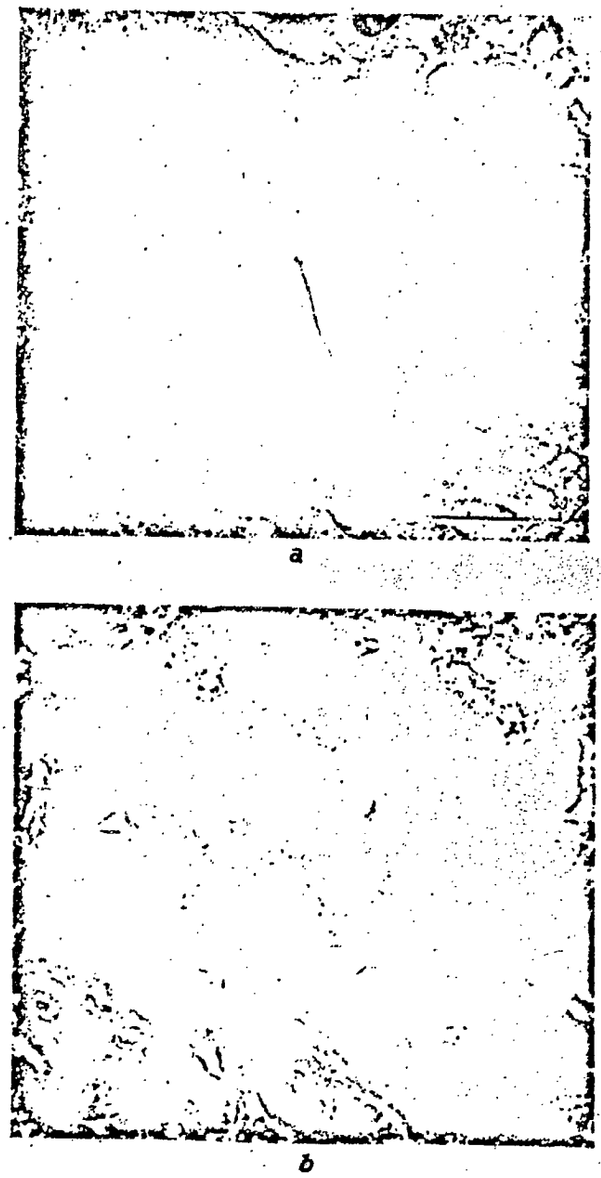

Abb. 20a und 20b. *T. o.*-Weibchen (600 g). a) Interrenalgewebe der exstirpierten I.O.-Hälfte b) Interrenalgewebe aus der 5 Tage im Tier belassenen Resthälfte. *b.K* blasige Kerne, *KB* Kerne mit Binnenkörper. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 20a and 20b. T.o. - Female (600 g.).

a) Interrenal tissue of the extirpated i.o. - half.
b) Interrenal tissue of the remaining half left in the animal for 5 days. b. K blistery nuclei, KB nuclei with interior bodies. Susa, E.H., S.F. (Microphotography).

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If larger parts of the i.o. are removed, approximately 75% in each case, increased activity is noted in the 25% remaining of the organ, which is so great that the nuclei and subsequently the cells may actually perish by the expbsive processes occurring during the development of incretion. Pictures of such tissues

which may be obtained in a way to be described later, will be presented in Chapter V. To prove the intensive activity of such remaining i.o. portions, we should like to refer to Illustration 21, as it represents the condition described in detail earlier of typical nuclei with "interior bodies" which can always be observed in such organs.

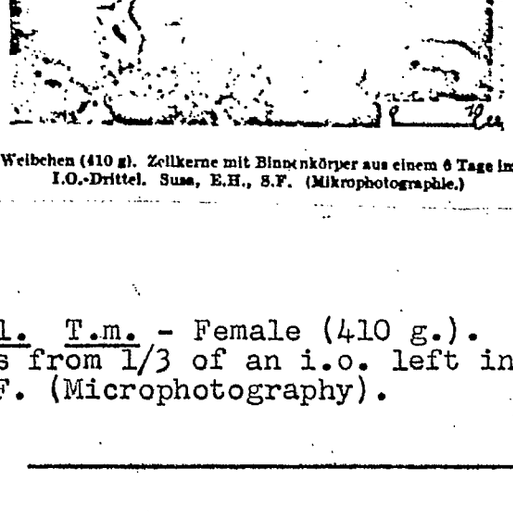

Abb. 21. T. m.-Weibchen (410 g). Zellkerne mit Binnenkörper aus einem 6 Tage im Tier belassenen I.O.-Drittel. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 21. T.m. - Female (410 g.). Cell nuclei with interior bodies from 1/3 of an i.o. left in the animal for 6 days. Susa, E.H., S.F. (Microphotography).

It should be remembered that it is possible through partial removal of interrenal tissue to stimulate the remaining portion to increased activity which is expressed in its cytological effects in the same way as indicated by increased activities in normal animals which occur during their sexual phases (compare Chapter IV, 5). The removal of 50% of the i.o. does not results in any conditions which might not also occur normally in the i.o.

5. The Interrenal Organs of Normal Torpedoes After Injections With Corticotropic Hormones.

The c.h. of the Promonta company (Dittus, 1939) which has been described in detail above, was used for the experiments aiming at stimulating the i.o. with c.h. As outlined in the introduction above, it was necessary to inject such a large dose of c.h. in order to bring about a stimulating effect upon the i.o., that the hypophysis of the experimental animal p.94 was unable to bring about a compensation for the artificially added stimulant by ceasing to secrete its c.h. A good criterion for the dose required in this case was provided by Dittus (1937) who described a test of melanophores and breathing frequency which permits the ascertainment of the stimulating effect of the i.o. which had occurred through the c.h. expressed by increased breathing frequency and more considerable expansion of the melanophores. It was possible in this way to arrive at a reliable and finely adjusted dosage.

At first the results of the experiments regarding animals prior to birth are to be represented, namely with regard to the 22 g. sister animal of the two T.o. - Embryos weighing 20 and 21 g., as described in Chapter IV, 2, which had been taken from the mother's body shortly prior to birth and were prepared separately. This animal was injected intraperitoneally in the following manner: 1 1/2 hours after the separate preparations had been made 0.018, 2 1/2 hours later 0.027, and after another 12 1/2 hours 0.022 ccm/g c.h. After 24 3/4 hours the animal was killed and the i.o. was fixed. Thus a total of 0.067 ccm/g c.h. were injected over a period of about 24 hours. For the

sake of explanation it should be stated that this solution contains 1 corticotropic unit (k.E.) according to Jores and Beck (1936) in 0.05 ccm of this solution, i.e. 0.05 ccm of this solution per gram body weight for 2 injections performed with an interval of 6 hours, and that this is said to have resulted in an increase of the weight of the adrenal gland by 50% in the case of infantile mice after 24 hours. The T.o. - Male indicated that - as described earlier (Dittus, 1937) - a strong increase of the breathing frequency occurred after the injections which would appear to point to a considerable stimulation of the i.o. It is particularly noteworthy that the third injection (of only 0.022 ccm/g) led to a considerably greater breathing reaction than did the second injection of 0.027 ccm/g, made 12 1/2 hours earlier, a phenomenon which was explained at that time by the strengthening of the functional performance of the organ. Illustration 22a now indicates that the i.o. of the control animal (T.o. - Male, 21 g.) was injected intraperitoneally within the same time intervals with the same amount of sterile sea water. The i.o. is in no way distinguishable from that of the animal (T.o. - Male 20 g.), which was fixed immediately and the histology of which was described in Chapter IV, 2. The microscopic picture of the i.o. of the animal injected with c.h. is represented in Illustration 22b, In the upper right hand corner of the picture we notice cells with cell-nuclei which are resembling those of the tissues of the control animal except for greater stainability. On the other hand, in the lower left hand corner we notice a series of cells with nuclei with great stainability and odd shapes. p.96 This phenomenon was observable in various regions of the i.o.

Under considerable enlargement we notice pictures of cells with maximum activities which are to be described here.

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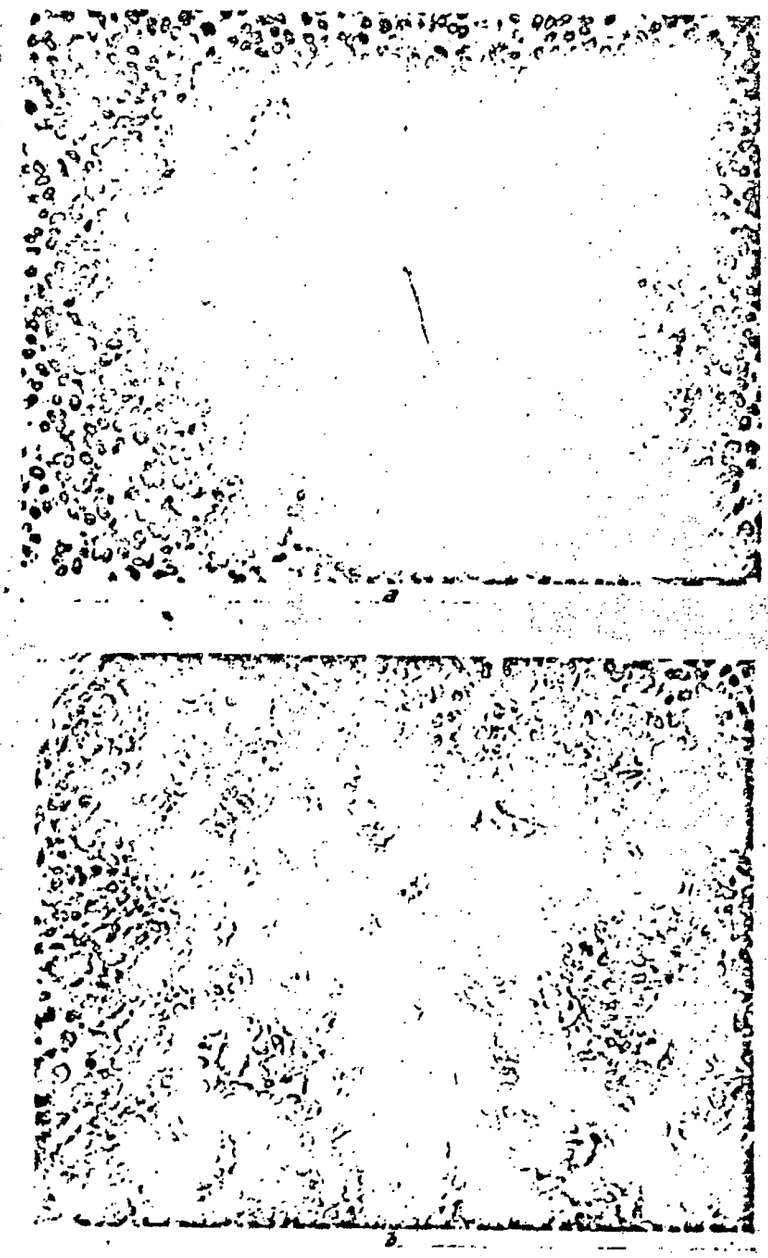

Abb. 22a und 22b. I.O. zweier kurz vor der Geburt freipräparierter *T. o.*-Embryonen (zwei Männchen, 21 und 22 g). a) Injiziert mit 0,068 ccm/g sterilem Seewasser. b) Mit 0,067 ccm/g k.H. 24¾ Stunden nach der 1. Injektion abgetötet. Susa. E.H., S.F. (Mikrophotographie.)

Illustrations 22a and 22b. i.o. of two T.o. - Embryos, prepared separately prior to birth (two males, 21 and 22 g.).
a) Injected with 0.068 ccm/g sterile sea water.
b) With 0.067 ccm/g c.h. Killed 24 3/4 hours after the first injection,
[illegible] Susa, E.H., S.F. (Microphotography).

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I should like to anticipate that, as compared to the animal which has not been treated, the Cytology of which is described in Chapter IV, 2, nothing new is to be reported in principle, but all the processes (with the exception of the

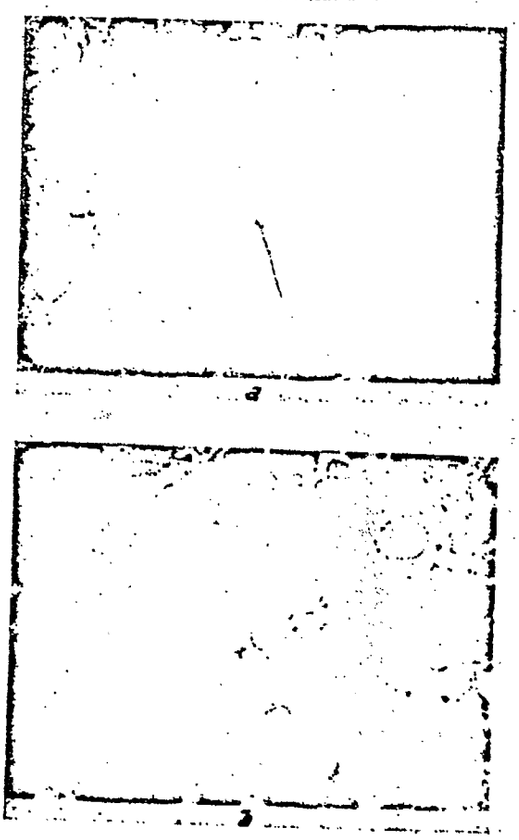

Abb. 23a und 23b. Dieselben I.O. wie in Abb. 22 in stärkerer Vergrößerung. *KV* Kernvakuolen, nucleale Blasen, *Kb* Kernbucht, *Kn* Kernnarbe, *M* Mitose, *bA* beginnende Amitose, *V* ins Plasma ausgetretene nucleale Blasen, *dV* sich dunkler färbende nucleale Blasen, *L* Lymphocyt. (Mikrophotographie.)

Illustrations 23a and 23b. The same i.o. as in Illustration 22, more magnified. KV nuclear vacuoles, nuclear vesicles, Kb nuclear indentation, Kn nuclear scar, M Mitosis, bA commencing Amitosis, V nuclear vesicles emitted into the plasma, dV nuclear vesicles staining darkly, L lymphocyt. (Microphotography).

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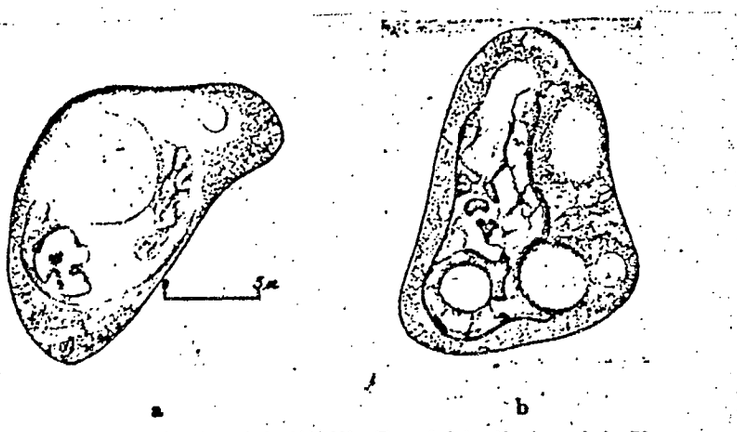
process of mitosis which is completely supressed in the case of this animal) are increased to an extreme degree which is clearly shown in Illustration 23a and 23b where under (b) the [illegible] i.o. stimulated with c.h. is represented whereas the normal i.o. is shown under (a). Initially an extremely great stainability of the nuclei is noted , which partly stain dark black with iron hematoxylin. According to the Feulgen-reaction, this phenomenon is again mainly due to an increase in nucleolar substance. Moreover, distinct nuclear vesicles are seen in many

p.97

of the nuclei, which partly hardly stain with iron hematoxylin, but do sometimes also assume distinct blue colour (dV in Illustration 23b). All the stages described in detail in Chapter IV, 2 with regard to the evacuation of these vesicles may be observed everywhere quite frequently; it is unnecessary to describe this again



a b

b. 24a und 24b. Dasselbe Tier wie in Abb. 23b. Kerne bei der Abgabe nuklealer Blasen.

Illustrations 24a and 24b. The same animal as in Illustration 23b. Nuclei discharging nuclear vesicles.

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in any greater detail. In contrast with the normal animal described earlier, the process of evacuation of entire vesicles, which initially remain in the plasma , assumed increased significance (Illustration 23b). One might gain the impression that the extrusion of nuclear substance into the plasma occurs so hurriedly and quickly that the vesicles are not emptied quietly but rather discharged in their entirety. Another factor contributing to this phenomenon might be the considerable size of the vesicles as compared to those in the normal animal, as a rule. Illustration 24a represents a nucleus in the process of emitting a vesicle. Two small emitted blisters are already lying

in the plasma, one of which stains relatively darkly which indicates a tougher colloidal condition of the content of the vesicle. In the nucleus itself another nuclear vesicle has been formed where darkly staining grains can be proved which probably originate in the adjacent dark nucleolar fragments. We also p.98 note several vesicles in the plasma in Illustration 24b; it may happen frequently, as becomes obvious in this Illustration, that two such blisters unite. One recently formed vesicle is still attached to one end of this nucleus whereas another one is already being formed in its centre. On the other nuclear pole a nuclear indentation is clearly visible, in front of which the vesicle is located which had just been emitted. It is often surprising how many such blisters one nucleus is able to produce. If the number of vesicles emitted is not too large, the nuclei would appear to recuperate after the secretion process, since we observe, in addition to the nuclei which collapsed due to the loss of substance, also nuclei which are round and full with a relatively well developed chromatin network, although such vacuoles lie in the cytoplasm. Very

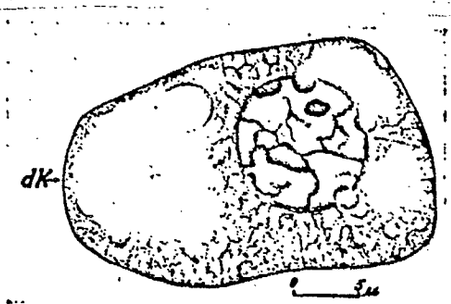

Abb. 25. Dasselbe Tier wie in Abb. 23b. Zweikernige Interrenalzelle. Degenerieren eines Kernes (*dK*) nach Abgabe vieler nucleoler Blasen. Der andere Kern zeigt Nukleolenextrusion.

Illustration 25. The same animal as in Illustration 23b. Binuclear interrenal cell. Degeneration of a nucleus (dK) after extrusion of numerous nuclear vesicles. The other nucleus shows extrusions of nucleoles.

frequently this process of strong secretion is leading, however, to the degeneration of the cell nucleus (dK), as illustrated in Ill. 25, where we can observe only chromatic, irregularly shaped fragments without clearly defining nuclear membranes of the nuclei after considerable extrusions of vacuoles.

In addition to this kind of emission of materials from the nucleus, extrusions of shaped nucleoles from the plasma can also be noticed everywhere, and this process is very intense in the case of this animal. Illustration 25 also shows such a nucleus next to a degenerated one having emitted vacuoles. In general these two processes, migration of vesicles and extrusion of nucleoles, do not occur in one and the same cell; it is interesting to note that in Illustration 25 the binuclear cell created by amitosis, the one nucleus only emits vacuoles while the other exclusively discharges nucleoles.

Very rarely can we observe cells which show that both these processes may occur simultaneously, such as in Illustration 26, where on the one side of the nucleus an emitted vacuole is seen lying directly in front of a distinct "nuclear scar", and where nucleoles are migrating into the plasma on the other side, and remain lying there to be transformed into granulations. p.99

An extreme form of evacuation of nucleolar substance into the plasma is connected with a degeneration of the nucleus. Nuclei are developed which contain very many nucleoles and practically no chromatin any more. Gradually the nuclear membrane disappears on one or on all sides, and the nucleoles are evacuated into the plasma so as to be transformed there

into the well-known granulations (Illustration 27).

Rather often one can also observe nuclei (Illustration 28) which are somewhat resembling the one shown in Illustration 8 (SK). The long appendage, which would seem to consist almost exclusively of nucleolar substance, has extruded two nucleoles. This long appendage is probably tied off, and disintegrates in the plasma, as may be concluded from pictures which show such a darkly stained form in various stages of dissolution, adjacent to a nucleus.

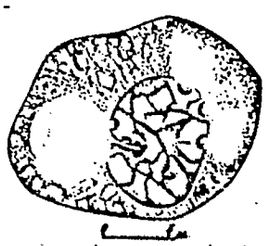

Abb. 26. Dasselbe Tier wie in Abb. 23b. Interrenalzelle, deren Kern die Extrusion von Nucleolen und das Austreten nuclealer Blasen zeigt.

Ill. 26. The same animal as in Ill. 23b. Interrenal cell with nucleus showing extrusion of nucleoles and emission of nuclear vesicles.

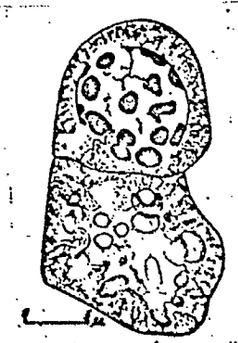

Abb. 27. Dasselbe Tier wie in Abb. 23b. Austritt von Nucleolarsubstanz ins Plasma nach Auflösung der Kernmembran.

Ill. 27. The same animal as in Ill. 23b. Extrusion of nucleolar substance into the plasma after dissolution of the nuclear membrane.

Abb. 28. Dasselbe Tier wie in Abb. 23b. Kern mit Fortsatz, der hauptsächlich aus Nucleolarsubstanz besteht. (Erklärung im Text.)

Illustration 28. The same animal as in Illustration 23b. Nucleus with appendage which mainly consists of nucleolar substance. (Explanation in the text).

The process of evacuation of nuclear vesicles is, however, the one observed most frequently, by far. In this context, I should like to point out that the formation of nuclear vesicles was also [illegible] frequently observable in the normal animal. This indicates that the stimulation with c.h. p.100 does not bring about any change in the mode or method of nuclear secretion, but merely an intensification of the form of secretion typical for this stage. As will be demostrated later, the stimulation of fully grown animals with c.h. does not result in any change either, but only in the intensification of its proper form, i.e. increased migration of nucleoles into the plasma through the intact nuclear membrane.

It is noteworthy that mitoses were never observed in the animal having had injections, whereas the one not treated, or the parallel experimental animal injected with sterile sea sea water, showed abundant mitoses (Chapter IV, 2). This fact will be discussed in greater detail later. Amitoses are found very frequently in the areas of the stimulated i.o. not yet showing any too pronounced secretion, whereas no amitoses are seen wherever the nuclei are fully occupied in producing secretion.

As has been described in Chap. IV, 3, the interrenal cells of normal animals shortly after birth, do not show any emissions of nuclear vesicles from the nuclei but only extrusions of entire nucleoles. The same observation is made in the case of stimulated animals. Illustration 29a shows the i.o. of a normal T.o.-male, weighing 11 g and killed 3 days after birth - as described above in Chapter IV, 3. The two other animals, brother and sister, a male weighing 10g, and a female of the same weight, were injected with 0.05 ccm/g c.h. each, 3 days after birth, and killed 24 hours post injectionem. The resulting i.o. is shown in Illustration 29b. In addition

to the longish strands of connective tissue, developed in the way usual for this stage, and proper orientation of the cells, occurring in the treated animal as well as in the control animal (compare chapter IV, 3), the treated i.o. shows a considerably greater stainability of the nuclei. Under greater magnification, an increase in the nucleolar substance, frequent emissions of nucleoles, as well as greater granulation of the plasma are observed as compared to the untreated animal. Thus no formation or emission of nuclear vesicles can be produced through i.o. stimulation in the case of these animals shortly after birth. This further substantiates the often expressed conception that the formation and migration of nuclear vesicles only occurs in animals before birth.

In comparing the reaction of the i.o. in response to the c.h. supplied, in animals shortly before and shortly after birth, it is striking that the cytodynamic effect is much more p.101 profound in the animal before birth than in the case of both animals after birth. However, we have to consider the fact that the animal before birth was injected with a little more c.h., i.e. a total of 0.067 ccm/g in 24 hours than the animals after birth who were supplied only with 0.05 ccm/g in 24 hours each. Moreover, since the total hormone supplied to the animal before birth was distributed over 3 injections, as against the one injection in the case of the animals after birth, a better "utilization" of the c.h. was probably involved. Even if these factors are taken into consideration, it would appear that the internal tissue of this animal before birth is changed more intensively and more profoundly than in the case of the animals p.102 after birth. It is true that there is some possibility that the animals still being in the mother's uterus receive some

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Abb. 29a und 29b. I.O. von *T. o.*-Wurfgeschwistern. a) Normales Tier, 3 Tage nach der Geburt. b) Am 3. Tage nach der Geburt mit 0,05 ccm/g k.H. injiziert; 24 Stunden p.inj. abgetötet. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 29a and 29b. I.o. from T.o. of the same litter.

a) Normal animal, 3 days after birth.
b) On the 3rd day after birth, injected with 0.05 ccm/g c.h; killed 24 hours p.inj.

Susa, E.H., S.F. (Microphotography)

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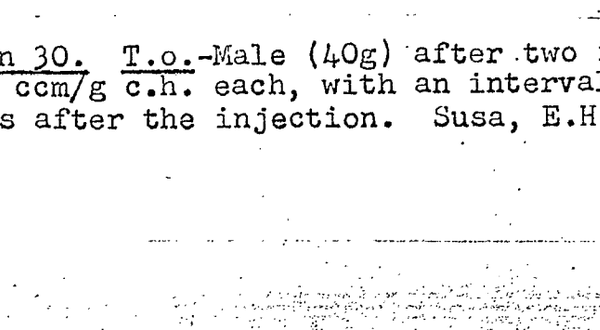

Abb. 30. *T. o.*-Männchen (40 g) nach zwei Injektionen von je 0,03 bzw. 0,02 ccm/g k.H. im Abstand von 6 Stunden. 24 Stunden nach der Injektion. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 30. T.o.-Male (40g) after two injections of 0.03, or 0.02 ccm/g c.h. each, with an interval of 6 hours. 24 hours after the injection. Susa, E.H., S.F. (Microphotography).

interrenal active substance by way of the uterine fluid, and that the i.o. of the uterine animal is thus not yet satisfying its own hormone requirements, although a distinct activity is present, as based on the fact ascertained and described above. On the other hand, the extraordinarily large amount of mitoses observed in the animals taken from the uterus, prepared and not stimulated, would seem to indicate that from the quantitative point of view their i.o. have not yet acquired the correlation of i.o.-weight to the weight of the corresponding animal, established for older animals. It would, therefore, be understandable that isolation of the animal, followed by injections, would constitute a greater strain on the i.o., than in the case of injections made on animals after birth, where the lack of mitoses would indicate that the quantity of interrenal tissue required for the body had been produced to the degree needed. It will be the object of further tests to determine whether or not the uterine fluid of pregnant torpedoes does contain interrenal active substance.

On the basis of the choice of the dose of c.h. supplied, and the duration of the supply, it is possible, in the case of animals weighing more than 35g, to obtain pictures of all transitional stages of i.o. functioning, from weak to highly active phases. Only a few examples are to be given here.

Illustration 30 shows the i.o. of a T.o.-male, p.103 weighing 40g, having been injected initially with 0.03 ccm/g, and with 0.02 ccm/g c.h. 6 hours later, and killed 24 hours after the first injection. For the sake of comparison, we should like to state in this context, that the suprarenal gland of the infantile mouse increases in weight by 50% under the same conditions; the weight increase is caused by the

increase in the cortex (i.o.) (Jores and Beck, 1936). As compared to the animals without injections (Illustration 11), the i.o. of this animal shows the symptoms of increased activity, described several times in the above text (considerable blood circulation, distinct formation of lobes, intensely stained nuclei, extrusions of nucleoles, and granulations in the plasma).

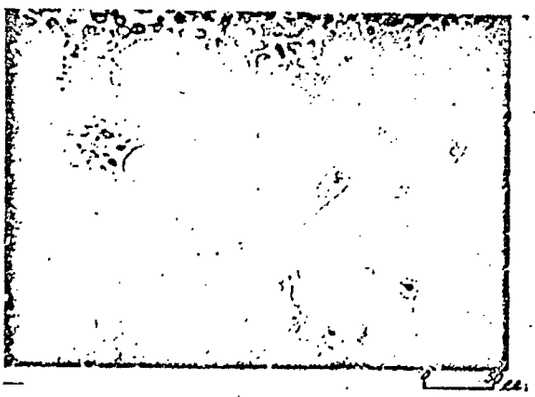

Abb. 31. *T. o.*-Weibchen (380 g) nach sechsmaliger Injektion von 0,015 ccm/g k.H. I.O. am 7. Tage nach der 1. Injektion. Susa, E.H., S.F. (Mikrophotographie.)

Illustration 31. T.o.-female (380 g) after having been injected six times with 0.015 ccm/g c.h. I.o. on the 7th day after the first injection. Susa, E.H., S.F. (Microphotography).

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The i.o. activity can be increased to a much greater extent, if e.g. the injections are continued for several days with smaller doses. Illustration 31 shows the organ of a T.m.-female, weighing 380g, having been injected with 0.015 ccm/g daily, for 6 days, and killed on the 7th day. The considerable activation of the i.o. indicates that doses in the amount of 0.015 ccm/g of this solution containing 1 k.E. (cort. unit) in 0.05 ccm cannot yet be compensated for by the regulating activity of the hypophysis. Physiological examinations (Dittus, 1937) also indicate that there is no compensation in the case of doses of 0.02 ccm/g c.h. If the hypothesis that the hypophysis has a regulating influence is correct, it may be concluded that

the amount of c.h. produced daily by the hypophysis of the normal animal is far below the amount of active substance contained in 0.015 ccm/g of the solution used by me.

Illustration 32 shows how intensive the emission of nucleoles from the nucleus may become, as it represents an interrenal cell of a T.m.-female weighing 70g, injected with 0.02 ccm/g c.h. on each of 3 consecutive days, and whose i.o. was preserved on the 4th day. p.104

With higher doses of c.h., i.o. conditions can be produced which are similar to the one shown in Illustration 12d. In the case of high doses and longer application, the exhausted blister-like nuclei and nuclei with "interior bodies", which have been described in detail, above, can always be seen. It is, in fact, possible to bring about a degeneration of the i.o. with not all too high doses of c.h., which is to be described in the following Chapter.

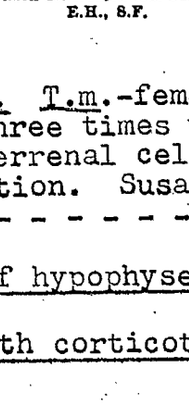

Abb. 32. *T. m.*-Weibchen (70 g) nach dreimaliger Injektion von 0,02 ccm/g k.H. Interrenalzellen am 4. Tage nach der 1. Injektion. Susa, E.H., S.F.

Illustration 32. T.m.-female (70g), after having been injected three times with 0.02 ccm/g c.h. each time. Interrenal cells on the 4th day after the first injection. Susa, E.H., S.F.

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6. The interrenal organs of hypophysectomized torpedoes after supplying them with corticotropic hormones.

The stimulating effect of the c.h. on the i.o. of torpedoes

becomes most obviously and unmistakably evident in the case of torpedoes whose hypophyses had been removed a somewhat longer time ago, therefore having atrophic i.o. before the injections. It had been demonstrated already earlier with regard to physiological effects depending on the i.o., that the i.o. of hypophysectomized animals strongly respond to artificially supplied c.h. (Dittus, 1939).

12 animals were examined, which were injected with varying doses of c.h., once or several times, 3 - 16 days after hypophysectomies. With this experimental schedule, depending on the amounts and number of the doses administered, it was possible to obtain pictures of f.o. functioning covering conditions ranging from weak activity up to extreme forms of incretion development. Some examples may illustrate this.

A T.o.-female, weighing 64g, supplied twice with 0.015 ccm/g c.h., with an interval of 6 hours, 6 days after hypophysectomy, showed that its i.o., 24 hours after the first injection, presented about the same condition as the i.o.-half of a T.o.-male left in the organism for 2 days (compare Illustration 19b).

A T.m.-male (40g), having had its hypophysis extirpated for 12 days, and having been subsequently injected three times with 0.03 ccm/g each, over a period of 3 days, showed a highly active i.o., similar to the i.o. of the pregnant female presented in Illustration 12d. We will forego giving further examples of this kind.

So as to be able to observe the condition of the i.o. shortly before and after the injection of c.h., on one and the same animal, in two cases test excisions of very small portions of the i.o. were made -/after a longer time removal of the hypophyses

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prior to the injections of the hormone hormone solutions - so that the atrophic condition of the i.o. after hypophysectomy had to become obvious. C.h. was then injected and the now stimulated i.o. examined. The piece of interrenal tissue taken out when the trial excision had been made was so small that it could be assumed that this would have no effect on the remaining portion.

Illustration 33a shows cells of the little piece of interrenal tissue taken out for testing purposes, 12 days after hypophysectomy of a T.o.-male weighing 490g; the interrenal cells present the typical atrophic condition after longer hypophysectomy, as described in chapter V, 2, above, which is expressed cytologically in the decreased, ungranulated plasma, faintly stained nuclei, and a lack of larger amounts of nucleolar substance. On the 12th, 13th and 14th day after hypophysectomy, the animal was injected intraperitoneally with 0.02 ccm/g c.h. each day, whereupon the i.o. of this animal was fixed on the 15th day. The resulting picture is presented in Illustration 33b. Adjacent to nuclei which still show a relatively distinct chromatin framework, are others with their "chromatin"-substance almost used up by the round about way of the nucleolar substance. Frequently we observe nuclei, each with a centrally located, larger nucleolar fragment from which threads of chromatin spread out towards the nuclear periphery like the spokes of a wheel, (Illustration 33b, lower right hand corner), as is frequently observable in partly interrenoprivic (compare Illustration 20b) or pregnant animals. Adjacent to them, we find large, blister-like nuclei which show all the stages already described in detail in Chapter IV, 5, with regard to the conglomeration of the stainable substance in the centre of the nucleus. As we

have pointed out there, the development of an "interior body" in the nucleus would indicate a restitution process (and in extreme cases also a degeneration process) after preceding extensive secretion. The heavy nuclear secretion is also manifested by the frequently observable migration of nucleoles into the plasma and the granulations in the plasma. Some areas with very few granules are seen in some cells, probably caused by the absorption of dissolved materials from the blood, as well as by the emission of incretion into the blood channel (Compare Chapter IV, 5). Cell borders were often dissolved. Amitoses could narely be seen; binuclear cells (on the left side in the Illustration) are not frequent either. Mitoses were never seen. In comparing Illustration 3 3a with Illustration 33b, the strong cytodynamic effect of the small dosis c.h. given (0.06 ccm/g in 3 days) becomes clearly obvious. p.107

The second animal, a T.m.-male weighing 60 g, showed a distinctly atrophic i.o. (Illustration 34a) 15 days after hypophysectomy, as the little piece excised would seem to indicate. On the 16th and the 17th day, 0.03 each, and 0.01 ccm/g c.h. on the 18th day were injected. The i.o. fixed on the 19th day after the hypophysectomy, i.e. 4 days after the first injection, had the appearance shown in Illustration 34b-d. Illustration 34b shows a region of greatest activity, characterized by increased nucleolar substance in many nuclei and frequent extrusions of nucleoles, disappearance of the chromatin and inflation of the nuclei, the strong granualtions in the plasma, increase in capillaries and the incretions which can be clearly proven in the blood vessels. Other regions of the organ clearly show all the stages of development of "interior bodies" of the nuclei, as represented e.g. in Illustrations 14 and 15. In some regions

the nuclei are inflated by fluid to such a degree that the nuclear membranes are partly touching each other, deforming each other and that the plasma between such nuclei is frequently greatly displaced (Illustration 34c and 34d - on the left side).

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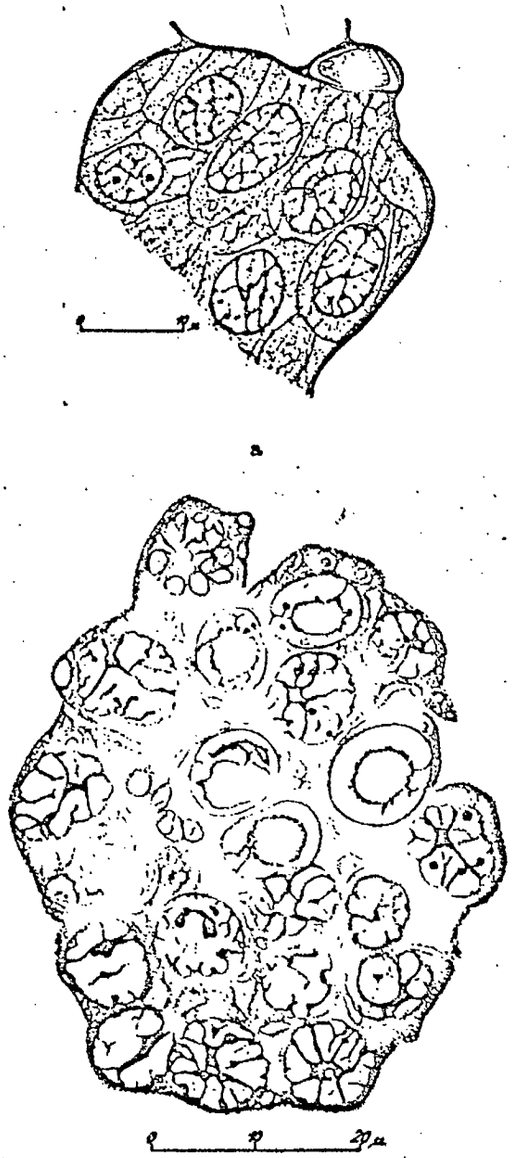

a

b

Abb. 33a und 33b. T. o.-Männchen (490 g). a) I.O. nach 12tägiger Hypophysektomie (Probeexzision). b) I.O. nach darauffolgender dreimaliger Injektion von je 0,02 ccm/g k.H. am 15. Tage nach der Hypophysektomie. Susa, E.H., S.F.

Illustration 33a and 33b. T.o.-male (490 g). a) i.o. 12-days-after hypophysectomy (test excision). b) i.o. after subsequent 3 injections of 0.02 ccm/g c.h. each, on the 15th day after the hypophysectomy. Susa, E.H., S.F.

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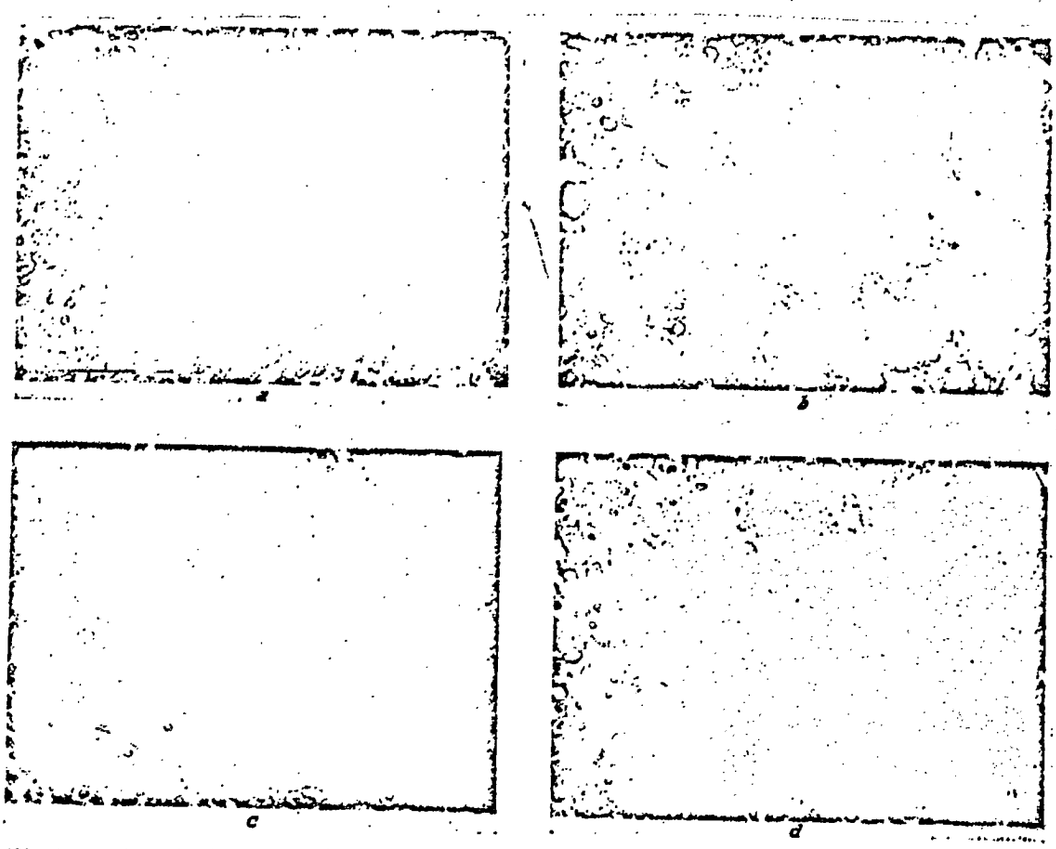

Abb. 34a—d. T, m.-Männchen (60 g). a) I.O. 15 Tage nach der Hypophysektomie (Probeexzision), b)—d) Verschiedene Regionen des I.O. 19 Tage p. op. nach Injektion von insgesamt 0,07 ccm/g k.H. Weitere Erklärung im Text.

Illustration 34a-d. T.m.-male (60g). a) i.o. 15 days after hypophysectomy (test excision). b)-d) Various regions of the i.o., 19 days p.op. after injection of a total of 0.07 ccm/g c.h. Further explanations in the text.

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Cont'd p.107

The entire stainable substances of these nuclei - staining black with iron hematoxylin, intense red with Azan - are located partly in the centres of the enlarged nuclear spaces, partly also adjacent to the nuclear membranes, in the form of well-defined homogeneous lumps. The process of absorption of dissolved materials by the nucleus, described above, has assumed pathological proportions here so that the degeneration of the nucleus is unavoidable. Illustration 34d

shows an area on the right side, where these inflated nuclei have collapsed completely and only the black shapes can be seen - and these bodies also partly dissolved completely. The plasma is still diffusely granulated; cell borders can no longer be determined. The objection that these phenomena occur in the portions of the i.o. damaged by the test excision, is not justified, as the areas affected by degeneration were located in various parts of the i.o., and were surrounded by healthy tissue areas similar to those represented in Illustration 34b. Illustration 34d, e.g., shows a center of degeneration which was diametrically opposite to the point of the excision and closely adjacent to the untouched kidney; in the upper right hand corner of this picture a portion of the connective tissue capsule can still be seen which separates the i.o. from the kidney. This objection can be refuted most unequivocally by the fact that hypophysectomized torpedoes having received p.108 strong doses through injections, show the same phenomena, although their i.o. had not been subjected to the test excisions. Thus it is possible to force the i.o. to such heavy secretion through doses of c.h. surpassing the "biological" framework, that the interrenal cells are unable to restore themselves after too heavy secretion, and perish. p.109 Unfortunately these experiments were not continued long enough so as to be able to observe the deaths of the experimental animals due to the complete degeneration of the i.o. brought about artificially. The two preceding pictures show clearly to how great an extent the cytodynamic effect of the c.h. acts upon the i.o. having become atrophic because of hypophysectomy.

In this context we should like to describe shortly the results of stimulation experiments with c.h. on the i.o.

of other animals. I should like to point out specifically that the cancelling out of atrophy of the adrenal cortex after hypophysectomy through hypophyseal anterior lobe extracts will not be dealt with here, since by supplying total extracts various incretion glands (the thyroid gland, gonads, etc.) might also be stimulated, and thus, in turn, act upon the adrenal cortex so as to make it impossible to determine a specific effect of c.h.

Unfortunately, the data about finer cytological changes of the mammalian adrenal cortex after supply with c.h. are rather scarce. Aside from quantitative findings which report enlarged suprarenal glands after supply with c.h. in the case of hypophysectomized or normal animals (Collip and co-workers, 1933; Anselmino, Hoffmann and Herold, 1934; Jores and Beck, 1936; Moon, 1937), mainly based on a considerable widening of the Zona fasciculatis, and a less pronounced one of the Zona glomerulosa, while the Zona reticularis does not undergo any noteworthy changes, (Anselmino and co-workers, 1934, 1934) - only a few exact cytological data are available. The cytological peculiarities after stimulation are said to manifest themselves in enlarged fasciculatis cells, which get a fine-grained homogeneous plasma. Moreover, the entire organ displays a more highly developed capillary network. As a further symptom the frequent occurrence of mitoses in the glomerulosa and outer parts of the fasciculatis is cited (Anselmino and co-workers), a phenomenon also especially described by Schmeckebier (1934) and Weber (1938), after stimulation with hypophyseal anterior lobe extracts containing c.h. The fact that my material did not show any increased frequency of mitosis occurrence after supply with c.h., is to be discussed in greater detail in the final Chapter.

In view of the processes occurring in interrenal cells of torpedoes stimulated with c.h., it may be assumed, on the basis of our present experience, that the Golgi-apparatus also has a part in forming incretion. Unfortunately I have not yet been able to extend my investigations in this direction. It is interesting that Reese and Moon (1938) were able to note a shrinking in the fascicularis cells of rats after hypophysectomy, and a hypertrophy of the Golgi-apparatus after supply of c.h. The typical and easily ascertainable symptom, (in addition to the widening of the cortex) caused by the c.h. is a considerable increase of the lipoid content, mainly being increased in the zona fascicularis, but also in glomerulosa and reticularis, whereas the hypophysectomy produces a decrease of the lipid content (Anselmino and co-workers, 1934); a fact used by Reiss and co-workers (1936) for setting up a testing method for c.h. Also Atwell (1937) found in the i.o. of the tadpole a decrease of lipid substances after hypophysectomy, which could be cancelled out by c.h., and even led to an increase in lipids. p.110

Conditions of lipids in the selachi an i.o. are to be discussed in the following Chapter.

VI. THE BEHAVIOUR OF THE LIPIDS IN NORMAL AND EXPERIMENTALLY CONDITIONED INTERRENAL ORGANS.

In examining the lipoid content (compare technique, Chapter III) of the interrenal cells of torpedoes, the conditions observed, are, from the very beginning, quite different from those in mammals as the torpedoe-tissue is not subdivided into zones. The examination of the lipids thus has to cover the special conditions in the individual cells, with varying

activities connected with the formation of basophilic granulations. It is obvious that the i.o. of adult animals, not showing any signs of any activities, contain practically the same amount of larger and smaller lipoid pellets in the interrenal cells. In the case of animals shortly before or shortly after birth, the amount of lipids is still somewhat smaller than in the case of fully grown animals. 5 days after hypophysectomy, the interrenal cells become distincly poorer in lipids, a phenomenon which permits the i.o. to be stained only slightly black with osmic acid, about 8 - 10 days post operationem.

Interrenal cells shortly prior to the formation of basophilic granualtions indicate an increased lipoid content as compared to the norm. These cells are completely laden with drops stained black by OsO_4. It is of no importance whether the state of readiness of the interrenal cell to form nucleolar-like granulations occurs in partly interrenopric animals or animals stimulated with c.h. - at the maturing of the germinal products. Any activity beginning in the interrenal cell is always characterized by increased lipid deposits.

There we find also in i.o. showing increased p. 111
activities cells which are laden with little drops to a much greater extent, in addition to neutral cells containing a normal amount of lipids. Adjacent to them - preferably close to the vessels - we also find a third type of cell, however, which contains very few lipids. Staining of osmic preparations with carmalum prove that these cells are full of nucleolar-like granulations. A large amount of these cells either poor in, or free from, lipids, were found in organs showing extreme activities, and thereby correspondingly strong basophilic granulations in the plasmas, as for instance the animals whose i.o. are represented in Illustrations 14, 20b and 33b. I was

never able to prove osmiophile drops in the vessels so that I had no evidence for any emission of lipids to the blood channel in unchanged form, although Fraser (1929) claims to have observed this process in Raja, and submitted microphotographies showing osmiophile substances in the i.o. capillaries. Biedl (1913), in his comprehensive presentation, does not consider the lipids as typical secretion products, either.

The fact that lipoid deposits in basophile granulated interrenal cells are reduced, was described earlier with regard to the i.o. of Ichthyophis (Dittus, 1936). At that time the opinion was expressed that the lipids participate perhaps in a transformed state in the formation of the granulations, and disappear for this reason; an assumption which is to some extent supported by the fact that the often considerable basophile amounts of granules cannot be - from a quantitative point of view - formed exclusively by the relatively small nucleolar substance emitted into the plasma, and that materials from the cytoplasm are, therefore, required for their development. Reichstein (1938) proved that the effective principles of the adrenal cortex, which make it possible for epinephrectomized animals to survive, are derived from sterol. As the lipids of the adrenal cortex or the i.o. are consisting mainly of cholesterol esters and cholesterol fatty acid mixtures (Biedl, 1913), it would appear likely, that they provide the sterol skeleton for the cortex hormone. Taking into consideration the facts determined in the microscopic picture, it would not seem to be wrong to assume that the lipids, together with the substances emitted from the nucleus, develop an incretion appearing in the form of basophile granulations, which contains (among other things) the "Kortikosteron" (corticosterone) which is able to keep interrenoprivic animals alive.

The opinion that the lipids participate in the development of basophile granulations is supported by another fact, p.112 also, to which reference has already been made (Dittus, 1936). In the mammalian suprarenal gland, cells are constantly being newly formed in the Zona glomerulosa and in the outer layers of the Zona fasciçularis, which becomes apparent through the occurrence of mitoses. The cells formed at the periphery, migrate up to the cortical core border, where they perish; the Zona reticularis thus represents the zone of cell destruction. It is interesting that the Zona fascicularis located between the Glomerulosa and the Reticularis, (the Zona fascicularis) is always the one richest in lipids, whereas only very few lipids are found in the Reticularis, but frequently basophile granulations instead. Due to
/the fact that in the case of the interrenal cells of torpedoes shortly prior to the formation of basophile granulations, an increase in lipid substances occurs, whereas a reduction, and even disappearance of the lipid substances is seen after the formation of the basophile granulations, it would seem quite obvious that the Fasciçularis in the mammalian suprarenal gland represents the layer in which the cells absorb or form all the materials including the lipids which will be required later for preparing the incretion, and that after these cells migrated into the Reticularis the definite secretion is formed and given off, whereupon the cells perish. Thus, in contrast with the prevailing opinion, the Fasciçularis would not seem to be the zone with the principal function, but rather the zone where the cells are being prepared for developing incretions, whereas the Reticularis would not only have to be considered as the zone where cells are destroyed but also as the zone where the incretion is definitely developed and emitted. The fact that suprarenal glands stimulated by c.h. showed mainly an extension

of the Fasciculoris, and no changes in the Reticularis, need not be regarded as a contradiction. The increased function of the Fasciculoris actually consists of the act of supplying larger amounts for developing secretions of capable cells, which is expressed by the widening, whereas an accordingly quicker emission of secretion and degeneration of cells would appear to occur in the Reticularis, and thus any widening of the Reticularis after stimulation with c.h. would not seem to be necessary. The opinion that every layer reacts in the manner typical for its function is further supported by the fact that the Glomerulosa and the outer layers of the Fasciculoris, which normally also represent the germinal zone, respond, after stimulation with c.h. on the whole solely with more frequently occurring mitoses as the mode of reaction appropriate for this layer.

VII. THE INTERRENAL ORGANS OF OTHER SELACHIANS. p.113

So as to supplement the findings presented above with regard to the i.o. of torpedoes, the i.o. of other species of selachians were also examined.

1. Sharks: The topography of the i.o. of any shark examined by me has already been described in Chapter II. The microscopic examinations of the i.o. of various species of sharks with resting gonads, resulted in the same picture, in each case.

The i.o. of Scyllium canicula was examined (on 15 animals, 7.5 to 305 g), Sc. catulus (12 animals, 15 - 800 g), Mustelus laevis (6 animals, 70 to 130 g), M. vulgaris (2 animals weighing 60 and 75 g), Carcharias glaucus (2 animals - postmortal: 1210 and 1650 g) and Galeus canis (1 animal - postmortal - length: mouth to tail: 1.38 m). In the cross section, the organ which is surrounded by a cover of connective tissue, shows the same

structure as the i.o. of the torpedo, except that the little lobes are usually less round and are rather developed like strands. Just as in the case of the i.o. of the torpedo, the individual interrenal cell is of polygonal shape, and the plasma is light and foamy after the usual non-fat-preserving preparation methods due to the extraction of the lipoid substances. The round or oval nuclei often show intensively staining chromatin frameworks, each having one or more nucleoles.

The i.o. of female animals with ovarian eggs of 1 - 3 cm in diameter, could be examined in the following cases: Scyllium canicula (3 animals), Sc. catulus (2 animals), and Mustelus laevis (2 animals). In each case the i.o. showed more or less clearly the changes typical for this stage as already described for the torpedo (Chapter IV, 5), i.e. an increase of the capillary network and a more pronounced development of the connective tissue in the i.o., the frequent occurrence of amitoses and intensively stained nuclei, increased nucleolar substance in the nucleus, and evacuation of this nucleolar substance into the plasma through the intact nuclear membrane, with the subsequent development of nucleolar-like granulations.

The same effect was obtained through injection of sufficient amounts of c.h. For this purpose sharks weighing from 20 to 40 g (Scyllium canicula: 6 animals; Sc. catulus: 3 animals, Mustelus laevis: 2 animals) were injected intraperitoneally, one to five times, with doses of 0.01 - 0.05 ccm/g c.h. each. The same i.o. picture resulted as already described in Chapter V, 5, in connection with the torpedo. For this reason we will not repeat the description and pictures in this context.

Also in the case of the sharks with totally removed

hypophyses, the following phenomena were noted about 7 - 8 days after the operations, just as in the case of the torpedoes: a p114 clear atrophy of the i.o. expressed by the obliteration of the lobated structure of the organ, the retrogression of the capillary network, a decrease in the "chromatin" and nucleolar substance of the nucleus, and the disappearance of the lipids. On the whole it may be said that the supplementary tests on sharks gave the same results as the experiments on torpedoes had brought, which were described earlier.

Fancello (1937) who examined the i.o. of Scyllium canicula and Sc. catulus, during the periods of resting and active germ glands, ascertained that the female i.o. assumes a more distinct lobated structure during the development of the egg, and that the cytoplasm of the interrenal cell as well as the granulation in the cytoplasm increases, and that, moreover, the nucleus becomes larger. Fancello did not describe any relation between the changes occurring in the nucleus and the occurrence of granulations. Grynfeltt (1902) believed that there was some relationship between the safranophile granules, which he found in the interrenal cells of Zygaena, and secretion; Grynfeltt was also of the impression that the nucleus of the interrenal cell participated in the secretion process. Unfortunately his reports do not contain any factual statements about these suspected connections.

2. Rajidae: Among the rajidae having resting gonads, the following were used for testing i.o.: Laeviraja oxyrhinus (3 ahimals, 990 - 2670 g), Raja batis (1 animal, 670 g), R. clavata (1 animal, 510 g), and mainly Raja asterias (15 animals, 30 - 685 g). From the histological point of view, the i.o. of the rajidae are essentially built like those of the torpedoes, although, at superficial examination, they would appear to

differ somewhat of those of the torpedoes. This impression is particularly due to the fact that the mostly round lobes building up the orban are much smaller and poorer in cells than those in the i.o. of torpedoes. Moreover, the framework of the i.o. composed of connective tissue is often hard to recognize because of always the/more pronounced interrenal cell granulations causing a relatively intensive staining of the cytoplasm (Illustration 35b). The lobated structure of the organ is more clearly visible in i.o. of most cases of/hypophysectomized [illegible] rajidae, because the granulations in the interrenal cells have disappeared almost completely, and the connective tissue consequently stands out much better as against the light interrenal cells (Illustration 35a). Moreover, the interrenal cells and the nuclei of the rajidae are smaller than they are in torpedoes.

Illustration 35b shows the i.o. of a Raja-asterias-female outside its germ gland activity. The lobated structure of the organ can be seen, as well as the dense capillary network filled with blood corpuscles, and the darkly stained interrenal cells with their clearly stained nuclei, due to the basophile granulations in the plasma.

7 - 8 days after their hypophyses had been removed, p.115
the animals showed distinct manifestations of atrophy; they then showed only very slight granulations , and accordingly, staining of the cytoplasm. Just as in the case of hypophysectomized torpedoes (compare Chapter V,2), the nuclei are also less stainable (Illustration 35a). (11 Raja asterias were p.116
hypophysectomized and their i.o. preserved after 4 - 18 days). Three female Raja-asterias (with ovarian eggs of about 2cm in diameter), however, showed all the symptoms of increased i.o. activities. Illustration 35c, which shows an intersection of the i.o. of such an animal, indicates a rich capillary

p.115

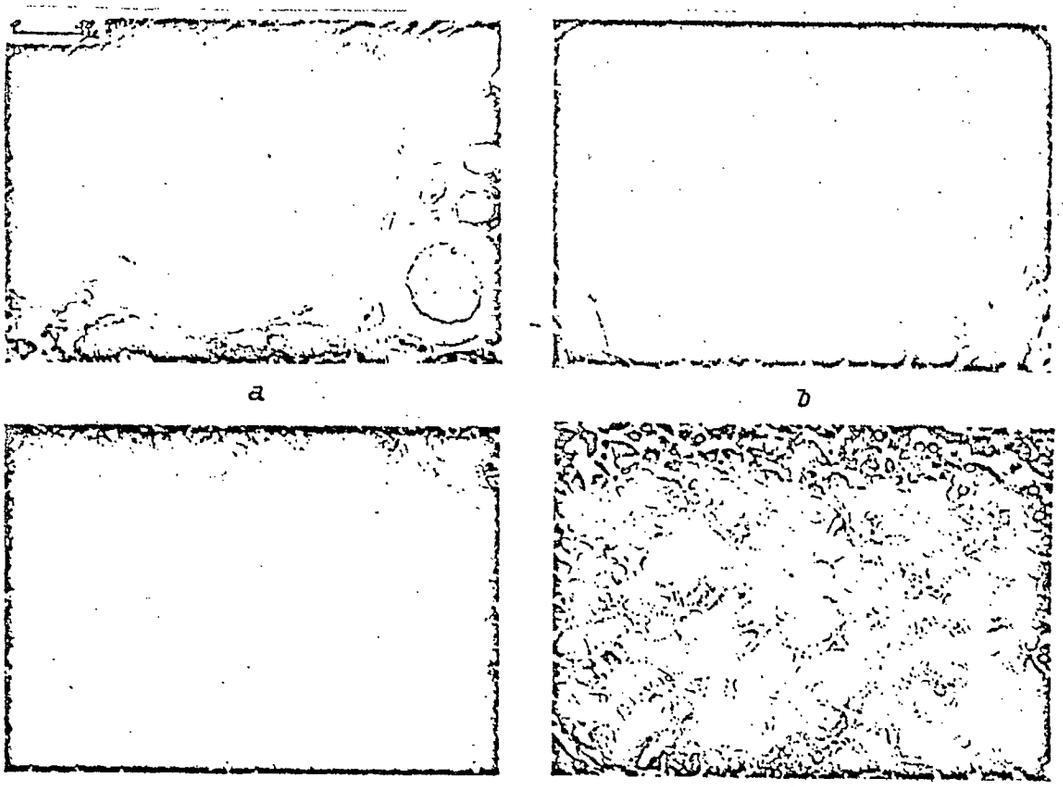

Abb. 35a—d. I.O. von zwei *Raja asterias*-Weibchen. a) (40 g) 14 Tage nach der Hypophysektomie. b) (520 g) Tier mit ruhendens Gonaden. c) (685 g) Tier mit Ovarialeiern bis 2 cm Durchmesser. d) (51 g) nach viermaliger Injektion von je 0,02 ccm/g k.H. am 5. Tage nach der 1. Injektion. Susa, E.H., S.F.

Illustration 35a-d. I.o. of two female Raja asterias.
a) (40g) 14 days after hypophysectomy.
b) (520g) Animal with "resting" gonads.
c) (685g) Animal with ovarian eggs up to 2cm in diameter.
d) (51g) After four injections of 0.02 ccm/g c.h. each, on the 5th day after the 1st injection. Susa, E.H., S.F.

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Abb. 36. I.O. desselben Tieres wie in Abb. 35d, stärker vergrößert.

Illustration 36. I.o. of the same animal as in Illustration 35d, under greater magnification.

network and a strongly developed framework of connective tissue. Some nuclei in the interrenal cells of these organs stain intensively. After the increase in nucleolar substance in the nucleus, which goes hand in hand with the utilization of "chromatin" substances, the extrusions of nucleoles through the nuclear membranes, and intensive development of basophile granulations in the plasma are noted.

Cont'd: p.116

These manifestations become still more clearly obvious in the i.o. of animals which had been injected with sufficient amounts of c.h. Illustration 35d and Illustration 36 show the i.o. of a female Raja-asterias 5 days after the first injection of four doses of 0.02 ccm/g c.h. each on four consecutive days. The granulations in the interrenal cells have become still more distinct. Everywhere migrations of nucleoles are seen, as clearly visible in Illustration 36, exhausted cells which have partly emitted their incretions and whose blistery nuclei with stainable substances located at the peripheries would point to the processes which have occurred. All the functional processes as described for the torpedoes are observable in every detail in the case of the rajidae. The only thing peculiar to the rajidae is the fact that interrenal cells in different stages are always lying next to each other, as also shown in Illustration 35d and 36, whereas in the case of the torpedoes larger organ areas would appear to work synchronally. 6 further animals showed the appropriate pictures after doses supplied at various intervals and in different amounts, in accordance with the treatment.

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It was obvious that the control tests carried out on sharks and rajidae fully confirmed the results obtained from the experiments on torpedoes.

VIII. FINAL OBSERVATIONS.

The present report which aimed at a more detailed cytological examination of normal and experimentally stimulated i.o. of selachians and at a more precise determination of the points affected by c.h. in the interrenal cells, resulted in the following findings:

It was proved that in the case of active interrenal cells the nuclei become intensively stainable at first due to increased "chromatin" substance, and that then nucleolar substance is formed gradually by transformation of the "chromatin", and that individual parts of the nucleolar substance pass through the intact nuclear membrane and extrude into the plasma. There, these fragments disintegrate, and their substances probably build - together with materials from the plasma - granulations staining intensively dark with iron hematoxylin, which then migrate into the blood channel and may therefore be considered as incretions of the i.o. On animals of the species called Torpedo marmorata and T. ocellata another mode of evacuation of nuclear substances into the plasma was noted shortly before birth as a characteristic method for this stage, i.e. the emission of nucleolar substance with the aid of vesicles. (Other processes occurring occasionally which also result in the evacuation of nuclear substances into the plasma, are described in detail in the text.) These facts present a new positive contribution to the disputed fact of the emission of morphologically comprehensible amounts of substances from the nucleus into the plasma.

The exhausted nuclei which are poor in substance undergo a restitution process which consists first in absorbing dissolved materials from the plasma, followed again by the development

of a nuclear structure typical for the "resting" interrenal cell. During this restitution process typical pictures of the nuclei can be distinghuished in interrenal cells after considerable activity, and these are described in great detail in the text.

During the development of the germinal products and pregnancy, increased incretion development in the i.o. is noted, which, in addition to the intensification of the processes just described, also results in a stronger development of the vascular network and a typical change in the connective tissue. This effect can also be achieved by partial removal of the i.o. thus forcing the portion of the i.o. remaining in the organism to become more active. With a hormone solution containing c.h., i.o. activities were also increased in normal animals; and it is to be stressed specifically that this increase in activity resembles completely, in the histological picture, those increased activities occurring normally in animals during their sexual phases or in partially interrenoprivic animals. p.118

Through sufficient supply with adrenal cortex active substance, an atrophy of the i.o. can be produced, since i.o. activity has become superfluous; and this can be achieved to a still greater extent by extirpation of the hypophysis and the elimination of c.h. connected with it.

By supplying doses of c.h. artificially, the atrophy of the i.o. of a hypophysectomized animal cannot only be eliminated but with proper dosage the activity of such an i.o. may be stepped up considerably to the extent of showing the same conditions as noted on pregnant animals. However, if the doses are too great, the processes are stepped up so much that they assume pathological proportions and lead to the degeneration of the i.o.

The increase of the basophile, nucleolar-like granulations during the formation of the sex products or after experimental stimulation of the i.o., or their decrease after extirpation of the hypophysis or artificial supply of adrenal cortex active substance proves also - in addition to the morphological findings with regard to the migration of these granulations into the blood - that these granulations have the nature of incretion.

Since results are identical for very active, normal animals (pregnancy) and animals stimulated artificially through the use of the hormone solution, and because of the fact that even small doses of the hormone used eliminate the atrophy of the i.o. having occurred after hypophysectomy, while e.g. larger doses of thyreotropic hormone did not produce any effects, it is concluded that the hormone solution actually did contain an active substance having a specifically stimulating effect upon the adrenal cortex, or the i.o., namely the c.h.; this is to be assumed all the more because this fact may be considered as proved due to other results mentioned in Chapter V,1. Also according to these results, a correlation between the hypophysis and i.o. is most probable in the following connection: increased emission of interrenal active substance represses the production p.119 of c.h. of the hypophysis, and vice-versa, an opinion which had already been presented by Jores (1937).

From the cytological point of view, the influence of c.h. is at first observable on the cell nucleus which shows first visible changes - changes already described above.

Amitosis is of great importance in the i.o. Since amitoses always occur in such i.o. which are shortly prior to or in a stage of increased activity, and because an extension of the surface of the nucleus naturally facilitates the emission of nucleolar substances, the significance of this process would seem to lie in an enlargement of the nuclear surface rather than in a propagation of cells, i.e. a growth

process; especially since the investigations proved that the number of cells with two and more nuclei is many times larger than the number of amitoses observed so that it may be concluded from this fact that the cell division occurs much later after the nuclear division. Therefore, the amitoses are not to be interpreted as "division amitoses", but rather as "reaction amitoses" (Benninghoff, 1922). Amitoses are always observed in active organs. However, in the case of strong stimulation, amitoses are repressed, as in this case each cell is apparently required for preparing incretion.

I found mitoses in larger number only in cases of untreated animals prior to birth; otherwise they can be seen only very rarely. While the occurrence of a large number of mitoses is always described in connection with the adrenal glands of pregnant mammals or animals stimulated with c.h., I could never observe this phenomenon in pregnant or stimulated animals. Quite on the contrary, injections of c.h. even put an end immediately to the mitoses in the i.o., as demonstrated by a T.o.-male prior to birth whereas the untreated parallel experimental animals showed an extraordinary amount of mitoses. It is not possible that the amount of the dose has any relation to the non-occurrence of mitoses, as the amounts used were the same as in the case of the mammals; moreover, cold-blooded animals always react to hormones to a lesser degreee than warm-blooded animals do.[1]

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[1] The possibility that the nuclei with "interior bodies" (represented in Illustration 15a and b) might represent clotted prophases, has to be exluded, as the various pictures of the nuclei forming a consecutive series do not support this opinion in any way. Moreover it would not be understandable why the mitoses of the selachians should be interfered with while they are occurring quite normally under the same conditions in the case of the selachians.

In my opinion the explanation for this is as follows: p.120 There is a polar orientation in the adrenal cortex of the mammal, i.e. the interrenal cells are first located in the outer development layer (Glomerulosa and the outer part of the Fasciculatis), and then migrate towards the interior in the course of their cell lives, while forming the incretions, and finally towards the innermost layer, the Reticularis, where they perish, where they actually have to perish, as their is constant supply from outside, and it is impossible for them to migrate from the interior of the adrenal gland. This polar orientation of the cortex, in which each cell has to pass through each layer, and in doing so undergoes the condition peculiar to the layer concerned, leads to the destruction of the cell in the inner layer, and to the replacement from the outside by mitotic divisions. In an organ which is not having any polar orientation, such as is the case for the selachian i.o., each cell passes through its cycle of development during secretion without having to perform substantial migrations (except for little shifts in relation to vessels) and is able to restore itself again, as there is no necessity for any degeneration as in the case of the adrenal gland of the mammal. The cells of the selachian i.o. are so to speak independent elements not dependent to any appreciable degree on the position in the total organ, while the fate of the cells within a specially-oriented organ such as the mammalian adrenal cortex is strictly determined and the independence of the individual cell in relation to its position within the entire organ has been lost.

This fact would also explain the lack of mitoses in the stimulated i.o. of the selachians tested. In their case the occurrence of mitoses is not connected with increased secretion activity, while this has to be necessarily so in the case of the mammalian adrenal glands.

It follows that the individual layers of the adrenal cortex have to be compared with the corresponding functional stages of the individual selachian interrenal cells - a concept which had been pointed out earlier (Dittus, 1936). Also the fact described in great detail in Chapter VI proves the accuracy of this conception, i.e. that the i.o. cells of selachians, shortly prior to the formation of nucleolar-like granulations, contain many lipids, and thus are similar to the Fasciculatis cells, whereas cells with granulations contain few, or no lipids, and resemble in their structure to the Reticularis cells, except for the fact that the reticularis cell has to perish whereas the interrenal cell of the selachian restores itself as a rule.

In the experimental schedules chosen by me, the i.o. initially responded to stimulation always with intensified formation of incretion. (Because of the reasons described above - Chapter IV, 2 - is initially also to be considered in the same context). It is probable that in the case of more prolonged but not too strong stimulus, a cell propagation of the organ through mitosis sets in so as to meet the continuous greater strain. p.121

IX. SUMMARY

1. The development and position of the interrenal system in various groups of selachii is examined; three different types could be established, which were called the scyllium-, the raja- and the torpedo-type.

2. The histology and cytology of the interrenal organ of normal torpedoes, from the time shortly prior to birth until sexual maturity of the animals, were described.

3. An incretion of the interrenal organ can be proven in the form of basophilic granulations, which are first located in the cytoplasm of the interrenal cells, and later enter the blood channel. The granulations are formed with the help of the nucleolar substance which left the nucleus and builds up these granulations in conjunction with materials from the cytoplasm. Probably the lipids in the interrenal cells also have a share in forming the granulations.

4. The emission of nucleolar substance to the cytoplasm may occur in various ways. The normal process is the extrusion of entire nucleoles through the intact nuclear membrane. In the case of animals shortly before birth, nucleolar substance is also emitted by means of nuclear vesicles. In rare cases there are also other ways of transmitting nucleolar substance to the plasma (see text).

5. The cells, and the nuclei respectively, undergo typical changes while the incretion is being formed and also in the course of their later phases of restitution. (See text for detailed description). Upon completion of the incretion development, such cells may also degenerate.

6. The interrenal organs show clear signs of shortly prior and after birth.
increased activity/. Animals from 35 g upwards, which are not in the sexual phase, have a low activity which essentially remains constant. During the development of the sexual products (in the case of the male as well as the female), and in the course of pregnancy, an increased functioning - often to a considerably increased degree - of the interrenal organ is noted.

7. In addition to these cytological changes in the p.122
interrenal cells (different nuclear conditions, evacuation of nucleolar substance, granulations in the plasma), an increased activity results in a typical transformation in the interrenal organ, which manifests itself in the formation of "little lobes", the appearance of a thicker capillary net, of a larger sinus, and in changes in the connective tissue.

8. Upon removal of the hypophysis, an atrophy of the interrenal organ is noted, mainly due to the lack of the c.h. in the body.

9. Through sufficient supply with adrenal cortex hormone, atrophy also sets in in the interrenal organ in normal animals.

10. Through removal of part of the interrenal organ, that part which is left in the body may be brought to high activity, whereby the reduction of the interrenal active substance in the organism, demonstrable after the removal, is overcome. An increase of the number of mitoses was not noted during the experimental period of time chosen.

11. Through sufficient supply with corticotropic hormone, added artifically, the interrenal organs of normal and hypophysectomized torpedoes can be brought to an in-

creased activity which may assume intensive forms and even become pathological in the case of appropriate doses. If the doses are too high, a degeneration of the organ sets in. An increase of the number of mitoses during the experimental period did not occur.

12. The increased activity of the interrenal organ, which had been induced experimentally (under 10. and 11.), occurs entirely according to the forms observed in normal animals.

13. It is concluded on the basis of cytological and physiological findings (Chapter V,1) that the hormone-solution contained a substance with a specific effect upon the interrenal organ (adrenal cortex), the corticotropic hormone.

14. The attack by the corticotropic hormone can be defined in the individual cell by typical nuclear transformations in addition to increased deposits of lipids into the cytoplasm.

15. While basophilic granulations are developed, the lipids disappear; they are probably used up in this process.

16. The comparison of the selachian interrenal organ, which is not polarly orientated, with the polarly-orientated mammalian interrenal organ shows that the individual stages of interrenal cell functions may be compared with the corresponding stages of the cells located in the different zones of the adrenal cortex. The behaviour of the lipids, of the connective tissue and of the basophilic granulations serves as a proof for the accuracy of this

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interpretation. At the same time this perception may serve to explain satisfactorily the difference between the interrenal organ and the mammalian adrenal glands in the behaviour of mitoses of normal and experimentally-treated animals.

17. Other selachians tested (sharks and rajidae) indicate the same conditions, in principle.

18. The present investigation lends new support to the finding that the over-production of an incretion gland depending on the hypophysis restrains the corresponding glandotropic hormone of the hypophysis, and the reduction of hormone production of a hormone gland depending on the hypophysis results in an increased emission of glandotropic hormone.

Finally I should like to express my sincere gratitude to the Deutsche Forschungsgemeinschaft (German research society) for its support. I also wish to thank Professor Dohrn for the hospitality extended to me during my stay at the Zoological Station at Naples in 1936 and 1938, and his Assistent there, Dr. G. Kramer, for his assistance.

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Translator's Note:

A book by Eugen Korschelt ("Das Haus an der Minne") is advertised on page 126 in the original text. p.125

Publishers: N.G. Elwert, Marburg. This is a biography by the well-known Marburg biologist, Eugen Korschelt.