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THE PROGRESSIVE ODOR OF ANTS.

ADELE M. FIELDE.

I. STATEMENT OF HYPOTHESES BASED ON RECENT AND FORMER EXPERIMENTS.

1. *The Specific Odor.* — The mother-ant transmits to her offspring the distinctive odor which is identical for ants of all ages and of both sexes within the species. This odor is appreciated among ants by organs near the proximal end of the funicle.[1]

2. *Progressive Odor.* — Female ants, including queens and workers, have, besides their specific odor, an odor which may be termed progressive. Queens of different lineage have different progressive odors. In a queen this odor is either unchanging or changes very slowly, and it is similar to that of her newly-hatched female offspring.

a. As worker-ants advance in age their progressive odor intensifies or changes to such a degree that they may be said to attain a new odor every two or three months. This progressive odor is appreciated among ants by organs in the penultimate joint of the funicle.[2]

b. Male ants have no progressive odor unless it be superficially incurred through association with workers; but the male carries latent in his spermatozoa the progressive odor of his mother. In other words the progressive odor is always recessive in the male ant.

c. The progressive odor of each new generation of females is

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[1] "Artificial Mixed Nests of Ants," A. M. Fielde, BIOLOGICAL BULLETIN, Vol. V., No. 6, November, 1903, p. 320.

[2] "Farther Study of an Ant," A. M. Fielde, *Proceedings of the Academy of Natural Sciences*, November, 1901, p. 531.

determined by the odor of the mother latent in her egg, and the odor of the father's mother latent in the spermatozoön. The progressive odor therefore changes in each generation of females.

d. The progressive odor manifest in female ants is the cause of the separation of ants of the same species into hostile colonies, and is of great advantage to the ants in their individual and their communal life.

3. *The Incurred Odor.* — An ant may incur from its associates an odor which is not inherent in itself, and which may be removed by washing. It may be transferred from ant to ant through air or through water. It arises from the substances that give the specific odor and the progressive odor and that create the nest-aura.

II. Recent Experiments with the Progeny of a Single Queen, Componotus Pennsylvanicus.

In 1901 I found that the odor of working-ants of *Stenamma fulvum piceum* changes with their age,[1] forty days being the minimum of time in which there occurs a change so great as to effect the behavior of ants of the same colony toward one another at their first meeting.

In 1902 my further experiments indicated that a cause[2] for the hostility of one colony toward another of the same species and variety is a difference in odor coincident with difference in the age of the colonies.

In 1904 my observations on several species of ants,[3] representing three subfamilies, gave further evidence of their change of odor with advance of age, and indicated that the odor of the queen is unchanging, or that her odor changes much more slowly than does that of the workers.

I have now had under observation for more than two years a colony of *Camponotus pennsylvanicus*, in which the assertion of a

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[1] "A Study of an Ant," *Proceedings of the Academy of Natural Sciences of Philadelphia*, July, 1901, p. 449.

[2] "Notes on an Ant," *Proceedings of the Academy of Natural Sciences of Philadelphia*, September, 1902, p. 609; "Cause of Feud Between Ants of the Same Species," Biological Bulletin, Vol. V., No. 6, November, 1903, p. 328.

[3] "Power of Recognition Among Ants," Biological Bulletin, Vol. VII., No. 5, October, 1904, p. 244.

progressive odor in the workers is definite and indisputable, the five successive broods included in the experiment being the issue of one queen.

The N Queen. — This queen was captured on Nonamesset Island, July 28, 1903. She was then deälated and was probably the mother of the hundred workers seen in her wild nest, and also of the ants that afterwards hatched from the many cocoons brought with her to the laboratory. She remained under my care and, unless another is indicated, she is the queen referred to in the herein recorded experiments.

The N1 Group of Workers. — Some of the captured workers were transferred to Dr. Irving A. Field, and they remained segregated in his care, usually at Harvard University, until the time of the experiment in which they appear. As no other than male offspring had appeared in this group during the two years of its separation from the queen mother, the workers composing it in August, 1905,, were certainly acquainted with the queen previous to her capture in July, 1903. Of the age of these workers of course nothing more was known than that it exceeded two years.

On August 6, 1905, I introduced into this nest,[1] where there were six major and five minor workers and about thirty larvæ from their own eggs, the queen-mother from whom these eleven workers had been separated for two years. The queen showed instant hostility, seized a major worker by one of its mandibles, braced herself on the sponge and held her prisoner there during the ensuing seven hours. All the other workers, sometimes six at a time, examined the queen meanwhile. They patted her with their antennæ, nabbed her gently, and licked her back and legs. Two of them, touching her body with their antennæ, appeared to dance for joy, shuffling their feet with great rapidity during several consecutive minutes. The queen then began to drag the worker that she had seized, and upon my releasing the latter, took a position near the larvæ-pile, as if to claim her incipient grandsons as her exclusive property, opening her mandibles at every worker who approached. Then followed a most curious and pro-

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[1] All the artificial nests referred to in this paper were of the Fielde pattern. See "Portable Ant-Nests," BIOLOGICAL BULLETIN, Vol. VII., No. 4, September, 1904.

longed effort on the part of the workers to placate the queen-mother. They surrounded her at all times, offering her regurgitated food. Whichever way she turned, there stood a humble servant with a proffered mouthful of pabulum. As many as seven workers simultaneously offered nourishment to her. Every worker of the eleven seemed bent upon wooing and winning her, and she was not for a moment left without attention. These efforts were unceasingly continued, and were meeting with a fair degree of success, when I removed the queen on the following morning.

This experiment showed that the workers all recognized the odor of their queen after two years of separation from it, and that the segregated workers had during the same interval acquired an odor unfamiliar to the queen, who had meanwhile met none of her daughters who were over fifteen months old. It also showed that major workers, having in this species nearly the same form and sometimes nearly the same bulk as has the queen, are like minor workers in having a progressive odor.

On August 7 I introduced into this N 1 group a marked major and a marked minor worker, daughters of the N queen, but many months younger than any of the ants in this group. The visitors were received with signs of curiosity, but with perfect amiability, though no younger sisters had been encountered within two years by these N 1 ants. The odor of the younger sisters was perfectly recognized by the eleven residents, and I removed the former.

I then introduced a young winged queen of the same species, *Camponotus pennsylvanicus*, but of an alien colony. The resident ants attacked her instantly and with exceeding virulence. In an instant she lost an antenna, one worker was pulling out her remaining antenna, and three others were dragging her by her legs. The scrimmage was fierce, and before I removed the intruder four of the residents had received injuries that resulted in their deaths. The residents had given to the alien queen a reception strongly contrasted with that accorded to their own queen-mother; while the havoc wrought by the alien queen indicated that, if unable to escape from the nest, she might have destroyed all the workers and have remained a fostering mother to their larvæ.

The N2 group. — During the first week in August, 1903, the queen deposited about a hundred eggs, and from these were reared five minor workers, denoted here as the N2 ants. These workers hatched between April 24 and May 10, 1904, and were therefore some fifteen months old at the time of the experiment here recorded. These workers had never met other ants than those of their own segregated group, and were therefore unacquainted with the odor of ants in any wise unlike themselves. They had never lived with the queen, had laid no eggs, and had the care of no young. On July 16, 1905, I put these ants into a new and very small nest where I had isolated the queen-mother without young. The five workers were wholly at ease with the queen, and hastened to evince their devotion in ant fashion; but the queen opened her jaws whenever they approached her, and was somewhat querulous in her behavior during the ensuing two days. The queen had lived during the previous five months with daughters, all minor ants, less than five months old, and her behavior indicated a difference in the odor of her younger and her older daughters. Her memory was manifestly less tenacious than that of the workers, who, on their part recognized in their queen the odor that had been their own in their infancy, fourteen months earlier.

The N3 group. — This group consisted of two major workers hatched in July, 1904, and four of their younger sisters, minor workers, over five months and less than a year old, all the issue of the N queen. The two majors were acquainted with sisters older than themselves, while the minors knew no sister older than these two majors. The members of this group had all lived with the queen, and had been separated from her and living in segregation since February 14, 1905. They had deposited no eggs, and they had the care of a few introduced larvæ. On July 18, 1905, I introduced into this group a sister nine months older than the oldest in the group. The majors, who had had acquaintance with sisters much older than themselves, did not attack the newcomer at all, while every one of the minors, never having met a sister so old as was the visitor, attacked, dragged and finally killed her.

It appeared that the behavior of the two major workers was dictated by memory, while that of the four minor workers was an effect of hostility created by the presentation of an unfamiliar odor. The major workers were either wanting in compassion, or else they lacked means of communicating with their younger sisters, for although they were each double the size of any minor ant in the group, they did not interfere in behalf of the victim.

The N4 Group. — The queen was transferred without eggs or young to a new nest on July 14, 1904. She laid no eggs thereafter until December, 1904, and from the eggs then deposited the five minor workers constituting the N4 group were hatched between February 19 and March 23, 1905. These workers were therefore four or five months old at the time of the experiment. On July 16, 1905, I removed the queen from the nest, leaving the five workers in charge of twenty larvæ, the issue of the queen's December eggs. Into this group of five minors, who had never met older sisters, I introduced one of the majors from group N3, now just a year old, and twice or thrice the bulk of any of the five residents. The introduced ant was instantly and violently attacked by three residents. This attack indicates that the major, like the minor ants, like in shape and size as they are to the queen, change their odor with advance in age, as do minor workers.

Having removed this visitor, I introduced a marked large minor worker, fourteen months older than the residents, a sister of theirs, hatched from eggs deposited by the queen in August, 1903. This visitor was likewise violently attacked, every one of the five residents manifesting hostility to her, and the next day I found her mangled body on their rubbish pile.

The N5 Group. — This group consisted of two minor workers, the issue of the queen's December eggs, sequestered in their cocoons and hatched on September 8, 1905. They were at once placed in segregation in a new nest, with a few larvæ and cocoons from their mother's eggs. Ten days later these ants drove away from their pile of young any member of the N4 group of sisters six months older than themselves.

In these experiments it appears that it is the age of the workers, not the age of the queen at the time when she deposits the eggs from which the workers issue, that determines at any date their progressive odor. All the ants engaged in this last mentioned experiment were certainly the issue of the eggs laid by the queen early in December, 1904. That there is similar progress in odor among ants of the same age and species is indicated by an immediate and amicable association of ants that are reunited after a period of separation so long as two years.[1]

Whether two mutually hostile groups could be created from among the worker-progeny of a single queen would depend on power of memory in the older workers. By segregating from the pupa-stage the broods of different summers, it would be found that the younger sisters would always be hostile to the older sisters, because the older sisters would present an unfamiliar odor to the younger. The hostility of the older sisters toward the younger would be nullified by their memory of the odors by which they had themselves been characterized at earlier periods in their own lives. If the younger sisters bore an odor which the older sisters, through the lapse of many years, should have forgotten, then the hostility would become mutual. It is certain that worker-ants can remember for years an odor with which they have once become familiar, and it is probable that they remember such odors as long as they live.

When ants of different groups meet amicably, either the members of these groups have the same odor, or else they have at some time in their lives been familiar with ants bearing the presented odor. If one group recognizes a familiar odor, while the other group discerns a strange odor, then those finding themselves among strangers will try to escape, or will make attack. There is no love at first sight between ants of different odors.

III. The Odor and the Sense of Smell in Male Ants.

Male ants apparently bear a specific odor, beside the odor that may be incurred during their residence with nurses in the home nest. I have introduced males of different species into the nests of *Stenamma fulvum*, *Cremastogaster lineolata*, *Myrmica rubra*, *Formica sanguinea*, *Formica Schaufussi*, *Camponotus pennsyl-*

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[1] "A Cause of Feud Between Ants of the Same Species Living in Different Communities," A. M. Fields, BIOLOGICAL BULLETIN, Vol. V., No. 6, November, 1903, p. 327.

vanicus, *Camponotus pictus*, *Camponotus americanus*, *Lasius latipes*, and *Lasius umbratus*, and all these males have invariably been killed within a day or two. If hybridization is to be effected among ants it will be necessary to cause the males and females to become acquainted with one another within a few hours after hatching. When hatched in the same nest, males of *Stenamma fulvum* pursue queens of *Cremastogaster lineolata* with the same ardor that they show in pursuing queens of their own species. In my mixed nests the failure of individuals of these two genera to mate was manifestly due to physical and not to psychic incompatibility.

In the summer of 1905 I had material in my stock of ants for experiments giving evidence that the male ant has at hatching the specific odor of his virgin worker-mother. My E mixed nest consisted of workers of *Camponotus pictus*, *Formica neogagates*, *Formica subsericea*, and *Stenamma fulvum*, all hatched during the last week of July, 1904, and kept in the same nest until the first day of January, 1905, when the *Stenammas* were segregated apart. They remained in segregation until August 22, 1905, when I put into their small nest, where there were ten workers and a few eggs, a fine male *Camponotus pictus*, the offspring of a virgin worker-mother who had shared the nest of these *Stenammas* until she was five months old. This young male was received by the resident *Stenammas* with evident pleasure. They licked him, regurgitated food to him, and rode on his back. He continued to live happily with them for many days. He bore a familiar specific odor, although hatched among segregated workers of his species, eight months older than any that these *Stenammas* had known; and this familiar odor made him welcome. His fate was in strong contrast to that of some of his brothers or cousins introduced into another nest. At the time of these experiments I had also a nest, marked D, of eleven *Stenamma fulvum* workers, that had hatched in a mixed nest during the last half of August, 1903, and had lived for several months with *Camponotus pennsylvanicus*, and *Formica subsericea*, but had never met a *Camponotus pictus*. These eleven *Stenammas* had lived in segregation since July 17, 1904, and were destitute of young, when on August 22, 1905, I introduced into their nest a newly hatched *Camponotus pictus* male, the offspring of a virgin worker, a brother or cousin of the one in the E nest above mentioned. These ants of the D nest at once began to harry him, and although he was eleven millimeters long and very sturdy, while none of the *Stenammas* were more than five millimeters in length, they harried his life out within two days. Repetitions of this experiment gave similar results in every case.

The eggs from which these *Camponotus pictus* males were produced were deposited by their virgin worker-mothers in May, 1905, five months after the said mothers were separated from the *Stenammas* that the said mothers had lived with during the first five months of their lives. It therefore appears that the male progeny of virgin workers have not the progressive odor which characterized their mothers. The males have, however, a specific odor, an odor recognized by the ants through certain joints of the antennæ, and this odor is doubtless the stimulus calling forth the exceeding care given by the workers to young males with whose specific odor they are familiar.

On August 26 I put into each nest, the E and the D nests above described, two males of *Stenamma fulvum.* These males, the first of their species ever encountered by these workers, wery treated alike in the two nests. They were so eagerly grasped be several residents at once that it seemed as if they must lose their lives through the determined efforts of the workers to retain them. They were not left free for several hours ; but so judicious were their virgin captors that no injury was done to the captives, and they lived in health and honor many days in these nests. In the E nest the *Camponotus pictus* male continued to be their associate.[1] In both the E and the D nests newly hatched

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[1] A deälated queen *Camoponotus pictus* captured alone in the open on July 5, was kept in isolation till August 15, 1905, when she received amicably into her small artificial nest two young males of her species, the offspring of virgin worker ants. She licked them, regurgitated food to them, and during the several days that they remained under my observation, remained in close companionship with them. Later on this queen also received in amiable fashion the virgin mothers of these males, the worker-mothers having been kept by me in segregation during their whole lives. As this queen was captured near the spot in which the workers had their origin a year earlier, these ants may all have been of one colony. This queen killed young males of *Formica argentata* and *Stenamma fulvum* introduced into her nest.

Stenamma workers, from the same colony as were these males, were immediately killed.

Since the males avoid, or are indifferent to, ants of other species than their own, unless hatched among such species in artificial nests, it appears probable that they discern the specific odor of other ants. But they probably lack the sub-nose that perceives the progessive odor of workers. Male ants of various species placed under observation in one of my artificial nests, grouped themselves according to species, but did not quarrel with males of species unlike their own. I infer that the only inherent odor of males is that of their species; but that they are the medium through which the progressive odor of their female progenitors is transmitted to the egg that produces a female, the progressive odor being latent in the males and reappearing in their female descendants. Only the egg receiving a spermatozoön would produce a female, and this female would be endowed with her paternal grandmother's tendencies in progressive odor, the progressive odor thus manifesting itself only in the female line of descent. The fact that the worker progeny of a queen, sequestered from the pupa stage, will receive their queen-mother or the queen-mother's sister with equal pleasure, indicates similarity of odor in the product of the same queen's impregnated eggs.

I venture to predict that there will be found in female ants secretory glands or organs that are wanting or are rudimentary in the male, and that these organs are the producers of the pressive odor. There must be in both males and females secretory glands or organs producing the specific odor which is common to both sexes. These diverse organs might be identified through the possession of both sets by the female and of a single set in the male. It is also probable that the male lacks the glands that secrete the scent whereby the female lays down her individual path from the nest, and he may also lack the sub-nose which discerns this path-scent. The male seems to be unable to lay a path, and, in a change of domicile by the colony, he is carried bodily by the females to the new nest. It is through his appreciation of the specific odor and his lack of perception of the progressive odor that the male is best fitted for his distinctive office in the ant world.

IV. The Progressive Odor of Queen Ants.

The change in the inherent, transmissible, progressive odor in a line of queens is probably slow and cumulative, but that such a change occurs is evidenced by the behavior of any segregated group of *Stenamma fulvum* workers, a species in which the queens generally remain in the colony in which they are produced. When workers from such a colony are segregated from the pupa-stage upward, it becomes difficult to find, in the wild nest, any queen that these segregated workers will accept as their own. In this species, I have reared worker-offspring from queens that were sequestered from all males except those of their own colony,[1] and these workers willingly associated with their worker-cousins. That the change of odor is but slight in a single generation is also shown by the fact that the worker-daughters of a queen, after having been segregated from their pupa-stage upward, and with no criterion of odor save that of their own bodies, will affiliate with their queen-mother at a first meeting, though they always examine her with exceeding care before rendering complete homage.

The gradual change in odor, through the introduction of the male element, from generation to generation, may be crudely represented by the use of letters as symbols of the odor of queens of the same species and variety.

The Roman numerals at the left denote successive generations of mated queens.

The letter *a* is used as a symbol of the odor characterizing two sister queens; the other letters as symbols of the odor inherited from the paternal grandmother.

I.	a,	I.	a,
II.	$a + b$,	II.	$a + l$,
III.	$a + b + c$,	III.	$a + l + m$,
IV.	$a + b + c + d$,	IV.	$a + l + m + n$,
V.	$a + b + c + d + e$,	V.	$a + l + m + n + o$,
	etc.		*etc.*

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[1] “Notes on an Ant,” *Proceedings of the Academy of Natural Sciences of Philadelphia*, December, 1902, p. 605.

The female descendants of sister queens would thus become more unlike in odor with every generation.

An odor providing the means of recognizing a maternal ancestor, or another descendant of that ancestor, may be dominant through more than one generation of females.

The fact that worker ants who have never met any queen will as joyfully associate with their queen-mother's sister as with their own mother, indicates that sister-queens have the same odor after mating that they had before mating, and that the first divergence in odor becomes apparent to the ants only in the offspring of sister-queens that mated with males capable of imparting unlike odors to their respective progeny. The worker ants, having attained the distinctive progressive odor characterizing their mothers' worker-offspring for the current year, may produce males who will each impart to his progeny the distinctive odor borne by all the female issue of the queen with whom he mates. Each generation in the line of queens would then depart farther from the odor of the queen ancestor, and we should find, as we do, colonies in which all the female inhabitants are inimical to all the female inhabitants of another colony. There would also be produced, as in colonies of *Stenamma fulvum*, where queens mate within the nest and remain to increase its population, the phenomenon of callows that, if segregated from the pupa-stage, refuse to affiliate with queens from the nest in which were deposited the eggs from which these callows issued.

During several years I have been interested in ascertaining whether adult, queenless workers would willingly accept a queen who was indisputably of another colony of their own species, and among many experiments I have never seen such an acceptance. If forced into association, escape of either party being made impossible, the workers may after a longer or shorter interval live peaceably with the alien queen, as they also may do with alien workers. But such forced alliances do not result in normal prosperity, even when a whole year is allowed for the cementing of friendship. So exacting are the ants concerning adherence to their standard of odor that they prefer a queenless state to the presence of an unknown ant-odor. Observations made by me in the summer of 1905 accord with my earlier ones. Eleven workers of *Stenamma fulvum piceum* had been inmates of one of my mixed nests, with *Camponotus pennsylvanicus* and *Formica subsericea*, all hatched between August 14 and September 3, 1903. The *Stenammas* had been removed from the mixed nest, and kept in segregation since June 23, 1904, and had never met a queen. On August 13, 1905, I introduced into this nest a young, winged queen of the same variety as these workers, on the twenty-fourth day after I had isolated her to ensure her freedom from incurred odor. The queen fled from the group of workers and constantly tried to escape. She was attacked whenever I forced her into the group of workers, and was caught and killed by them on the ninth day of her sojourn. A deälated queen introduced later, from the wild colony to which these workers originally belonged, was also killed by them.

Since ants possess so discriminating a sense of smell, and are so exacting concerning an adherence to the criterion established for their nest, and since even those ants who have had an extended experience in ant-odors, and who have been queenless for two years, refuse to affiliate with a queen of an alien odor though of their own variety, we may hardly expect that they will voluntarily associate with queens of another species. During the summer of 1905 I introduced queens of other species into segregated, queenless groups of adult *Stenamma fulvum*, of *Formica neogagates* and of *Formica Schaufussi*, that had had their sense of smell highly educated by long association with workers of two or three different species of ants, but in every case the introduced queen was killed within a few days, in spite of her constant efforts to keep aloof from the workers.

In no species of ant have I found workers that would tolerate the presence of any queen of unfamiliar odor, nor any queen that would willingly remain among workers of unfamiliar odor. Although all species of ants have not been thus tested we may well assume that what is shown to be a fundamental trait in a few species will manifest itself in all species of the tribe.

V. EFFECTS OF THE PROGRESSIVE ODOR IN THE COMMUNAL LIFE OF ANTS.

Since the queen is ordinarily the earliest occupant of the ant-nest, and since her callow young have the same odor as herself, the odor of her earliest nest must at first be the same as is that of the queen. Probably this odor is at all times dominant in the permanent nest; but as the progressive odor of the workers is gradually added thereto, the nest-aura would be thereby modified. The change in the nest-aura, cumulative with the age of the colony and the increase of the inmates, would be so gradual that all habitants of the nest would at all times find it familiar and therefore congenial. The greater dominance of the queen's odor in the earlier nest may be the cause of the persistence with which many workers cling or return to the old habitation even after the majority of the colony has for sound reason removed to a new abode.

It appears probable also that diffused ant-odor is in direct ratio to bulk of ant-body, and that a cause of the common activity of workers in adding the lesser to the larger pile of brood, sometimes even against the inhibitory effects of light, is due to the more manifest odor of the larger pile.

I have at different times during several years observed in my artificial nests a most curious phenomenon among ants that had long lived amicably together. Several or many workers were seen standing around one ant as if holding a court of inquiry concerning this associate. Sometimes the associate is proscribed, sometimes rent limb from limb. This extraordinary behavior is probably due to the victim having attained a progressive odor that is obnoxious to many other inmates of the nest because unknown to them. This might happen to an aged ant whose horde of companions were all young. It might also happen that in prowling for food, or in raids made on the nests of aliens, the worker ants would bring in alien young for food, and that this much licked and tended young would incur the progressive odor of the nurses. At a later period the introduced ant might produce a progressive odor unlike that of the multitude inhabiting the nest, and it would therefore be doomed to destruction. Ostracisms or violent deaths, such as sometimes occur in nests where amity has long prevailed, are probably to be explained by the attainment by some of the inmates of a new and therefore an alarming progressive odor.

There may be seen among ants of the same variety, and even in the same individual, all degrees of attraction and repulsion towards other ants at a first meeting with them. Such manifestations range all the way from cuddling, caressing, cherishing devotion through indifference and inattention, distrust, suspicion, animosity and enduring, ferocious enmity. The inciting cause is doubtless the progressive odor of the visitor, and the practical end is the preservation of the chemical standard of the nest.

Whatever the action of the ants, it is always more obvious when there are numerous young in the nests, and when the nest-aura is well established.

During five years of fairly constant study of ants I have seen no evidence that their antennæ are the organs of any other sense than the chemical sense, and I am convinced that any statement concerning the behavior of ants may well be distrusted if it ignore the dominance of the olfactory sense over the conduct of the ant, the ant's almost inconceivable minuteness of discrimination in odors, or the ant's marvelous memory of odors that have been encountered. Only when ants are segregated from the pupa-stage, and full record kept of every experience of theirs in meeting other ants, can the investigator truthfully declare that ants behave in a certain manner in the presence of other ants. Moreover, as every ant acts on personal experience and individual memory, the ants should be considered singly as well as in groups and communities, when a theory of their behavior is to be enunciated. But when the total history of an ant is known, the investigator may accurately predict the behavior of that ant toward other ants. There is exceeding uniformity of behavior among ants having an identical history.

The progressive odor of the worker ants is manifestly an advantage in their communal life, since it furnishes the means whereby every ant can recognize its home and its fellow-citizens, avoiding nests and communities other than its own. The uses of this odor within the colony may also be numerous, and it may determine the distribution of labor in the ant community.

Through the male element, it probably differentiates the odor of queens of the same species, enabling the workers to find, to defend, and to cherish their own queen.

It differentiates ants otherwise alike ; determines their distribution in separate communities ; dictates the behavior of members of one colony toward those of another colony, and in connection with an acute sense of smell and a powerful memory, is a dominant factor in the life of the individual ant and in the structure of the ant-colony.

REACTIONS TO LIGHT AND MECHANICAL STIMULI IN THE EARTHWORM PERICHÆTA BERMUDENSIS (BEDDARD).

E. H. HARPER.

Recent work concerning the behavior of earthworms has related chiefly to their reactions to light. Since the contributions of Hofmeister and Darwin, and that of Hesse ('96) there have been a group of recent papers by Parker and Arkin, Miss Smith, Adams and Holmes, which have been devoted chiefly to the directive influence of light. In the present state of the discussion of this subject the current theory of tropisms has been called in question, according to which the earthworm is oriented directly by light. Holmes has shown that light induces a general state of activity leading to random movements of which those toward the light are checked and those away from it continued, this resulting in final orientation.

This paper aims to show that random movements are a feature of less strong light, tending to disappear with the increase of intensity, and are replaced by direct orientation in very strong light. It is also shown experimentally that the earthworm is more sensitive in the extended than in the contracted state, and that this has an important bearing upon the production of random movements. The explanation given of this is that when extended the sensitive elements of the skin are expanded over a greater surface. This is shown to have a bearing upon the production of random movements as follows: Locomotion consists of a succession of extensions and contractions and as each extension begins in a state of lower sensibility the anterior end may be projected toward the light, only to be checked when its increase of sensibility with extension makes the stimulus appreciated. Movements away from the light are not so checked. In stronger light the sensibility of the worm when contracted is sufficient to suppress movements toward the light at the outset. In such light the worm appears to be orientated without trial movements. It is important that the worms be kept in the dark before all experiments, as their discrimination diminishes and random movements begin again when this is the case.

It is shown that the reactions which are typical of the life in the burrow are more definite and controlled by weaker stimuli than reactions in the open, and this may be expressed by saying that the earthworm's organization is more highly adapted for life in the burrow. Reactions in the axial direction are definite and more sensitive to stimuli than lateral movements in response to light.

The genus *Perichæta* is noted for its agility, and of its special reactions the leaping movements are the most notable.

Description of the Species.

Perichæta, the eel-worm, as it is called by gardeners, is an exotic genus of earthworms which is said to be quite commonly established in greenhouses in the old world, and also in gardens in parts of France, where they have been introduced, it is said, from the east. The only mention of *Perichæta* having been found in this country, that has come under the writer's notice, is that of Garman, who reported a species of *Perichæta* as becoming established in greenhouses in Urbana, Ill. The writer found *Perichæta bermudensis* (Beddard) in a greenhouse in Evanston, Ill. In suitable conditions of soil these worms flourish in great abundance.

The genus *Perichæta* is noted for its activity. The squirming movements which have given it its name of eel-worm are a striking exhibition of agility. This sort of movement is not confined to *Perichæta*, but is developed in the genus to an extent not found elsewhere. By alternate contractions of the longitudinal muscle bands it makes a series of leaps, by which it may waltz about for quite a distance. It reacts in this way when handled or disturbed, as when uncovered from its burrow.

The worms are of rather large size. They are found often measuring nine inches in lengh when killed fully extended. They are rather pointed at both ends. The continuous circles of setæ on each segment give the name to the family. The clitellum is a complete band or girdle encircling segments 14–16. A large pair of spermiducal glands shine through the opalescent skin behind the clitellum, making a conspicuous mark. The dorsal pores are very prominent, exuding an abundant yellowish mucus. The everted buccal cavity is used as a proboscis, and is thrust out constantly in its feeling movements. The blood vessels are prominent, shining distinctly through the skin. The very numerous, minute, diffuse nephridia are a feature which, along with the continuous circles of setæ, have caused considerable discussion as to whether these conditions are primitive for earthworms or secondarily derived.

The Theory of Tropisms.

The orientations to light and other stimuli, which are among the most striking phenomena in the behavior of the lower animals, have received various explanations. After the first anthropomorphic explanations of these movements, based upon likes and dislikes, there came an apparent revolution of ideas bringing in explanations of seemingly great simplicity. As the physiology of plants, particularly of the higher plants, had made considerable progress towards a solid physico-chemical basis, there was a transference of conceptions based upon plant physiology to the realm of animal behavior and the orientations of the lower animals were illuminated by analogies drawn from plants. For example, we find the assertion of identity between heliotropic phenomena in plants and animals. The mechanism of the tropism was not a reflex according to this conception, but was a unique form of movement to be added to the classification of animal movements into reflex, instinctive and voluntary.

The current theory of phototropic or -tactic phenomena as applied, for example, to the earthworm, was that when light strikes one side of the animal so as to cause unequal stimulation of the two sides, it changes the tone of the muscles on the side affected. The muscles of one side are thus either relaxed or their tension is increased according as the animal happens to be positively or negatively phototropic. It is bent away from or toward the source of stimulation by the direct action of the environment upon the protoplasm. The tropism is accordingly regarded as a peculiar kind of forced movement, dependent upon the chemical nature of the protoplasm.

Jennings has shown in the case of the Protozoa and also the Rotifera that the tropism theory gives an untrue explanation of the mechanism of orientation. These animals are not directly swerved away from or toward the source of stimulation, but they have their peculiar methods of reaction and orientation in the direction of the stimuli is effected by a sort of "trial and error" method.

Reactions of Earthworms to Light.

Since Darwin's account of the habits of earthworms there has been a series of papers devoted chiefly to the directive action of light upon these forms. Parker and Arkin, Miss Smith, Adams and Holmes have studied the reactions of earthworms crawling over surfaces, exposed to light stimulation from one side.

Parker and Arkin observed the head movements of worms placed at right angles to the direction of the light and determined that 65 per cent. of the movements were indifferent, *i. e.*, straight ahead, 30 per cent. were away from the light and 4 per cent. toward it. They regarded the various head movements in different directions as due to a variety of chiefly undefined causes in addition to light and since 4 per cent. were toward the light they assume that as many of the negative responses would be due to other causes than light. So subtracting 4 per cent. from 30 per cent. the remaining 26 per cent. they regard as the measure of the negative phototactic response. Adams showed in addition that the earthworm is positive to very weak light.

The observers mentioned did not consider the question of the mechanism of orientation. Holmes takes up the current tropism theory and questions its explanation of the mechanism of orientation for these animals. He shows that the various extension movements appear to be of a simply random character, due to a general stimulation by light. The way in which orientation is effected he describes as follows. Movements that are toward the light are checked and the animal draws back and usually moves in the opposite direction. Movements away from the light do not lead to further stimulation and so are prolonged farther, and as a final result of such random movements, the worm gets into the direction of the rays, in which position the stimulation of the sensitive anterior end is least, and it then continues to move straight ahead. Any swerving from this path leads to an increase of stimulation and hence is corrected. Holmes regards none of the movements as forced by light. All are random in direction but certain favorable ones are followed up and unfavorable ones checked by the increase of stimulation resulting from them.

Holmes proposes his theory of the "selection of random movements" only as one factor in phototaxis, not wishing to exclude the possibility of a slight amount of directive influence in the light. His reason for so doing is based on the observation of himself and the other experimenters alluded to that there is an excess of negative turnings over positive ones. Of course if the movements of the animal are random there should be an equal number of movements in the positive direction as in the negative, when one considers only the first movements occurring after stimulation. Holmes counted a number of first movements and found them about as equally divided between the positive and negative side as could perhaps be expected (23 : 27). Parker and Arkin found an excess of negative movements over positive of 26 per cent. Miss Smith (on the same basis of reckoning) found an excess of 39 per cent. and Adams, using different intensities of light, found that the excess was greater with an increase in the intensity. If the observers did not count only the first movements after stimulation but also many subsequent movements, the excess of negative movements is not against the supposition of their random character. It may be well for clearness to suppose a case. Of one hundred first movements after stimulation (when the worms are placed at right angles to the light) there should be an equal number of positive and negative, if they are purely random. But according to the theory, negative movements tend to be continued while the positive ones are checked and may be followed by negative movements. This would give rise to an excess of negative movements in any large number that were counted. Holmes says that the excess of negative movements may be due to one of three causes—accident, failure to count many of the slight positive movements which are easily overlooked, or to a slight orienting tendency of the light. Holmes undoubtedly has in mind first movements only, when he assumes that an excess of negative movements is against the supposition of their random character.

Holmes's theory of the "selection of random movements as a factor in phototaxis" is thus based upon observational evidence which is easy to verify. It is easy to observe that the movements toward the light are apt to be checked and the movements away to be more prolonged. It is less easy to note in weak light, as the final result of orientation takes longer in that case.

Absence of Random Movements in Negative Phototaxis in Very Strong Light.

All of the experimenters referred to used artificial light except Miss Smith, who used diffuse daylight. Since all of them but Holmes took for granted the direct orienting power of light, they did not care to put the matter to a crucial test. It would seem that a test of the orienting power of light would require the use of lights of various strength, and especially of very strong intensity, since the perceptive power for light is so poorly developed in the earthworm. A test of the orienting power of direct sunlight is a very easy thing to make. Place the earthworm upon a sheet of wet paper in a beam of direct sunlight from a window. The light may be passed through a water chamber. The results are sufficiently obvious as to leave no doubt of their general nature. *Perichæta* is oriented directly away from the light, when placed at right angles to the rays. The first effect is a turning of the anterior end away from the light and by a series of turns the worm gets into the oriented position and crawls directly away. Usually the result is produced without a false movement. It is immaterial whether heat effects are excluded by passing the light through water or not. A species of *Lumbricus* was experimented with and behaved in the same manner.

If the sheet of paper is turned as the worm turns, so as to keep it at right angles to the rays the worm will travel in a circle continuously. To show the difference between the orienting effect of sunlight and that of an ordinary artificial light the following experiment was tried. By using a sort of searchlight consisting of a tube of asbestos paper surrounding a 32 c.p. incandescent light and narrowed to a small aperture, the light was so manipulated by the hands as to keep it constantly directed upon the anterior end of the worm, with the worm at right angles to the rays. In this way the worm was kept under constant stimulation and caused to turn through one complete revolution and the time required was noted. The process of turning was slow and was effected by a series of readjustments involving many trial movements in the opposite direction. Most commonly about two minutes was necessary. In twenty such cases the average time required was five minutes, the greatest time, twenty minutes.

In the beam of sunlight as before stated the worm turns continuously without trial movements. The difference in behavior in the two cases is so striking that the occurrence of an occasional positive random movement in the sunlight is plainly seen not to affect the general result. When the worm is exposed to the sunlight, if a passing cloud obscures the sun, random movements begin to appear. Miss Smith, who used diffuse daylight from a north window, observed that the worm moves in a general direction away from the light, but in an uncertain manner. Adams, using a graded series of artificial lights, showed that the per cent. of negative movements increased with the intensity. Adams did not observe the whole process of orientation since he placed the worms in an illuminated box and observed the direction of their movement after an interval of stimulation. Holmes used artificial light of only one strength. A Welsbach burner was also used to give an intermediate intensity between those before mentioned. Worms were used that had been kept in the dark and they were brought suddenly into this powerful light. They all moved away from the light with very little appearance of random movements. At each forward extension they would turn a little away from the light so that their path appeared like a curve. It is not meant to be stated that there were no random movements. But there could be no hesitation in saying that there was a decided difference in their reaction under the stronger light. Fresh worms would by a series of turns get into the oriented position frequently without a noticeable random movement. If the worms were kept in the light for some minutes, they lost sensitiveness and their random movements began to be evident.

OCCURRENCE OF POSITIVE PHOTOTROPISM.

When using a 32-candle-power incandescent light it was noticed that some individuals behaved positively. About 6 per cent. of 200 worms tested showed the positive reaction. But at a few inches distance from the light these worms would apparently become negative. Heat effects were not excluded however. The following is a typical instance. An earthworm crawling on a table moved straight toward a 32-candle-power incandescent light until within a few inches, when it began to swerve and without pausing moved in a continuous curve away from the light until it was in the line of the rays, when it continued to move in a straight line away from the light.

DIFFERENCE IN THE SENSIBILITY OF EARTHWORMS TO LIGHT IN THE CONTRACTED AND EXPANDED STATE AND THE BEARING OF THIS FACT UPON THE PRODUCTION OF RANDOM MOVEMENTS.

The conclusion reached is that earthworms are oriented directly by light, but owing to their low degree of sensitiveness their movements are uncertain except in very strong light. The influence of light produces a number of noticeable effects upon the behavior. First, there is a state of general stimulation or restlessness inducing locomotion. Second, in light not strong enough to produce direct orientation the worm projects its anterior end in any direction. If toward the light, the worm after stretching out its anterior end will again retract it as if stimulated. If the worm is checked only after making an extension movement toward the light, the conclusion would seem to be that the anterior end is more sensitive when extended than when in the contracted condition. One may test this conclusion by further experiment. If a light is flashed suddenly upon a contracted worm the influence of the stimulus seems to affect it gradually, leading after an interval to movements. The extended anterior end responds far more quickly to sudden changes of stimulation. The basis for this difference in reaction must be in the fact that when the head is extended the sensitive elements in the skin are spread out over more surface than in the contracted state. A simple experiment will illustrate this fact. If an earthworm is crawling on a moist paper it may be shaded by the hand or otherwise. When the worm crawls to the edge of the shadow and thrusts out its anterior end into the light it is jerked back suddenly. But if the light be thrown upon the worm when contracted, there is no sudden response, but only a gradual awakening to stimulation, as evidenced by subsequent movements. The bearing of this observation upon the movements of the worm would seem to be as follows : The worm contracted is like an animal with its eyes partly closed. It extends its head at random, thus gradually receiving the full stimulation upon its surface. If the movement is toward the light, this causes it to contract more or less and so check stimulation. If the movement is away from the light, the oblique illumination produces less stimulation and the movement is more prolonged. An animal with eyes, as a crustacean, or an insect, is of course so organized that movements toward the light may be checked, as it were, at the outset, in the case of negatively phototactic animals.

It is to be observed that the earthworm begins these random movements while in the contracted state. After extension it draws up its body by means of the longitudinal muscles and is therefore in the contracted state. It then advances again, and at each advance there may be a random change in direction. Thus the worm begins these random movements when in the contracted state and under minimum stimulation. The nature of its locomotion and of the sensitive elements in its skin necessitates the alternation of states of low and high sensitiveness. The random movements of an earthworm under light stimulation are consequently of an entirely special character, due to causes inherent in its structure.

To recapitulate, three situations in regard to light have been described, with their characteristic reactions. First, in weak light, second in strong light and third in a situation involving change of light intensity.

The stimulus of a change in intensity causes the animal to draw back its anterior end slightly and it then usually alters its course. When crawling under the influence of sufficiently strong light, it bends its head away from the light at each successive advance, until it gets into the oriented position. In light not strong enough to have the directive effect its extension movements are random, an advance toward the light being checked and orientation being brought about by following up of favorable random movements. There are only two responses in reality, the checking or drawing back of the head involving the symmetrical use of the longitudinal muscles of both sides, and the turning response, involving the longitudinal muscles of only one side, that opposite to the source of stimulation. The two responses may also be combined.

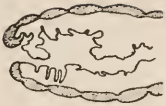
The Anatomical Basis for the Difference in Sensitiveness to Light in the Extended and in the Contracted State.

The text figures introduced are intended to make clear the reason for the difference in the sensibility to light of the anterior end in the contracted and extended state. Hesse, who has worked on the organs of light perception of the lower animals, has shown the structure of the light cells in many species of earthworms and has worked out their distribution segmentally. He shows that these cells are most numerous on the first segment, and especially on the prostomium (which is fused with the first segment in *Perichæta*) and that their number diminishes rapidly on each segment as we go farther back. It is consequently the very tip of the animal (the posterior tip as well) which is most important for the perception of light, although light cells are found in small numbers over the whole length of the body.

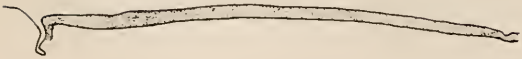
The sections of *Perichæta* (Figs. 1 and 2) show that the first segments are subject to great extension and contraction. It was not possible to get the worm fixed in the fullest state of either extension or contraction. In Fig. 2 it is seen that the first segment is partly inrolled into the buccal cavity in the state of contraction. For further demonstration of this point the epithelial layer alone, of the first segment, is represented in the extended and the contracted state in Figs. 3 and 4. It is seen to be greatly thickened as well as inrolled when contracted. The effect of this on the light cells is seen by comparison of Figs. 5 and 6. The light cells are on the basement membrane. The thickening

1

2



3



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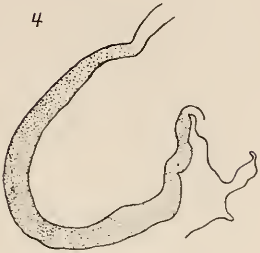

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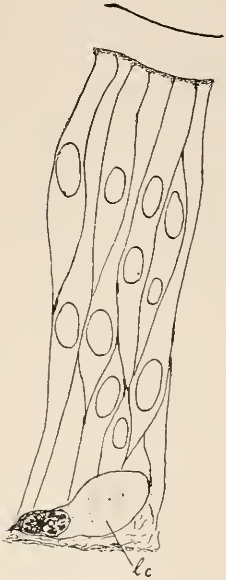

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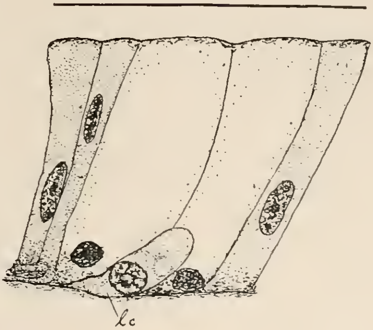

The figures illustrate certain features of the worm in the extended and in the contracted state. The states of extension and contraction represented are not the most complete possible.

FIGS. 1 and 2 are sagittal sections of the first five segments of an extended and a contracted worm respectively. (*a*) body wall, (*b*) everted buccal cavity slightly protruding, which is used as a proboscis. It is to be noted that the first segment, which is the most sensitive to light, is partly inrolled in Fig. 2

FIGS. 3 and 4 are sections of the dorsal epithelium of the first segment of an extended and contracted worm respectively in the same plane as the last.

FIGS. 5 and 6 give sections of epithelium in the extended and contracted condition. (*l. c.*) light cell.

of the epithelial layer must of course tend to cut off light from these cells. The inrolling of the most sensitive region is another important factor.

The Bearing of Experimental Results upon the Habits of the Earthworm.

It is a truism that in all experiments upon animals the relation of the experimental results to the normal life of the animal should be kept in mind. The behavior of the earthworm has not been systematically studied as a whole except by Darwin. It is obvious that all the experimenters mentioned have studied the reactions of the earthworm in only one phase of its activity, and that phase is not what we should call the normal life of the worm. It is as if the experimenters had chosen the situation of the earthworm as we find it crawling on the sidewalks after a heavy rain as being its typical mode of life. None would probably admit this sooner than themselves, and doubtless they have regarded certain facts as too obvious to require mention. Does the fact that the normal life of the earthworm is carried on in a burrow affect our view of the experimental results obtained ? Now the earthworm does spend a portion of its life, during the night time, crawling on the surface of the ground in search of leaves, and also during sexual activity it is less mindful of the light, as is stated. The earthworm leaves its burrow rather reluctantly. Darwin describes the earthworm as retaining the posterior end in the burrow while making searching movements in all directions in search of leaves. In drier weather we know that worms burrow deeply and seldom are found near the surface, depositing their castings in old burrows instead of on the surface.

If the worm is at home almost exclusively in the burrow we should expect those responses which are typical of the burrow life to be better organized and more definite than its activities when crawling in the open. The movements which are typical of the life in a burrow are mainly in the line of the axis of its body. Are these movements and the responses which control them of a more definite nature than its lateral movements ? We may first consider the typical burrow movements in response to light. These may be imitated easily by using a screen to shade the worm or portions of it while crawling on a moist surface, preferably covered with a thin layer of dirt.

If a worm which has been kept in the dark is placed on the moist surface, and the screen is suddenly moved so as to expose the anterior end to light, it contracts the anterior segments slightly, sometimes so slightly as to be barely noticeable, and crawls backward into the shadow. If the posterior end be illuminated in the same way the worm crawls forward into the shade, but after a noticeably longer interval. A slight twitching of the posterior end may be noticed at first, if the light is suddenly turned on. The worm always crawls forward when stimulated posteriorly, If a worm is crawling backward it can always be reversed by stimulating the posterior end. Crawling backward is of course the method by which the worm comes to the surface to eject castings. The two sorts of responses described are of the kind called "photopathic" as distinct from phototactic, and they serve of course to inhibit the worm from leaving its burrow in the light. These "photopathic" responses are very definite and the stimulus calling them forth may be quite weak light. Adams has shown that in very weak light *Allolobophora fœtida* is positive and he suggests that the worm leaves its burrow in response to the stimulus of very weak light upon its anterior end. These observations show that the movements of the worm in its burrow are very definitely controlled by the light, so far as they may come in contact with it by their more sensitive anterior and posterior ends. The middle of the worm is less sensitive to light but its sensitiveness may be shown in the following way.

If the worm is placed on the moist surface exposed to full light from overhead (a 32-candle-power incandescent was used, at a distance of 15 inches) and a screen is then brought over the posterior part of the body leaving the anterior end exposed, the worm does not draw back as when the anterior end was suddenly illuminated. Instead it begins to make random movements in various directions. It may crawl farther out into the light, thus bringing the middle portion into stimulation. This movement is however checked before the more sensitive posterior end is exposed. After a noticeable latent period showing the lower sensitiveness of the middle portion, the worm crawls back under the shade rather quickly, but usually not completely. The commonest way in which the worm gets under the shade is as follows: It makes all sorts of random movements in every direction, and tries to burrow into the thin layer of dirt, until it accidentally gets the tip of the anterior end under the shadow of the screen. It then at once is oriented, so to speak, and crawls completely under the glass. It may crawl under as if circling around a post. The imaginary post may be exposed to the light so that the posterior part has to crawl forward into the light to get around the post. Usually, however, the anterior end travels faster so as to jerk the middle part under the screen at once.

These so-called "photopathic" reactions are consequently very definite and predictable because they are adaptations important in the normal life of the worm. As compared with the random lateral movements we see that they are controlled by weaker stimuli and are more definite. The anterior and posterior ends are more sensitive than the middle for the obvious reason that the ends alone come into contact to any great extent with light stimulation.

The lateral movements, which are typical of life outside the burrow, are as we have seen of a random nature and less definitely controlled. The worm "dashes back like a rabbit into its burrow," to use Darwin's expression, under a weak stimulus. But when crawling on the surface the same strength of stimulus produces only a general irritation and swaying random movements occur which lead to orientation away from the light only after many trials. With a higher intensity of light the worm is oriented more quickly. Thus we see that a very high stimulus is required to produce a direct sidewise movement away from the light while a very weak stimulus will cause it to move back into its burrow away from the light. The random lateral movements are aptly described by Holmes as "inconsequential vermiculations." But this description does not apply to the movements which are typical of its burrow life. The worm is as definitely adapted to the burrow and as little adapted for life in the open as some other burrowing animals of higher rank that could be mentioned. However this statement must be modified when we consider that a worm exposed to the light on the ground does not trouble to make random movements but begins to burrow into the soil immediately. After heavy rains we see them washed out of their burrows, and crawling in unwonted places when they are unable to burrow.

REACTIONS TO MECHANICAL STIMULI.

Perichæta goes through its peculiar jumping movements only under mechanical or similar stimulation, never under the influence of light, so far as we have observed. When touched with a needle on the anterior end it contracts the anterior segments slightly and may begin to crawl backward or it may go forward, lifting its head and making various random movements before settling on any direction. With a slightly stronger stimulus the anterior end turns away slightly from the stimulus. Increase the stimulus and the worm may contract the longitudinal muscles of the opposite side so as to jerk the body around 90 or even 180 degrees, and so give it a new direction. Or the worm may go off into a whole series of jerks, so that there is a complete gradation between the extent of the responses, depending upon the stimulus. More important as determining the extent of the reaction is the condition of the worm. Well-fed worms in fresh condition, when just dug out of their burrow, spring around in the liveliest fashion. If handled they give a series of movements which must make it difficult for an enemy, a bird, for instance, to pick them up before they get a chance to crawl under cover. When stimulated they exude an abundant yellowish mucus. Whether this is an offensive secretion to its enemies is not known to the writer. When a point in the middle of the worm is stimulated the body recoils away from the stimulus at that point and there is a slight swelling due to contraction of the longitudinal muscles, like the contraction and shortening of the anterior end under stimulation. Occasionally the worm may move violently toward the stimulus, but this seemed to be due to an overstimulation producing a complex of effects rather than a simple reflex.

The leaping movements of *Perichæta* are certainly the best examples of random movements that are afforded. They are exclusively adapted to those chance circumstances when the worm gets out of its burrow. They lead in no definite direction, though they may carry the worm to a considerable distance and enable it to distract the enemy. They are a conspicuous example of the character of most movements of the earthworm, which belong to its limited life outside the burrow.

Conclusions.

The method of reaction to light of the earthworm is far removed from the sort of "trial and error method" of the Infusoria, as analyzed by Jennings. Its avoiding reaction in strong light is of the nature of a definite reflex which causes it to turn directly away from the stimulus, if the whole body is in the light, or to retreat into its burrow, if only the anterior end is stimulated, or go forward if the posterior end alone is stimulated. Methods of trial and error in reaction to light and other ordinary stimuli have clearly been supplanted by more definite responses in all but the Protozoa and certain other low types of animal life. The earthworm's reactions to stimuli, mechanical, thermal, chemical, are in general such as its nervous system and musculature would lead us to expect. The occurrence of random movements in response to all but very strong light is the outcome of the undeveloped condition of its organs of light perception, not to the want of a nervous system and musculature adapted for such simple reflexes. Diffuse organs of light perception may not respond definitely to a localized stimulus unless it is a very strong one. The trial and error method of its responses to relatively weak light are exceptional in character in comparison with its reactions to other ordinary stimuli. Its archaic type of end organs for light gives rise to a type of behavior which is to be regarded as primitive. For the trial and error method is clearly supplanted in the ascending scale of animal life, by reactions of a definite nature, in the case of the simple responses to the ordinary stimuli.

Summary of Results.

1. *Perichæta bermudensis* (Beddard) is an exotic earthworm found sometimes in greenhouses. Its active habits are one of its chief characteristics.

2. The body is less sensitive to light when contracted than

when extended, owing to the fact that when extended the sensitive elements are spread out over a greater surface and become more susceptible.

3. In locomotion, as there are alternate extensions and contractions, there is an alternation of the condition of lower and of higher sensibility. This is important particularly in the sensitive anterior end.

4. As the worm begins each extension in a condition of lower sensibility, it may project its anterior end toward the source of light. This movement is checked as soon as the increased sensibility of the extended anterior end appreciates the stimulus. Movements away from the light do not meet such a check and so are prolonged farther. Orientation is the result of a trial and error method.

5. In strong enough light, random movements toward the light are suppressed altogether, and the worm appears to move directly away from the light without noticeable trial movements. This applies to worms which have been kept in the dark and are in a perfectly fresh condition, as after a time they lose their discrimination and begin to make random movements.

6. Movements in the longitudinal direction are typical of the normal burrow life of the animal, and the axial movements initiated by the anterior and posterior ends are more definitely controlled by the stimulation of light and by a weaker stimulus than are the lateral movements. Lateral movements tend more to be random and are directed only by stronger stimuli because the organization of the worm is chiefly in adaptation to a burrowing life and not to an open air life.

7. The characteristic leaping motion of *Perichæta* is a conspicuous example of random lateral movements, adapted to life outside of the burrow. All gradations may be observed between the ordinary reaction to a slight local stimulus by jerking back, and also bending the body away, if the stimulus be stronger, up to a complete series of leaping movements.

8. Reactions to mechanical stimuli, as well as to other stimuli, chemical, thermal and electric, show that the worm is like other animals as highly organized as itself in responding to a local stimulus of an injurious nature by contracting and bending away

in a definite "avoiding reaction." In this respect the effect is like the response to very strong light. Consequently we see that the random reactions to weaker light have a special explanation and are only an apparent exception to the general form of negative response.

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SOME POINTS REGARDING THE BEHAVIOR OF METRIDIUM.

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It has recently been shown that the reactions of many sea anemones are modifiable, in dependence on a variety of internal conditions (Jennings, 1905). The purpose of the work here presented was to determine how far similar relations hold for *Metridium marginatum.* Since *Metridium* is the commonest of our sea anemones, and the one most used in investigation and instruction, it is important that its behavior should be well known. The work was suggested by Dr. H. S. Jennings, and carried out under his direction at the Marine Biological Laboratory at Woods Hole.

I. Changes in the Reactions to Certain Sorts of Food Bodies.

The point to which experimentation was first directed was the interpretation of the results of certain experiments of Nagel (1892) and Parker (1896). In Parker's experiments alternate pieces of meat and filter paper (soaked in meat juice) were given to the tentacles of one side of the disk of *Metridium.* It was found that while the meat was swallowed each time with equal readiness, the time taken in swallowing the paper increased, and after three or four trials the animal no longer ingested the paper, though the latter contained each time the same amount of meat juice as at first. After reaching this result with the right side of the disk, the same series of experiments was performed on the opposite side of the disk of the same specimen. It was found that the left side had not become modified by the experience of the right side. It at first took the paper, then by the same gradual change seen previously on the right side, it came to refuse the paper. A series of records of the times required for swallowing the meat and paper in such an experiment by Parker are given in the following table :

	Right Side.	Left Side.		Right Side.	Left Side.
1. Meat	85 sec.	45 sec.	9. Meat	70 "	35 sec.
2. Paper	80 "	90 "	10. Paper	—	85 "
3. Meat	50 "	45 "	11. Meat	40 "	30 "
4. Paper	90 "	—	12. Paper		95 "
5. Meat	40 "	45 "	13. Meat		35 "
6. Paper	105 "	55 "	14. Paper		—
7. Meat	50 "	35 "	15. Meat		35 "
8. Paper	—	105 "	16. Paper		—

What is the cause of the change of behavior in these experiments? Several possibilities suggest themselves:

1. Jennings (1905) found that in *Aiptasia* similar effects were produced, and in this case the result was evidently due mainly to changes in the state of hunger. The very hungry *Aiptasia* took meat and paper readily, but after feeding a short time it refused paper, and later it came to refuse meat also. The animal could be caused to refuse paper more readily by feeding it meat alone than by feeding paper alone or by feeding the two in alternation. It was evident that the changed reaction toward paper was due to loss of hunger. We must inquire whether this factor plays a part in *Metridium*.

2. Nagel (1892) referred the changes in reaction toward paper to a process corresponding to what we call *judgment* in higher animals; he held that the animal discovers by experience that the paper is unfit for food, and thereafter refuses to take it. Such a process in so low an animal would of course be of great interest, and the evidence for it needs to be examined carefully. Nagel held that it was owing to the lack of close nervous interrelation of parts in these animals that the experience of one side is not transmitted to the opposite side.

3. Parker sums up the phenomena shown in these experiments as follows: "The successive application of a very weak stimulus is accompanied, not by the summation of the effects of stimulation, but by a gradual decline in these effects, till finally the response fails entirely" (Parker, 1896, p. 116).

In my experiments I attempted to test these different possibilities, and to work out in a systematic way the various factors which modify reactions in *Metridium*.

The taking of food has been well described by Parker (1896). It is important to note that ciliary action plays a large part in the taking of food by *Metridium*. Muscular movements of the tentacles, disk, and œsophagus also plays a part, but a less important one than in the anemones studied by Jennings (1905). The tentacles bearing bits of food are bent toward the mouth, and the cilia of the tentacular surface carry the food toward the mouth opening. The cilia of the œsophagus are usually beating outward, but when food enters the mouth the stroke of the cilia of the part in contact with the food becomes reversed, so that the food body is conveyed inward (see Parker, 1896, 1905). I have found that the reversal of the œsophageal cilia is frequently caused in *Metridium* by indifferent solids, such as filter paper, so that such bodies are ingested.[1] There is much variation in regard to this matter, some individuals take filter paper readily, others slowly and only at times, others not at all. As we shall see later, this depends largely on the degree of hunger.

We will now take up experimentally the various possibilities of modification above distinguished. In studying these matters, it is most important that specimens which are fresh and in good condition should be used, otherwise clear results will not be obtained.

1. *Hunger.*—Conditions of hunger and satiety affect the food reactions of *Metridium* in a most decided way. When the animal is very hungry (and in good condition otherwise), the column is extended, becoming long and slender, while the disk is widely spread, and the tentacles extend a considerable distance beyond its edge. If the animal remains contracted, it can often be induced to extend by placing a piece of clam meat or some meat juice on the infolded disk. Two or three applications of food will often cause the most obstinately contracted specimens to expand beautifully. If now a piece of mussel of considerable size (having an area equal to the cross section of the column, with a thickness of three or four millimeters) is brought near the edge of the disk, so as to come in contact with the tips of two or three tentacles, a decided reaction is produced. The tentacle tips adhere to the meat, and the tentacles and adjacent parts of the disk contract quickly, so that the piece of meat is drawn inward. It

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[1] This was possibly due, as Parker (1905, 1905*a*) has set forth, to the paper's having been touched by the fingers; this matter was not tested.

thus comes in contact with many other tentacles, these bend down upon it. Then all bend over toward the mouth, while at the same time this portion of the disk contracts. Thus the food is brought nearer the center of the disk. The mouth meanwhile opens, and the food is passed into it, partly by the ciliary action of the tentacles, partly by muscular contractions of tentacles, disk and mouth. The latter factors play a more important part in the reaction of a hungry specimen to a large piece of food than does ciliary action.

After ten or a dozen good-sized pieces of meat have been swallowed, the reaction becomes much slower. If the meat is brought in contact only with the outer tentacles, these no longer react, and such food is not taken. If placed on the inner tentacles, the meat is slowly transferred to the mouth, where it is swallowed. The animal is frequently in this condition when brought into the laboratory; food placed on the outer tentacles is refused, while that on the inner tentacles is taken.

The reaction of the tentacles becomes slower as more food is taken, so that the process of ingestion takes a much longer time than at first. Finally, all the tentacles cease reacting to food, and it is not carried to the mouth. But if the meat is placed by the experimenter directly on the mouth, it is ingested. This appears to take place almost alone through the action of the cilia; the reversal of the cilia seems to be more nearly independent of the physiological states of the animal than are the contractions of the muscles. The mouth never reaches a condition where it rejects pieces of mussel placed directly upon it. So much food may be taken that the body becomes puffed out to form a swollen sack, yet new pieces are forced inward. Large pieces of meat may however be refused even when placed directly on the mouth, the lack of assistance from muscular contractions appearing to make it impossible for the cilia to draw them inward. The feeding may be carried so far as to cause internal disturbance, resulting in the disgorgement of the food. But immediately after such disgorgement the mouth will take new food. Sometimes when the animal is nearly filled, a large piece of meat placed on the mouth is partly swallowed, then partly disgorged by a convulsive movement, then the swallowing is resumed. This may happen repeatedly.

The loss of reaction on the part of the tentacles after much food has been taken is not due to fatigue resulting from their activity in taking food. This is demonstrated by the following facts: (1) The animal may be fed from one side of the disk till it is satiated. Now meat given to the opposite side of the disk is not taken, though the tentacles of this side have not been active, and so cannot have become fatigued. (2) Seven hours after the animal has been fed all it will take, the tentacles still refuse to take food, though they have had this period for recuperation. Actual fatigue, as we shall see later, lasts but a few minutes.

The effects of hunger and satiety are further seen in the reactions of *Metridium* to indifferent bodies, such as bits of filter paper. These are commonly taken readily by hungry specimens of *Metridium*. The tentacles react to them, just as to meat, so that they are carried to the mouth. Here they cause the reversal of the ciliary movement in the same way as does meat, so that they are carried inward. But after the animal has been fed a considerable quantity of meat, it will no longer take filter paper. First the outer tentacles refuse it, later the inner tentacles, and finally the mouth. A piece of filter paper placed squarely on the mouth no longer causes the reversal of the stroke of the cilia of the œsophagus, so that it is not carried inward.

The fact that the reversal of the cilia under such stimuli depends on the physiological state of the animal is one of much interest. It shows that the cilia are not entirely independent of such states, as some other facts would seem to indicate.

Seven hours after the animals had been fed an abundant meal of mussel meat, they still refused to take filter paper, though before the meal paper was taken readily.

Thus it is clear that the state of the processes of metabolism is in *Metridium*, as in other sea anemones, a most important factor in determining behavior under mechanical and chemical stimuli. But it is equally clear that this will not explain the results of Parker's experiments, described in the first paragraphs of this paper. Parker found that after one side of the disk has refused to take paper, the other side still accepts it. In repeating

Parker's experiments, I fed in one case six successive regions of the disk, each till it had rejected food; the next would then take it as readily as at first. This refusal then cannot be due to a general lack of hunger, and we must examine the other explanations that have been given.

2. "*Judgment.*" — If the animal comes to reject paper through experience of the fact that the paper is not good for food, there must be some way in which this experience is obtained. It might be supposed that this comes through swallowing the food; being indigestible, its effect after swallowing might cause the animal thereafter to reject it. This was tested by preventing the swallowing of the paper. The animal was fed meat and paper in alternation, as in Parker's experiments. But after the paper had been carried to the mouth and was passing down in the œsophagus, it was removed with a fine pair of tweezers. This is easily done without disturbing the animal. Thus the bits of paper never reach the digestive cavity. Yet the animal comes to reject them as quickly as before. After a few alternations of meat and paper, only the meat being completely swallowed, the animal ceased to take the paper, while it still accepted the meat. Hence the effect of the paper after it reaches the digestive cavity is not the cause of its rejection.

Furthermore, nothing like a contrast or comparison between the meat and the filter paper is necessary in order to induce the rejection of the paper. If successive pieces of paper were fed alone to the anemones, they soon came to reject these as before. In such cases it is noticeable that the animal takes a larger number of pieces of paper than when the paper is fed in alternation with meat. The number of pieces required is about the same as the number of pieces of meat and paper together that result in rejection of the paper when the two are given in alternation. This fact throws some light on the cause of the rejection, as we shall see later.

3. *Repetition of Weak Stimuli, till Effect Fails.* — Is the loss of the positive reaction to the paper due to the general fact that weak stimuli, when repeated, gradually lose their effect? This was tested by excluding this factor from the experiments, in the following way. A given specimen was first tested and found to

accept filter paper (in some cases plain, in others soaked in meat juice). After this first test, the same region of the disk was fed successive pieces of meat, which were all readily taken. After eight to twelve pieces of meat had been accepted, a piece of filter paper like that originally accepted was given to the same region of the disk. *It was not accepted.* This experiment was repeated with many specimens, always with the same result. This result was likewise reached if the animal was not allowed to complete the swallowing of the meat, the latter being removed after it had passed into the œsophagus. This, of course, shows conclusively that loss of hunger is not the cause of the change of reaction toward the paper.

The placing of meat juice on a certain region of the disk causes the food reaction, as Parker has shown. If this experiment is tried successively a dozen times in the same region of the disk, the animal comes to reject filter paper in this region, as in the experiments described in the foregoing paragraph.

Thus it is not necessary that weak stimuli should be repeated in order that the animal shall reach a state in which it fails to react to them. Repetition of strong stimuli (meat) causes failure to react to weak stimuli just as readily as does repetition of the latter. Repetition of strong stimuli alone, of weak stimuli alone, and of the two in alternation, all have the same effect ; the animal ceases to react to weak stimuli.

In all cases in which meat is fed to a given region, moreover, the reaction to strong stimuli ceases some time later than that to weak stimuli. After giving a certain region sixteen to twenty pieces of meat, meat is no longer accepted here, though other regions of the disk take it readily.

4. *Fatigue.* — The facts brought out in the foregoing paragraphs seem to make possible a clear interpretation of the rejection of the paper. It is evidently a case of plain fatigue. After stimulating a certain region of the disk a number of times, it ceases to react — first to weak stimuli, then to strong stimuli — though other parts react as before. The same results are produced whether the successive stimuli are all strong or all weak, or partly strong and partly weak. It appears evident therefore that it is the reaction of the animal, not the precise character of

the stimulus, that causes the fatigue. This is perhaps what should be expected when the nature of the food reactions is taken into consideration. In taking food the region in contact with the food produces a very large quantity of mucus, enveloping the food body. It is not surprising that successive immediate repetitions of this excessive production of mucus gradually exhausts the region. As is usual in fatigue, strong stimuli may produce reaction for some time after weak ones have failed.

The fatigue thus caused usually lasts only two to five minutes. After this period has elapsed the fatigued region is frequently as ready to take food as before — provided the animal is still hungry.

Nagel and Parker have held that the result of their experiments "illustrates the extreme looseness, or even independence, of the nervous activities of the two sides of the animal" (Parker, 1896, p. 116) — since the effects of the experience of one side are not transmitted to the other side. With the recognition that these results are a simple matter of fatigue, they perhaps cease to have any bearing on the question of the closeness or looseness of nervous interconnection. In the highest organisms, as man, fatigue induced by repeated contractions of a finger of the left hand, in erogographic experiments, is not transmitted appreciably to the right hand. But the experience gained by touching a hot iron with the left hand would nevertheless later prevent the right hand from touching it.

II. Other Modifications in Behavior.

A very peculiar modification of behavior is seen in the following: A specimen refuses to take filter paper, though it still takes meat. After it has thus refused paper, two or three pieces of meat are given in succession, and taken readily. Now the bit of paper is again placed on the disk, and it too is swallowed. Clearly, the uninterrupted taking of a number of pieces of meat changes the physiological condition of the animal in some way, preparing it for the taking of any object with which it comes in contact. (After a larger number of pieces of meat, the paper is refused, as we have before seen.)

Acclimatization to weak stimuli is readily demonstrated in fresh, active specimens of *Metridium*. If a light stream of water is directed with a pipette against the expanded disk, the animal contracts strongly. Waiting till it has again expanded, the stream of water is directed upon it as before. This time it does not react. In specimens that are not in good condition, this change of behavior cannot be seen. The animal does not contract at all save under strong stimuli, and if such are repeated, it contracts as at first.

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THE INFLUENCE OF THE NERVE ON THE REGENERATION OF THE LEG OF DIEMYCTYLUS.

CECIL SHEPARD HINES.

The following experiments were carried on at the suggestion of Professor Morgan, for the purpose of ascertaining whether regeneration in the leg of *Diemyctylus* is dependent on its connection with the nervous system, as has been found in the case of other urodeles, or whether the supposed result may not have been due to unintentional injury to the blood supply. The hind limb was chosen for operating on account of its larger size. The general course of procedure was to cut the nerve in the upper part of the leg *without injury to the artery*, and then amputate the leg at the knee joint. After a period of a little more than three weeks the new part can be clearly recognized as a dark protuberance sharply contrasting with the lighter color of the surrounding skin.

In the first lots the nerve was cut as near the proximal end of the femur as possible. A longitudinal slit through the skin was made with a sharp knife. The muscles were then separated until the nerve was brought into view. Care was taken not to injure the blood vessel which closely adheres to the nerve and is almost inseparable from it. If the operation were performed without injury to the blood vessel and the leg showed a resulting paralysis it was amputated at the knee. For comparison an equal number of salamanders had the leg cut off without injuring the nerve or blood vessel. The results obtained from the first series seemed to show that regeneration in *Diemyctylus* was in no way dependent upon the nerve. The proliferation of new material began as soon in those in which the nerve had been cut as it did in the checks. Nor did the amount of material regenerated seem to be affected in any way. There were, it is true, great variations in the rate of regeneration, but these seemed to arise from purely individual differences, and to bear no definite relation to the presence or absence of the nerve connection. In two checks operated upon on the same day and kept in the same aquarium, so that external conditions could play little, if any, part in the result, an interval of ten days or more might occur between the earliest and latest appearance of proliferation. The results obtained after cutting the nerve at this level may have been due to the presence of collateral nerve-connection sufficient to give the required stimulus to the tissue ; for as was later clearly shown, the nerve is an important factor in the regeneration.

In the succeeding series the nerve was cut through the pelvic girdle close to the backbone, in the hope of more completely cutting off the nerve supply of the limb. The incision necessarily went through into the body cavity as the nerves given off from the spinal cord lie close to the inner wall and are covered only by cœlomic epithelium. The operation is by no means as serious as would be imagined, since the wound heals completely in three days. During this time the animal acts in a perfectly normal manner except for the injured leg. Instead of using separate individuals for checks as before, both legs of the same individual were amputated, but the nerve was cut on one side only. The males are much more difficult to operate upon than the females, on account of their greater muscular development, whereas in the female the pelvic girdle stands out prominently and the body wall is thin. In the males the girdle is not visible externally and is overlaid by a thick musculature which renders operating upon the animal at this point difficult.

On November 26 a set of seven salamanders was operated upon as described above. They were not again observed until the forty-fifth day. At that time both legs of each individual had proliferated material to a greater or less extent. The side used as a check could be identified in every case but one, by its far greater amount of proliferated material.

In the exception the animal assumed a peculiar green color, evidently from a disease, and at a later period both legs were entirely sloughed off. This set was continued under observation until the eighty-third day, at which time there was still a decided difference between the appearance of the two sides, although by no means as pronounced as before. The check showed in each individual the foot plainly differentiated, while in only two instances was this the case upon the other side, and even in those cases to a much less extent.

Later I operated upon at least thirty salamanders in several lots. Some of these were starved while others were well fed. The abundance or lack of food did not seem to be a factor in the rate of regeneration. Salamanders which were reduced almost to "skin and bones" showed the same comparative amount of regeneration as well-fed individuals. The influence of food did show itself, however, in the amount of material proliferated. During starvation an individual shrinks greatly in size and proliferates much less material for the same relative amount of regeneration as a well-fed companion.

To ascertain whether the blood supply was a factor in regeneration the artery of several individuals was cut just above its entrance into the leg, the nerve being left intact. The result showed that the leg regenerated at the normal rate. However, not much stress can be laid upon this experiment, owing to the rapidity with which a sectioned blood-vessel heals.

That the circulation in the leg may have continued to some extent after the operation in those individuals whose nerve as well as artery had been sectioned near the backbone was shown conclusively by the following experiment. A salamander was taken and a cut made in the pelvic region as before. Then a vein was severed in the lower part of the leg. This continued to bleed freely for a considerable time, as would not have occurred had the total blood supply been cut off. The collateral blood supply probably still brought blood to the limb. Similarly the collateral nerve supply may in the first series of experiments have sufficed to keep the regeneration up to the same rate as in a limb in which the nerve was not cut.

In a number of cases regeneration did not set in at all on the side on which the nerve had been cut. At least, after a period of two months and a half there was not the least sign of proliferation, while in the normal course of regeneration the new part appears in about twenty-five days. This lack of regeneration is probably due to the distal end of the nerve being displaced, and in consequence the regenerating nerve was unable to grow down its old path along the degenerated nerve, but was turned aside. Consequently not even a retarded regeneration occurred.

The most important work bearing on the question of the relation between the nerve and the regeneration of the leg is that of Wolff.[1] He found that if the nerve-cord were destroyed in the region of origin of the leg nerve that the leg regenerated at the normal rate. Since the spinal ganglion was left after this operation, its presence may have sufficed to produce the result. In fact, nerves were found in the new foot. In order to remove the ganglion also, a piece of the spinal column was cut out including cord and ganglion. Six individuals that survived this operation showed that after the first proliferation of new material had taken place growth came to a standstill for a time and then began again. The result suggests, Wolff thinks, that the standstill was due to the lack of nerve connection, while the renewal of growth was due to the reëstablishing of a new nerve connection. In fact the disabled leg showed some signs of having regained its power of motion.

Wolff discusses the question whether the period of standstill may not have been due to the lack of function or activity of the leg while its later growth was due to its regaining its locomotor function. He argues that this is probably not the case, but that the nerve connection is directly responsible for the result.

A student of Busfurth's, R. Rubin,[2] has obtained similar results. What part the nervous connection plays in these cases is still obscure. Morgan and Davis[3] have found that for the regeneration of the tail of the tadpole, the presence of the notochord and not the nervous system is the important factor.

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[1] *Virchow's Archiv*, CLXIX., 1902.

[2] *Archiv Entw. Mech.*, XVI., 1903.

[3] *Archiv Entw. Mech.*, XV., 1903.

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AN EXAMINATION OF THE METHODS FOR THE MICROCHEMICAL DETECTION OF PHOSPHORUS COMPOUNDS OTHER THAN PHOSPHATES IN THE TISSUES OF ANIMALS AND PLANTS.

R. R. BENSLEY.

(From the Hull Anatomical Laboratory, University of Chicago.)

The microchemical reaction for the detection of phosphorus in the tissues of animals and plants introduced in 1898 by Macallum ('98) is a modification of that devised in 1893 by Lilienfeld and Monti ('93). These investigators attempted to demonstrate the distribution of phosphorus in tissues by subjecting the latter for some time to the action of a solution of ammonium molybdate in nitric acid, after which they were treated with a solution of pyrogallic acid. The nitric molybdate reagent was supposed to liberate the phosphorus from its organic combinations, to convert it into orthophosphoric acid, and finally to precipitate the latter as the yellow phosphomolybdate of ammonium. The further treatment of the tissues with pyrogallic acid had for its object the reduction of the ammonium phosphomolybdate to a lower oxide of molybdenum, which, according to Lilienfeld and Monti had a brown or black color. In this way the pale yellow precipitate containing the phosphorus was converted into a dark-colored precipitate which could be easily studied under the microscope.

The importance of a microchemical reaction which would enable us to determine accurately the distribution of the compounds of phosphorus not only in the tissues but also in the parts of the cells of the tissues can hardly be overestimated. It is therefore not surprising that the results of Lilienfeld and Monti have been subjected to careful experimental examination by a number of other investigators.

Raciborski ('93) in 1893, in his review of Lilienfeld and Monti's article, showed that the reaction of ammonium phosphomolybdate with pyrogallic acid resulted in the production of a green compound, while ammonium molybdate gave by reduction with the same reagent a brown compound. He concluded that the brown reaction of Lilienfeld and Monti was due to ammonium molybdate mechanically imbibed by the section, and not to ammonium phosphomolybdate.

Heine ('96), also, showed that phosphorus-free histone, prepared from the thymus, formed with the nitric-molybdate reagent compounds from which the ammonium molybdate could not be removed by washing in water, and in which it could be detected by the use of reducing compounds. For this purpose he employed stannous chloride.

Macallum confirmed Raciborski's observations as to the color compounds produced by the reduction of ammonium phosphomolybdate and ammonium molybdate respectively, and showed that ammonium molybdate could not be removed from tissues which had been treated with the nitric-molybdate reagent even by washing for several months in changes of distilled water. Macallum perceived the necessity of substituting for pyrogallic acid, which gives colored compounds with both the phosphomolybdate and the molybdate of ammonium, some reagent which would discriminate between these two compounds, and give a color reaction with phosphomolybdate alone. This condition he found to be fulfilled by zinc chloride, previously introduced for this purpose by Polacci ('94), which gave a green color with the phosphomolybdate but did not act on ammonium molybdate. Owing, however, to the fact that zinc chloride acted very slowly, he finally adopted as a reducing agent phenylhydrazin hydrochloride, which, according to him, made a very marked distinction, in the absence of alcohol or of caustic alkali, between the molybdate and the phosphomolybdate compounds. It gave with the former in powder the brown oxide at once, in solution, a brownish precipitate, which appeared at once, or later, according to the strength of the solution. On a solution of the molybdate containing nitric acid, *e. g.*, that used as the reagent for phosphoric acid, it had no apparent effect on the molybdenum compound, although, in a few minutes, a soluble, reddish, aromatic compound might be formed in the solution. On the other hand, with phosphomolybdates, either in the presence or in the absence of ammonium molybdate, or of nitric acid, or of both, it gave at once the dark green oxide of molybdenum.

Concerning the use of these reagents on tissues Macallum says: "On the molybdate and phosphomolybdate compounds distributed in animal and vegetable tissues, the phenylhydrazin hydrochloride acts as it does on these in the test-tube. It is not necessary to free the tissue preparations from ammonium molybdate." He recommends washing the preparations for a minute or two in a dilute solution of nitric acid after which they are transferred to the reducing solution, which in less than two minutes, brings out the green color where the phosphomolybdate compound occurs, but a faint yellow reaction where ammonium molybdate alone is present.

The technique of the reaction is as follows: Fresh tissues or tissues hardened in alcohol were used. Pieces of tissue or thin sections in the case of hardened material, were placed, for a period varying from ten minutes to forty-eight hours, in a solution of ammonium molybdate in nitric acid, prepared by dissolving one part of pure molybdic acid in four parts of strong ammonia, and adding thereto, slowly, fifteen parts of nitric acid, sp. gr. 1.2. After the nitric molybdate reagent has acted for a sufficient length of time, the preparations are washed in water or in dilute nitric acid, and treated with a ¼ per cent. solution of phenylhydrazin hydrochloride, which reduces the phosphomolybdate to a green-colored oxide of molybdenum.[1] The tissues may be then dehydrated, cleared in oil of cedar and mounted in balsam.

According to Macallum, inorganic compounds of phosphorus

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[1] Although Macallum speaks of a green oxide of molybdenum being formed by this reaction, it is probable that the bodies formed belong to the blue oxides. The green color obtained at the beginning of the reduction of phosphomolybdate of ammonium in vitro is due to the yellow background of unreduced molybdate, that obtained in the tissues to the associated xanthoproteic reaction (vide infra).

are first affected, then lecithins, and finally the organic compounds of phosphorus. Where it is desired to demonstrate the distribution of the latter he recommends the preliminary removal of the lecithins by repeated extraction with hot ethyl alcohol in a Soxhlet apparatus.

In the original form devised by Lilienfeld and Monti or in the form of Macallum's modification this reaction for phosphorus has been extensively employed. Macallum's original paper dealing with the reaction contains a considerable number of contributions dealing with the distribution of organic compounds of phosphorus in various tissues, and the reaction has been applied to the solution of special problems of this nature by Sherrington ('94), Gourlay ('94), Scott ('99), Held ('95), Bensley ('03), Wager ('05), Richter ('05), and many others. It is therefore of the utmost importance that every detail of the reaction should be carefully tested to exclude all possible sources of error.

Recently I have obtained results which have led me to suspect that the reaction obtained by Macallum's method is not wholly due to the formation in the tissue of ammonium phosphomolybdate, but that other compounds of molybdenum may be present which are capable of reduction to the blue oxide by means of phenylhydrazin hydrochloride. For example, I observed that the peripheral portions of sections gave uniformly a deeper and more diffuse reaction than the central portions. This result was first noted in the tips of the villi in sections of the small intestine, and was ascribed to the presence of inorganic phosphates absorbed from the food. Later it was noted that the same result was obtained in sections of the liver, pancreas, and other organs. Furthermore, I observed that freshly prepared solutions of the nitric molybdate often gave a strong reaction in the tissues after very short periods of immersion. For example, sections of the fundus region of the stomach of the rabbit, treated with warm, freshly prepared nitric molybdate reagent for ten minutes, then washed in water and reduced by means of a one per cent. solution of phenylhydrazin hydrochloride gave a diffuse bluish green reaction together with a strong reaction in the nuclei and in the granules of the parietal cells. This result was clearly not due to phosphorus compounds of any sort, because the same nitric molybdate reagent after having been kept several days, during which it had deposited copious crusts of molybdic acid, gave, when applied to sections from the same source, no such result, the reaction proceeding in the slow progressive manner characteristic of the phosphorus reaction as described by Macallum. Again, in his investigation of the nature of the granule cells of Paneth, Mr. Klein, working under my direction, found it necessary to employ formalin in the fixation of the tissues in order to preserve the granules. In preparations of this material treated by Macallum's method, we were surprised to obtain a strong reaction in the fibrils of the collagenic tissue of the tela submucosa.

Clearly, the intense marginal reaction obtained in sections and the early reaction obtained when freshly prepared solutions of the nitric molybdic reagent were used could not be due to phosphorus. These anomalous characters of the reaction as applied to sections could only be explained on the assumption that, after the treatment with the nitric molybdic reagent, there existed in the tissue compounds of molybdenum other than phosphomolybdates, which gave the blue reaction with phenylhydrazin hydrochloride.

On account of the fact that the difference between freshly prepared and older solutions of the nitric molybdic reagent is to be found in the amount of molybdic acid contained, it seemed probable that the extraordinary results of the reaction, as described above, were due to the absorption of this substance by the tissue elements. Accordingly, I undertook experiments to determine the behavior of solutions of molybdic acid in reaction with phenylhydrazin hydrochloride, as well as the capacity of the tissue elements for absorbing it from its solutions. Later it was found necessary to reinvestigate the reaction obtained by treating ammonium molybdate in solution, and phosphomolybdate of ammonium suspended in water, respectively with solutions of phenylhydrazin hydrochloride.

Although the experiments were started with the expectation that a portion of the reaction would be found to be due to absorbed molybdic acid, I still thought at that time that the fundamental assumption was true, upon which the reaction was based, namely, that the organic phosphorus was liberated from its combinations by the reagent, converted into orthophosphoric acid and immediately precipitated *in situ* as ammonium phosphomolybdate. As a result of the experiments, however, I have been forced to the conclusion that the whole of the reaction obtained by Macallum's method is due to compounds of molybdenum other than phosphomolybdate and that the phosphorus of the tissues is not concerned in the production of the reaction at all.

For the purpose of testing the reactions of molybdic acid with phenylhydrazin hydrochloride, I prepared two soluble molybdic acids. The first of these was prepared by the method recommended by Ullik ('67). Barium molybdate, prepared by precipitating a warm solution of ammonium molybdate with barium chloride washing thoroughly with hot distilled water, and drying the precipitate on a water bath at 100° C., was suspended in water and decomposed with its equivalent of sulphuric acid. The solution was then filtered, tested for barium, sulphuric acid and chlorides, from which it was found to be free, and the total acidity was determined by titration with a normal solution of sodium hydroxide, using phenolphthalein as indicator. Assuming that the solutions contained a molybdic acid having the formula $H_2Mo_2O_7$ the concentration of the solutions obtained by the method described above was in the case of one preparation 5.25 per cent., in another 7.52 per cent. The second molybdic acid was colloidal molybdic acid prepared by the process recommended by Graham (64), except that ammonium molybdate was employed instead of sodium molybdate. A solution of ammonium molybdate in hydrochloric acid was dialyzed for several days against distilled water, until free from chloride. The resulting solution was then titrated against normal soda solution using phenolphthalein as indicator. According to Sabanejew ('90) the molecular weight of the molybdic acid prepared by Graham's method as determined by lowering of freezing point is 620 corresponding to the formula $H_2Mo_4O_{13}$. Assuming that the same compound was obtained by the dialysis of the solution of ammonium molybdate in hydrochloric acid the solution obtained contained 6.73 per cent. of colloidal molybdic acid. From these solutions were prepared the various solutions mentioned in the succeeding experiments.

Solutions of both molybdic acids give, when treated with phenylhydrazin hydrochloride an immediate blue reaction which gradually deepens in color and a blue precipitate forms.

Sections of tissues fixed in alcohol, cut in paraffin, and fastened to the slide by the water method were placed in each of the solutions of molybdic acid. From time to time sections were removed from the solution, rinsed in water, and tested with a 1 per cent. solution of phenylhydrazin hydrochloride. It was found that the molybdic acid was taken up by the tissues from both solutions and was detectable in them by the blue reaction obtained by reduction with phenylhydrazin hydrochloride. In sections treated with pure solutions of soluble or of colloidal molybdic acid the strongest reaction was obtained in the collagenic fibrils which were deep blue. A slight diffuse reaction was obtained in the cytoplasm of cells, and a somewhat stronger reaction in the nuclear chromatin. The amount, however, of molybdic acid taken up from dilute pure solutions was not great except as regards the collagenic tissue. The experiments show, however, that molybdic acid may be taken up from its solutions by tissues and may be detected in these by the blue reaction produced by treatment of the sections with phenylhydrazin hydrochloride.

Under the conditions of the Lilienfeld-Monti-Macallum reaction molybdic acid occurs in the solution associated with nitric acid as well as with ammonium molybdate, ammonium nitrate, and the products of dissociation of all these compounds. Accordingly, the effect of the presence of acids on the absorption of the molybdic acid from its solutions by tissues was tested. Sections were placed in solutions of soluble and of colloidal molybdic acid to which five per cent. of nitric or of hydrochloric acid had been added, and were tested from time to time with phenylhydrazin hydrochloride. I found that the addition of either nitric acid or hydrochloric acid to the solutions of molybdic acid produced a remarkable increase in the capacity of sections for combining molybdic acid, which was again detectable by the blue or green reaction obtained by reduction with phenylhydrazin hydrochloride. With the mixture of nitric and molybdic acids the reaction obtained after reduction was a deep greenish blue color in the nuclear chromatin, a faint greenish blue in the cytoplasm, and a deep blue in the collagenic fibers. Except for the strong reaction in the connective tissue, the result obtained by treatment of sections with a solution of molybdic acid containing nitric acid, followed by reduction in one per cent. phenylhydrazin hydrochloride was exactly similar to the so-called phosphorus reaction obtained by the procedure recommended by Macallum. It may be noted that the color obtained by the use of molybdic acid containing nitric acid followed by reduction differed from that produced by reduction of molybdic acid by phenylhydrazin in the test tube, inasmuch as the former gives a greenish blue color, the latter a pure blue. This difference was obviously due to the yellow background afforded by the xanthoproteic reaction. As in the phosphorus reaction, the absorption of the molybdic acid was progressive, the reaction after eighteen hours being much stronger than after three hours.

Similar results were obtained with solutions of molybdic acid containing hydrochloric acid, except that the molybdic acid was taken up much more rapidly from the hydrochloric solution than from the nitric solution, and that the resulting reaction was blue rather than greenish blue, owing to the absence of the yellow zanthoproteic reaction. A further difference exhibited itself in the fact that sections left for some time in the solutions developed the blue color *without the use of any reducing agent*, the organic compounds of the tissue evidently acting as reducers. In the presence of nitric acid this, of course, could not occur because of the strong oxidative action of this compound.

Thus, in sections treated with solutions of molybdic acid containing either hydrochloric, or nitric acid, followed by phenylhydrazin hydrochloride, results were obtained which were the exact counterpart of the results of the so-called phosphorus reaction, although there could be little possibility of the formation of precipitates of ammonium phosphomolybdate in the tissues. Curiously enough, the anomalous characters occasionally observed in the reaction obtained by Macallum's method were to be found in the molybdic acid material, that is to say, the more intense diffuse reaction of the outer portions of the sections and the deep reaction in the connective tissue. It seemed clear from these experiments that a portion, at least, of the result obtained by the procedure of Macallum was due to absorption of molybdic acid from the nitric molybdate solution. There was, however, some possibility that even this reaction with molybdic acid solutions depended on the presence of phosphorus and its liberation as phosphoric acid. It might be supposed that this phosphoric acid reacted with the molybdic acid to produce phosphomolybdic acid which was in turn precipitated by the albumens of the tissues. Or it might even be supposed that ammonium phosphomolybdate was formed, the ammonium ions necessary to the reaction being furnished by the albumens. That this is not the case, and that the reaction obtained by the use of molybdic acid solutions is in no way dependent on the phosphorus content of the tissue, I think the following experiments will show.

In studying the reaction of solutions of molybdic acid with phenylhydrazin hydrochloride, I found that the addition of nitric acid to the mixture retarded the reaction and if a sufficient quantity were present prevented it altogether. Accordingly, experiments were undertaken to determine the limits of this reaction with molybdic acid, ammonium molybdate, and ammonium phosphomolybdate, respectively, in the hope that a sufficient difference in the behavior of these compounds would be discovered to enable one to employ a solution of phenylhydrazin hydrochloride containing enough nitric acid to inhibit the reduction of molybdic acid and of ammonium molybdate while permitting the reduction of ammonium phosphomolybdate, and thus discriminate between these compounds occurring in tissues treated by Macallum's methods.

I found that with a constant concentration of phenylhydrazin hydrochloride, the amount of nitric acid required to prevent the reduction to the blue oxide of molybdenum varied directly with the concentration of the molybdic acid but was constant for any given concentration.

I found, moreover, that the blue reduction was invariably obtained in solutions of ammonium molybdate containing nitric acid, when they were treated with solutions of phenylhydrazin hydrochloride, provided the amount of nitric acid did not exceed a certain amount, which, as in the case of molybdic acid, was constant for a given concentration of the other two constituents but varied directly with the concentration. This fact made it necessary to determine the conditions of this reaction with ammonium molybdate if solutions of the hydrochloride containing nitric acid were to be used for the reduction of the sections, because in trying to eliminate the reduction of the molybdic acid by using a solution of the hydrochloride containing nitric acid, a new source of error might be introduced, inasmuch as the nitric acid would make ammonium molybdate available to the blue reduction.

The experiments to determine the limits of these reactions were made in test-tubes in much the same way as experiments in hæmolysis are carried out. In each of a series of test-tubes was placed, from a pipette graduated in fiftieths of a cubic centimeter, a measured quantity of the solution of molybdic acid. To this was added a quantity of nitric acid solution of known strength increasing by increments of one tenth of a cubic centimeter from tube to tube. Sufficient distilled water was then added to make the contents of each tube up to 9.5 c.c., and finally 0.5 c.c. of a two per cent. solution of phenylhydrazin hydrochloride was added to each tube. In such a series of tubes, if a sufficient range of concentrations of the nitric acid was included the tubes at one end of the series would give the blue reduction, those at the other would at first show no signs of reaction, but after several hours would develop a brownish color. At some point in the series two tubes, side by side, differing from one another only in the nitric acid content, would present the one a blue, the other a brown color. When solutions of nitric acid containing 32 grammes of nitric acid per 100 c.c. were employed, that is, when the difference in the contents of two tubes amounted to .032 gr. of nitric acid, the contrast in color between the tubes which marked the limit of the reaction was a very striking one. Attempts to define this limit more accurately by the use of more dilute solutions of nitric acid, and accordingly smaller increments, of nitric acid from tube to tube did not result more satisfactorily. For example in a series of tubes in which the increment of nitric acid was 0.012 gr. from tube to tube, the transition was distributed over several tubes, those immediately preceding the first tube free from the blue precipitate containing a slight blue precipitate which subsided after several hours leaving a brownish supernatant fluid. Thus, the possible error in determining the proportion of nitric acid necessary to prevent the blue reaction is considerable, although the results are accurate enough for the purposes of this investigation, as subsequent statements will show.

Reactions of Soluble Molybdic Acid with Phenylhydrazin Hydrochloride in the Presence of Nitric Acid.

Strength of Molybdic Acid in Fractions of Normal.	Percentage of Phenylhydrazin Hydrochloride.	Percentage of Nitric Acid Necessary to Prevent Blue Reaction.
0.02	0.1	1.38
0.04	0.1	2.26
0.06	0.1	2.94
0.08	0.1	3.27

Reactions of Solutions of Ammonium Molybdate with Phenylhydrazin Hydrochloride in the Presence of Nitric Acid.

Solutions of ammonium molybdate when treated with phenylhydrazin hydrochloride give, as stated by Macallum, a brown color, and a brown precipitate slowly forms in the solution. In the presence of nitric acid, however, provided the latter does not exceed a certain amount which varies with the concentration of the molybdate, the reaction consists in the production first of a blue color and finally of a deep blue precipitate. If the amount of nitric acid exceeds this maximum no reaction occurs at first, but a brown color slowly develops in the solution. The following table gives the concentration of nitric acid necessary to prevent wholly the blue reaction with several concentrations of molybdate.

Percentage of Ammonium Molybdate.	Percentage of Phenylhydrazin Hydrochloride.	Per Cent. Nitric Necessary to Prevent Blue Reaction.
0.5	0.1	3.14
1.0	0.1	4.40
1.5	0.1	5.03
2.0	0.1	5.66

REACTIONS OF PHOSPHOMOLYBDIC ACID AND PHOSPHOMOLYBDATE OF AMMONIUM WITH PHENYLHYDRAZIN HYDROCHLORIDE.

Phenylhydrazin when added to a solution of phosphomolybdic acid or to crystals of ammonium phosphomolybdate suspended in water gives at once the reduction to the blue oxide. The reaction under these conditions proceeds so rapidly that it is difficult to follow the steps. The resulting body is not green in color as described by Macallum but blue, the green color which is first seen when phenylhydrazin hydrochloride is added to the crystals of ammonium phosphomolybdate being simply due to the yellow background afforded by the unreduced phosphomolybdate. After a few minutes the reaction proceeds still further and a soluble blue-violet compound is formed.

In the presence of nitric acid, the reaction proceeds somewhat more slowly, although it is less sensitive to the presence of nitric acid than the corresponding reactions with molybdic acid and ammonium molybdate. With crystals of phosphomolybdate suspended in water and 0.1 per cent. of phenylhydrazin the concentration of nitric acid may be increased to 36 per cent. without the reduction to the blue oxide being prevented. Even in the presence of this great amount of nitric acid the reduction of the phosphomolybdate reaches a maximum within ten minutes after the phenylhydrazin hydrochloride is added. The reaction between phenylhydrazin and phosphomolybdic acid proceeds in much the same way, and is similarly much less sensitive to the presence of nitric acid, than the corresponding reactions with molybdic acid and ammonium molybdate. For purposes of comparison the experiments were made the results of which are presented in the subjoined table.

Percentage of Phosphomolybdic Acid.	Percentage of Phenylhydrazin Hydrochloride.	Per Cent. Nitric Acid Necessary to Prevent Blue Reaction.
0.1	0.1	6.55
0.2	0.1	9.82
0.3	0.1	13.10
0.4	0.1	14.74

On comparison of this table with the preceding one it will be seen that the reduction of phosphomolybdic acid having a concentration of 0.1 per cent. will proceed in the presence of nitric acid having a concentration sufficient to prevent reduction in a solution of ammonium molybdate of 2 per cent. strength.

While it would have been difficult to draw from these experiments conclusions as to the probable behavior of the compounds of molybdic acid in the tissues when treated with solutions of phenylhydrazin hydrochloride containing nitric acid, yet they suggested a possibility which was capable of being proved experimentally that the compounds of molybdic acid and molybdates found in the tissues would fail to react to phenylhydrazin hydrochloride in the presence of an amount of nitric acid which would have no effect on the reduction of ammonium phosphomolybdate.

In order to test this question, sections of the liver of Necturus prepared after fixation in alcohol and fastened to the slide by the water method were treated with Macallum's nitric molybdate reagent, a solution of soluble molybdic acid in 10 per cent. nitric acid, and a ten per cent. solution of phosphoric acid, respectively. The two first mentioned solutions were allowed to act for three hours at 37.5° C. followed by eighteen hours at ordinary room temperature. They were then tested with a 0.1 per cent. solution of phenylhydrazin hydrochloride and found in each case to give a strong reaction corresponding in its characters and distribution to the phosphorus reaction of Macallum. The reaction obtained in the sections treated with the solution of molybdic acid was much the stronger. Other sections from the same lot were then treated with solutions containing 0.1 per cent. of phenylhydrazin hydrochloride and varying known quantities of nitric acid, in each case for a period of fifteen minutes. The sections from the molybdic acid solution and from the nitric molybdate reagent were treated side by side in the same solution, and for purposes of control a section which had been soaked in phosphoric acid and then treated with the nitric molybdate reagent was also put at the same time in the solution, so that it was possible to observe the effect of different concentrations of nitric acid on the reduction of sections treated with molybdic acid, or with the nitric molybdate reagent, and of sections containing ammonium phosphomolybdate artificially introduced. It was my expectation that a low concentration of nitric acid would suffice to abolish that portion of the reaction which was due to ammonium molybdate and to molybdic acid, and that a considerable residuum of the reaction would be found unaffected by even high concentrations of nitric acid and could thus be interpreted as a true phosphorus reaction. I was quite unprepared for, and greatly disappointed at the actual result of these experiments, namely, that relatively low concentrations of nitric acid abolished the reaction altogether.

With a concentration of 3.27 per cent. of nitric acid, phenylhydrazin hydrochloride 0.1 per cent., the molybdic acid sections and the nitric molybdate section showed no reaction after three minutes' treatment, although the section containing ammonium phosphomolybdate artificially introduced gave a maximum reaction in less than one minute. After fifteen minutes' action, a very faint reaction was obtained in the nuclei, both in the molybdic acid section and in the nitric molybdate section. When the concentration of the nitric acid reached 16.37 per cent., the phenylhydrazin remaining the same, the reaction was not recognizable after fifteen minutes' treatment although sections containing ammonium phosphomolybdate artificially introduced reduced to a maximum depth of color in the same solution in five minutes. I have repeated these experiments many times, always with the same results. It is significant that the reaction disappeared at exactly the same point as regards concentration of nitric acid in the molybdic acid section and the nitric molybdate section.

Only one conclusion is possible from these experiments, namely, that sections after treatment with Macallum's reagent for this length of time did not contain appreciable quantities of ammonium phosphomolybdate. Thus the fundamental assumption on which the reaction of Lilienfeld and Monti and of Macallum is based falls to the ground. It is obvious that if the phosphorus of the organic compounds is liberated at a point short of the destruction of the recognizable structures of the cell, it is not, at all events, precipitated *in situ* by the nitric molybdate reagent.

As a result of these experiments I am of the opinion that the reaction obtained by Macallum's procedure is entirely due to the formation of compounds of molybdic acid with the albumens of the tissue and not in any respect to the formation of ammonium phosphomolybdate at the expense of the organic phosphorus.

The facts on which the conclusion is based are, briefly, as follows:

The essential conditions of a successful phosphorus reaction are, first, that the phosphorus may be liberated from its organic combinations at a point short of the destruction of the recognizable structure of the cell; second, that the liberated phosphorus be precipitated at once at the point of origin as ammonium phosphomolybdate; third, that the reducing substance employed to make the phosphomolybdate visible for microscopic study act on phosphomolybdate and on no other compound of molybdenum which may be present in the tissue.

Phenylhydrazin hydrochloride does not meet the third condition because it reduces to the blue oxide of molybdenum, soluble molybdic acid in the test tube as well as molybdic acid combined with the tissue constituents in sections.

Phenylhydrazin hydrochloride also produces the blue oxide when treated with ammonium molybdate in the presence of nitric acid, provided that the latter does not exceed a certain concentration which is constant for constant concentrations of the molybdate.

Nitric acid affects the reduction of molybdic acid, ammonium molybdate, and ammonium phosphomolybdate, by phenylhydrazin hydrochloride in the same way, namely, retards the reduction, but to different degrees, inasmuch as low concentrations of nitric acid *prevent* the reduction of the two former to the blue oxide, while high concentrations of nitric *merely retard* the blue reduction of the phosphomolybdate. Accordingly, if phosphomolybdate is formed at the site where a reaction is obtained by the method of Macallum, the reaction ought to be elicited by treatment of the sections with solutions of phenylhydrazin in having a high content of nitric acid. This, however, the experiments show is not the case. Even low concentrations of nitric acid eliminate the greater portion of the reaction, and the reaction is entirely abolished by a nitric acid content which has little effect on the reduction of phosphomolybdate of ammonium artificially introduced into sections for purposes of control. Furthermore, the reaction is abolished at the same concentration of nitric acid with sections treated with Macallum's nitric molybdate reagent as with sections treated with a pure solution of molybdic acid. These facts dispose finally of the first and second essential conditions of a successful microchemical reaction for organic phosphorus, for it is clear that if the sections after treatment with the reagent contain no phosphomolybdate of ammonium, that the organic phosphorus has either not been liberated from its compounds, or that, if it has, it has not been precipitated at the moment and at the point of liberation. If these conclusions are correct, it is also obvious that there is no hope of a real phosphorus microchemical reaction being obtained by the employment of the nitric molybdate reagent.

These conclusions do not, of course, apply to the identification of phosphates by the nitric molybdate reagent, in cases where the characteristic crystal form of the ammonium phosphomolybdate can be recognized under the microscope.

In conclusion, it may be mentioned that in making the experiments to determine the effect of nitric acid on the reduction of the molybdenum compounds by phenylhydrazin hydrochloride it is important to employ solutions which are free from nitrous acid, which reacts with the phenylhydrazin and reduces its concentration.

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ON THE LOCOMOTION OF A SEA ANEMONE (METRIDIUM MARGINATUM).[1]

J. F. McCLENDON.

Last winter while studying some animals in the marine aquaria of the University of Pennsylvania I noticed that the anemones, after being placed at the bottom of an aquarium, would creep up the side of the glass to a more favorable position. Their method of progression is similar to the ordinary creeping of a snail,[2] consisting of a succession of waves that travel from behind forward, but in the anemone the waves are larger and not so rapid or regular. The accompanying photographs were taken of an anemone creeping up the side of an aquarium, with its distal end inclined forward, probably to test the water into which it was advancing. The undulations of the foot progress in the direction of locomotion. If the functionally posterior end of the

FIG. 1. *Metridium marginatum* seen through the glass, up which it is creeping. The lower side of the photograph has been outlined.

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[1] Contribution from the Zoölogical Laboratory of the University of Pennsylvania.

[2] For a more complicated form of locomotion in some snails see A. J. Carlson : "The Physiology of Locomotion in Gasteropods," BIOL. BULL., Vol. 2, January 1905, pp. 85–93.

foot be watched closely it will be seen to let go at several points (below in Fig. 1) and slip forward. This contraction is carried forward and on reaching the center of the foot, the contracted portion rises up from the glass, forming a wave that deepens as it approaches the "anterior" end (Fig. 2, above). On reaching the "anterior" edge the wave is retarded by the firmer attachment of the edge, which releases locally, breaking the wave into segments (Fig. 1, above). A wave requires about a minute to traverse the foot of the anemone, and before it has disappeared, another commences.

FIG. 2. *Metridium marginatum* about half a minute later than Fig. 1.

I threw a number of anemones into an aquarium to observe their actions. They threw out acontia, which caught hold of any solid near them and contracted until some portion of the foot touched the object and caught hold. One anemone sinking to the bottom and resting on its tentacles contrived to right itself by suddenly contracting and expelling water from its mouth. I observed this once or twice subsequently, but rather think it a coincidence than a common reflex.

BIOLOGICAL HALL, UNIV. OF PENN.,
December 5, 1905.

THE SIGNIFICANCE OF SCUTE AND PLATE "ABNORMALITIES"[1] IN CHELONIA.

A CONTRIBUTION TO THE EVOLUTIONARY HISTORY OF THE CHELONIAN CARAPACE AND PLASTRON.

H. H. NEWMAN.

CONTENTS.

PART I.

I. INTRODUCTION.

1. *Statement of the Problem.*

During a residence of several years on Lake Maxinkuckee, Marshall County, Indiana, my attention was repeatedly attracted to the large numbers and variety of species of tortoises that are found in the lake and in its accessory streams, swamps and pools.

In the spring of 1903 I began a study of the habits, variations,

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[1] The word "abnormalities" in the title is used for lack of a better one, and includes supernumerary scutes and plates, deficiencies in these structures and cases of fusion. The word "diversities" might have been used with equal appropriateness.

etc., of these species, involving the collection of large numbers of individuals of all sizes. One of the most striking phenomena that came to light was the prevalence of many kinds of scute abnormalities, consisting for the most part of supernumary scutes on the carapace and plastron. As examples multiplied I became aware of a marked degree of regularity in these abnormalities, the same supernumerary scutes occurring in exactly the same locations time after time.

Diversity in scutes had been noted by two observers. Gadow ('99) studied *Thalassochelys caretta* (L.), a species with no fixed number or arrangement of scutes, and Parker ('01) found two abnormal specimens of *Chelopus insculptus* (Le C.) on the basis of which he published a paper on correlated abnormalities in the scutes and the bony plates.

It seemed, then, that this phenomenon needed further investigation and the collection of large numbers of abnormal specimens was begun in the hope of reaching a rational explanation of this very prevalent diversity. Careful study has convinced me that these abnormalities are to be considered not as meaningless anomalies but as examples of systematic atavism in the sense of deVries. From this standpoint it seems possible to throw some light on the phylogeny of *Chelonia*.

The color patterns are intimately associated with the scutes and throw much light on their phylogeny. Consequently a brief consideration of chelonian coloration has been appended.

2. *Nomenclature. The Normal Plate and Scute Pattern.*

The following description and appended drawings (Plate I., Figs. 1 and 2), although referring particularly to an adult female specimen of *Graptemys geographica*, will apply to any genus of the Emydidæ. Fig. 1 represents the dorsal and Fig. 2 the ventral aspect.

The armor of tortoises consists of two elements, bony plates and horny scutes, which for brevity will be referred to as *plates* and *scutes*. Dotted outlines are used for the plates and solid outlines for the scutes. In labeling, small letters are used for plates and capital letters for scutes.

A. Plates. — There are in the *carapace* (Fig. 1) five longitudinal rows of plates, a single median and two paired rows. The median row has been variously designated as dorsal, vertebral and neural. In this paper the term *neural* will be invariably used. The neural row consists of the following elements : an anterior plate of large size called the nuchal (*nu.*), eight neurals (*n.* 1–8), two procaudals (*pr.* 1 and 2), and posteriorly the pygal (*p.*).

Lateral to the median row are the paired costals (*c.* 1–8), directly overlying the eight pairs of ribs.

Bordering the carapace on both sides and extending from nuchal to pygal are the marginals (*m.* 1–11).

The plates of the *plastron* (Fig. 2) are nine in number — the paired epi- (*e.*), hyo- (*ho.*), hypo- (*hp.*), and xiphi- (*x.*) plastrals, and the unpaired endo-plastral (*en.*).

The hyo- and hypoplastrals articulate directly with the fourth, fifth and sixth marginals and form the so-called "bridge" between the dorsal and ventral armor.

B. Scutes. — On the *carapace* (Fig. 1) there are, as in the case of the plates, five longitudinal rows of scutes that receive the same names as the plates. The median row, *neurals*, consists of a small anterior element, the nuchal (*NU.*), and five large *neurals* (*N.* 1–5). There are four pairs of large *costals* (*C.* 1–4). Twelve pairs of *marginals* (*M.* 1–12) completely surround the carapace with the exception of the small space occupied by the nuchal.

The scutes of the plastron (Fig. 2) are twelve in number, consisting of six pairs of large flat elements named from anterior to posterior end as follows : gulars (*G.*), humerals (*H.*), pectorals (*P.*), abdominals (*A.*), femorals (*F*), and anals (*AN*).

At the angles made by the junction of the pectorals and abdominals with the marginals are two pairs of small triangular scutes called respectively axillaries (*X.*) and inguinals (*I.*). These constitute all that remains in the Emydidæ of the *inframarginals*, a row much more prominent and complete in more primitive families.

No other plates or scutes occur normally among the Emydidæ, but for the sake of completing the nomenclature, it should be mentioned that one species, *Macrochelys temmincki*, possesses an additional pair of rows of scutes between costals and marginals, called *supramarginals.* Traces of a median ventral row of scutes are found normally in some species — and I have given the name "*interplastral*" to this row. A single median scute occurs normally in the anterior part of the plastron of certain specialized groups and receives the name *intergular.*

II. Discussions of the Present Status of the Question Concerning the Morphology of the Chelonian Armor.

The frequent abnormal occurrence of traces of the inframarginals and interplastrals in *Graptemys geographica*, *Chrysemys marginata* and *Chelydra serpentina* led me to review the literature relating to the evolutionary history of the chelonian carapace and plastron.

For nearly a century the chelonian armor has offered to morphologists a problem of unusual difficulty, and, although much has been written on the subject, its derivation is still unsettled. The question has been attacked from the three standpoints of paleontology, embryology and comparative anatomy.

1. *Paleontological* data are far from conclusive. It is not possible to go into this phase of the subject at all fully. Baur in 1887 published a brief summary of the more valuable paleontological data in an article entitled "On the Morphogeny of the Carapace of the Testudinata." A brief statement of the substance of this paper will, perhaps, serve to show the inadequacy of the paleontological evidence in this case.

The condition seen in the Dermochelydæ is considered to be the most primitive. Fossil remains of this group agree closely with the existing *Dermochelys coriacea* in the possession of "a pavement of small osseous plates extending over the whole shield, jointed to one another by more or less fine sutures. The number of these plates is much larger than that of the other Testudinata, which is never more than 70." This pavement of osseous plates is not united with the internal skeleton, as are the plates of other Testudinata, but has an independent dermal origin. "That the carapace of the Dermochelydæ is homologous to the carapace, without internal skeleton, of the rest of the Testudinata, there is no doubt." The fusion of the dermal pavement bones with the ribs and vertebræ is, according to Baur, proved by a specimen of *Eretmochelys imbricata*, a fossil species in which are found "small polygonal plates of the same shape as those of Dermochelys, suturally connected with the third, fourth, fifth and sixth costal plates." "A form between the Dermochelydæ and "*Thecophora*" (Dollo) is represented by the oldest known turtle, *Psephoderma alpinum*, H. v. Meyer, from the Triassic of the Bavarian mountains, preserved in Munich. In this highly interesting specimen, never mentioned in monographs on the Testudinata, we have certainly not less than 193 plates suturally united." According to Zittel's Paleontologie, Baur later expressed the opinion that *Psephoderma* may not be a chelonian at all, but perhaps a nothosaurus. Thus doubt is cast upon the best link in the chain of evidence. That all the principal groups of *Chelonia* were in existence in the earlier Mesozoic ages and that Palæozoic *Chelonia* are entirely unknown are familiar facts. So our attempts to reconstruct an ancestral condition must be made largely on the basis of embryology and comparative anatomy.

2. Nor is *embryological* evidence of chelonian phylogeny at all conclusive. The best and most recent study of the developmental history of the chelonian carapace and plastron was made by Goette in 1899. He summarizes the previous literature on the subject and shows that the main question at issue is that of the character of the neural and costal plates. Some authors, principally paleontologists, have maintained that these structures have a dermal origin and hence arise independently of the internal skeleton. Others hold that these plates are mere outgrowths of the ribs and spinal processes of the vertebræ. Goette favors the latter view and presents as evidence of its correctness a series of very careful embryological studies.

Suspecting that there might be some flaw in Goette's work, I repeated much of it, using the embryos of *Chelydra serpentina* and *Graptemys geographica*, and have satisfied myself that the neural and costal plates actually do originate as outgrowths of a differentiated tissue that surrounds the neural and rib cartilages. Whether this differentiated tissue be true periosteum, as Goette affirms, or simply a somewhat denser portion of the connective tissue that fills the space between the epidermis and the cartilaginous skeleton, is not certain. Haycraft ('99) maintains the latter view, but his paper is far from convincing.

As to the remaining plates of the carapace — nuchal, procaudals, pygal and marginals — there is no difference of opinion. All agree that they are of true dermal origin.

Thus it would seem that the plates of the carapace have a dual origin — the neurals and costals being periosteal ossifications while the nuchal, procaudals, pygal and marginals are dermal ossifications.

The carapace, then, as it exists to-day is not a simple structure but consists of a complex of at least two independent systems of bones.

Accepting the evidence of embryology as to the origin of the neural and costal plates, it remains to determine whether the dermal ossifications are, as Goette believes, mere supplementary structures that have come in to supply the deficiencies of the periosteal system, or are remnants of a once more or less complete dermal carapace that has in large measure been rendered superfluous by the broadening-out of the ribs and neural processes. The latter view would involve the former existence of complete rows of dermal bones overlying the vertebræ and ribs. Embryological evidence seems contrary to this view, as no dermal ossifications are found in the costal or mid-neural regions. It is possible that we may in this case overestimate the evidence of embryology as a guide to phylogeny. The great antiquity of the chelonian carapace is undoubted and in highly specialized structures that have attained a marked morphological fixity we should not be surprised to find great condensation in development, so that two structures formerly independent in origin — such as dermal and periosteal plates — may originate simultaneously so as to form only one inseparable structure. It seems quite plausible, then, that the rapid secondary broadening of ribs and neural processes has crowded out or appropriated the primordia that formerly went to form the dermal carapace and that only in places where the ribs and neural processes fail to reach the dermis do the true dermal bones have a chance to appear.

The fact that the nuchal plate appears before the ribs and neural spines have even commenced to broaden out and that the procaudals and the marginals follow before the neurals and costals are completely organized, points to the antiquity of these dermal structures and indicates that the neurals and costals are of more recent origin.

3. *Comparative anatomy* furnishes us much valuable evidence. In the family Trionychidae, for example, we have a series of forms that show a gradual reduction of a portion of the dermal armor. Fossil Trionychidæ are well known in which are shown a nuchal, a procaudal and a nearly complete set of marginal plates. Such a form was figured by Dollo in 1884 and named by him *Pseudotrionyx*. Another fossil species discovered by the same palæontologist and named by him *Emyda granosa*, lacks the procaudal and the marginals from the anterior half of the carapace. A third form, *Emyda Ceylonensis*, possesses a nuchal and several marginals at the posterior part of the carapace. The extreme limit of reduction is seen in *Aspidonectes spinifer*, which possesses only the nuchal plate as the last remnant of the dermal carapace.

It will be noted that the order of the appearance of these dermal ossifications in ontogeny is just the inverse of the order of disappearance in phylogeny. The latest elements to be formed in ontogeny are the first to disappear in phylogeny. This is just what we would expect if we consider that there has been a gradual shortening of the developmental process, a gradual elimination of the latest stages. The Trionychidæ show clearly that there is a marked tendency to reduce the system of dermal bones and it is not difficult to imagine that earlier reduction has taken place in which the dermal ossifications of midneural and costal regions were lost.

What evidence have we that such dermal ossifications overlying neural processes and ribs actually existed? O. P. Hay ('97) in an important paper dealing with the evolution of the chelonian carapace and plastron, describes and pictures an incomplete carapace of a fossil form named *Toxochelys serrifer*. Three ossicles occur above and overlapping the neural plates and occupy positions coincident with the keels of the second, third and fourth neural scutes. These ossicles have the general form of the tubercles seen on the dorsal ridge of the tail of *Chelydra serpentina*, and this suggests that the ossicles of *Toxochelys* are merely a continuation forward of a series of tubercles that must have been present on the tail.

Hay suggests that the keels seen especially in the young of modern *Chelonia* are the representatives of ancient dermal tubercles that formed the chief armor of ancestral forms. That in most cases these dermal ossicles have ceased to form independently of the deeper and more vigorous bony layers is perhaps to be expected as the result of condensation in developmental processes.

The degree to which modern species exhibit keels is extremely varied. Some highly specialized forms show none, or at most one, even in very young specimens, while one very primitive species, *Macrochelys temmincki*, possesses seven distinct keels on the carapace and four rows of flat scutes on the plastron. This multiplicity of keels is evidently a very primitive condition and naturally suggests to Hay the condition seen in *Dermochelys coriacea* in which twele well-marked keels are found, each keel consisting of rows of dermal ossifications that are lager and more prominent than the remaining intermediate ossicles that form the continuous pavement of the test. This peculiar aberrant chelonian is taken by Hay, following Baur and others, as the hypothetical ancestral type from which our modern chelonians have been derived by a process of simplification.

A survey of the field reveals the fact that the nearest approach to this condition of twelve rows of keels is seen in *Macrochelys temmincki*, which possesses seven distinct keels on the carapace. The four rows of flat scutes on the plastron may once have been keeled, for keels on the plastron are known in both extinct and living groups. The total number of keels or keel equivalents in *Macrochelys* is then eleven, one short of the supposed ancestral condition. The missing keel is the mid-ventral one and is represented in certain groups by intergulars. Thus all of the ancestral keels find representatives among modern species.

Hay seems to have been the first observer to suggest the importance of the scutes as factors in the evolution of the carapace. Previous authors have confined their attention to the bony structures, considering the scutes as of little significance. Hay's view of the rôle of the scutes may be stated briefly as follows: The probable ancestral condition is that seen in *Dermachelys*, the skin of which is found to be broken up into small polygonal areas, larger in the keels than elsewhere. These areas coincided with the osteodermal plates that are or will be developed in the skin. As the deeper elements of the carapace (neural and costal plates) increased in protective efficiency, the dermal structures were in many regions rendered superfluous and disappeared. In some cases the scutes were lost with their corresponding plates, in others the lost plate left its trace in the keel of the scute. The direction of growth of each of the existing series of scutes shows the direction of encroachment on other rows now lost.

This exposition of Hay's seems to me to be the most rational yet advanced, yet I believe that he fails to appreciate the evidence of embryology and thus introduces undue complexity. In the first place, he considers the nuchal plate as a fascia bone instead of an ordinary dermal plate. In the second place he states that the neural and costal plates are of the same character as the nuchal. Embryology shows that the nuchal plate is as true a dermal bone as are the marginals, while the neurals and costals are true periosteal expansions. It seems to me more rational to suppose that the dermal ossifications of the mid-neural and costal regions have undergone a complete suppression identical with that indicated by the series of Trionychidæ described above, rather than that they have become indistinguishable by fusion with the rib and neural fascia bones, as Hay calls them.

If we remove the scutes and underlying dermis from the carapace of a specimen of *Chelydra* we find that the long tubercles on the neural and costal plates bear no constant relation to the plates themselves, but are nevertheless clearly of a piece with them. It was natural for Hay to suppose that these bony tubercles were produced separately and then fused with the underlying plates. I have been able to trace this matter to a conclusion in the young of *Chelydra*, with the result that I have seen all the stages of ossification in the carapace and know that the tubercular keels on the neural and costal plates are produced by gradual thickenings of the growing plates. These thickenings send out branching processes that gradually displace the dermal connective tissue of the tubercles and fill the space with bone. Complete ossification of these tubercles does not occur until the animals are several years of age.

III. Description and Discussion of Abnormalities.

That a process of reduction both in the number of rows of scutes and in the number of scutes in surviving rows has taken place seems highly probable. From this standpoint I made a systematic study of all the abnormal specimens that showed traces of these lost rows or lost scutes. Inframarginals of all grades of prominence were found in specimens of *Graptemys geographica* and *Chrysemys marginata*, while interplastrals were found more rarely in the same two species. It will be noted that both of these recurring rows are plastron rows which probably means that the carapace has reached a high degree of fixity with reference to number of rows. Yet many abnormalities are found that indicate that the reduction in the number of scutes in a row was of comparatively recent occurrence.

These abnormalities will be discussed under three heads: (1) Inframarginals, (2) interplastrals, (3) supernumerary scutes in a row.

1. *Inframarginals.*

The occurrence or non-occurrence of inframarginals has formed the basis for separating the Thecophora into two great groups. Gadow in his volume on Amphibia and Reptiles gives Boulanger's key for classifying *Chelonia*. In this the two groups are characterized as follows:

1. Pectoral shields separated from the marginals by inframarginals — Chelydridæ, Platysternidæ, Cinosternidæ.

2. Pectoral shields in contact with the marginals — Testudinidæ, Chelydidæ, Pelomedusidæ.

It is evident that the more primitive families possess as normal factors this row of scutes while the more specialized families *normally* lack this row. When, however, dozens of specimens of *Graptemys* and *Chrysemys* possess this row in more or less perfect form, I am forced to consider this phenomenon as a well-marked case of systematic atavism. In view of the fact that no such anomalies have been previously described, it seems worth while to tabulate those in my collection — an easy task in view of the fact that the scutes occur in definite places. In any species, such as *Chelydra*, that possesses this row normally, there are typically three scutes in the row, one in contact with the axillary, one at the angle of contact of the pectoral and humeral and the marginals, and one abutting on the inguinal scute. These three scutes may be designated respectively as I., II. and III. Out of 476 specimens of *Graptemys geographica* examined, I found 31 with traces of inframarginals varying all the way from three large scutes on each side to one small one on one side. The tabulation below gives the number of the specimen, the sex, the length and breadth of carapace in millimeters, the occurrences of inframarginals on the right and left sides separately. Three general sizes are distinguished, which although quite arbitrarily laid down may serve to give a more definite idea of the amount of variation that occurs. These sizes are designated as large, medium and small.

At Woods Hole this summer I found two specimens of *Nannemys guttata* and three specimens of *Chrysemys picta* with well marked inframarginals.

It will be readily seen that in both species the middle scute is much the commonest recurrence, and this is natural if we consider that in *Chelydra*, and other species with well developed inframarginal rows, the middle scute is always the largest. The largest and most vigorous scute would probably persist longer and hence be most likely to recur as an atavistic reminiscence. The fact that no. III. is next in prevalence in *Graptemys* and no. I. in *Chrysemys*, indicates that the order of suppression of the other scutes of the row was subject to individual and group variation.

In the species *Chelydra serpentina* the inframarginal row is in a highly variable condition. Many stages in the reduction of numbers of scutes are to be seen in different individuals. The middle scute, corresponding to no. II., is always the largest, and the adjoining ones are next in size and would correspond to no. I. and no. III. Frequently there are two or three smaller scutes both in front of and behind the large central scutes, but

GRAPTEMYS GEOGRAPHICA.

No.	Sex.	Length in mm.	Breadth in mm.	Right Side.	Left Side.
1	F	98	81	Small II.	Medium II.
2	F	189	139	Small II.	
3	F	200	171	Small II. Large III.	
4	M	109	82		Medium I.
5	F	75	63	Large II.	Medium II.
6	?	63	57	Medium III.	Medium III.
7	M	82	70		Small II.
8	?	63	56	Large II.	
9	F	75	63	Small III.	
10	?	60	54	Small II.	Medium II.
11	?	57	51	Large II. Large III.	Large II. Large III.
12	F	195	147	Large III.	Large II. Large III.
13	F	160	118	Medium III.	Large III.
14	F	99	82	Medium I. Medium II. Medium III.	Medium I. Medium II. Medium III.
15	F	94	82	Large II. Large III.	Large II.
16	F	134	112	Large I. Large II.	Large I. Large II.
17	F	101	76	Medium I. Large II. Large III.	Large II. (See Fig. 44.) Large III.
18	M	88	69	Medium I.	
19	M	95	73	Large II. Large III.	Large II.
20	?	56	51	Small II.	Small II. Small III.
21	?	55	50	Medium II.	Medium II.
22	?	57	53	Large II.	Large II.
23	?	62	54	Large II.	Large II. Large III.
24	F	80	65		Medium II.
25	M	60	50	Large II.	Medium I. Medium II.
26	?	51	48	Medium II.	Medium II. Medium III.
27	?	58	53	Small III.	
28	M	66	54	Medium II.	Medium I.
29	M	110	78	Medium III.	
30	M	97	77		Small II.

they shown signs of suppression and in the majority of specimens are of insignificant size. The axillary and inguinal scutes of the Emydidæ, etc., correspond, I believe, to two of these smaller scutes that are undergoing suppression in *Chelydra*. They have persisted in the Emydidæ probably because they were needed to fill in the angles between the plastrals and marginals. *Aroma-*

CHRYSEMYS MARGINATA (188 specimens examined).

No	Sex.	Length in mm.	Breadth in mm.	Right Side.	Left Side.
1	F	104	80	Medium II. Medium III.	
2	F	116	88	Small II.	
3	F	102	81	Large I. Large II.	Large II.
4	M	85	67	Medium I. Medium II.	Small III.
5	F	90	73	Small II.	Small II.
6	M	96	72	Large I.	
7	F	98	80		Large I.
8	?	55	50	Medium I. Medium II.	Large II.
9	M	72	58	Small II.	Small II.
10	F	113	86	Large II.	Large II. (See Fig. 43)

A tabulation of the above results shows :

Graphemys :

	Right Side.	*Left Side*
I.	4 scutes	5 scutes
II.	20 "	20 "
III.	13 "	10 "
Total :	37 "	35 "

Chrysemys :

	Right Side.	*Left Side.*
I.	4 scutes	2 scutes
II.	8 "	4 "
III.	1 "	1 "
Total :	13 "	7 "

chelys odorata (Fig. 53) shows a curious survival of inframarginals, having invariably only two scutes, one large and the other very small and vestigial. From its position, separating the pectoral shields from the marginals, I would homologize this large scute with no. II. and the vestigial scute with no. I. Complete suppression of the inframarginal row has occurred in the terrestrial genera of the Emydidæ.

2. *Interplastrals.*

The occurrence of traces of the interplastral row are not nearly so frequently found as those of the inframarginals. Yet they are sufficiently numerous and definite to note in this connection. Traces have been found in *Chelydra*, *Graptemys* and *Chrysemys.* In preparing a list of these occurrences it will be convenient to number the places where such scutes might occur, *A*, *B*, *C*, *D* and *E*, beginning at the anterior end. Two specimens of *Chrysemys marginata* have extra scutes at *A* (Fig. 47). Two specimens of *Chelydra* have extra scutes at *C* (Fig. 45). One specimen of *Graptemys* has extra scute at *D* (Fig. 48). One specimen of *Graptemys* has a pair of extra scutes at *E* (Fig. 46). The primitive condition was probably one in which a scute was present at each point of union of four plastron scutes, but the fact that even in the tail of *Chelydra* this row is either partially or wholly wanting indicates the rather uncertain character of the row. In the specimens listed above scutes are found occurring in four places out of a possible five. No doubt a larger collection would serve to fill in this gap.

I consider these recurrences as true reversions to ancestral conditions; and that they come under the head of systematic atavism I see no reason to doubt.

How the typical number of scute rows seen in our modern tortoises has been acquired has, perhaps, been sufficiently discussed and it now seems necessary to consider the processes that have brought about the reduction of the number of scutes in a row — for it is beyond dispute that such a reduction has taken place.

3. *Supernumerary Scutes in a Row on the Carapace.*

The literature on this subject is limited to one paper, Gadow's much-discussed "Orthogenetic Variation in the Scutes of Chelonia," that was published in Willey's Zoölogical Results in 1899. The author gives a very interesting account of the conditions found in the common loggerhead turtle, *Thalassochelys caretta.* He has gathered together a miscellaneous assortment of some sixty-nine specimens of various sizes, principally newborn, from many parts of the world. On the basis of this collection he comes to the conclusion that scute reduction proceeds along certain definite lines. His observations, however, are limited to reductions in the neural and costal rows. According to Gadow, the ideal ancestral condition is one in which the neural and costal bony plates determine the number of scutes. The author's idea is that there was originally a scute for each of these plates.

Starting with this ideal condition as stage I., he finds the nearest approach to it in specimen 1, that has 8 left costals of which 2 are vestigial, 8 right costals of which 1 is vestigial, and 8 neurals. The greatest reduction is that seen in specimen 26, which has 5 left costals of which 1 is vestigial, 4 right costals, and 7 neurals of which 1 is vestigial. This latter specimen is reduced below the normal for the species, which is arbitrarily said to possess 6 neurals and 5 pairs of costals. This condition is said to be the goal toward which every young *Thalassochelys caretta* is striving.

The following stages are mapped out in diagram, following Gadow, to show the sequence in scute reduction in the chelonian carapace:

Stage I. — Hypothetical, eight neurals and eight pairs of costals. Neurals and costals lie in the same transverse plane and coincide with neurals and costal plates.

Stage II. — Eight neurals and eight pairs of costals, the latter fitting with their inner angles dovetailed between two successive neurals. Rearrangement probably brought about by the partial reduction of one pair of costal scutes. This reduced pair is probably the second.

Stage III. — Eight neurals and seven pairs of costals, the original second costals suppressed, original third becoming second, etc.

Stage IV. — Seven neurals and seven pairs of costals, but fifth neural and fourth pair of costals (original fifth), in a state of reduction.

Stage V. — Six neurals and six pairs of costals, owing to complete suppression of fifth neural and fourth (original fifth) pair of costals.

Stage VI. — Six neurals and five pairs of costals, brought about by fusion of last two pairs of costals into one or, perhaps, by suppression of one pair. This is the normal condition in *Thalassochelys.*

Stage VII. — Six neurals and four pairs of costals. Normal condition in the majority of tortoises to-day, brought about by suppression of first pair of costals.

Stage VIII. — Six neurals and four pairs of costals, first neural (nuchal) greatly reduced.

Stage IX.— Five neurals and four pairs of costals, first neural (nuchal) suppressed as seen in pleuroderous tortoises.

Beyond this last stage chelonians have not ventured yet, at least normally.

The order of loss in scutes is according to Gadow: (1) No. 2 costals, (2) no. 7 neural, (3) no. 5 neural and no. 4 (original no. 5) costals, (4) no. 7 or 8 costals (by fusion or suppression), (5) no. 1 costals, (6) no. 1 neural.

Gadow's paper, while most suggestive, must be criticised in several particulars, but before proceeding to the criticism it will be necessary for me to produce the data that to a large extent form the basis of the criticism. The data are derived from a collection of a large number of abnormal specimens, principally of two species, *Graptemys geographica* and *Chrysemys marginata.* Gadow worked on a species that is normally abnormal — if such an expression be permissible. He selected the commonest condition and arbitrarily called it normal. As a matter of fact, there is no normal or fixed condition. The species *Thalassochelys caretta* is evidently in a highly variable state as to scute number and arrangement, and no stability has as yet been attained. The species I have studied have, on the contrary, reached an advanced state of stability. Yet a sufficiently large number of abnormalities occur to give one nearly as many examples as Gadow had. Out of 476 specimens of *Graptemys*, varying from embryos to adults and taken at random, there occurred 48 specimens with supernumerary carapace scutes, while 188 *Chrysemys* yielded 8 such abnormal specimens. Four other species belonging to widely diverse groups yielded one abnormality apiece. It seems probable that abnormalities of exactly the kind that I have found so plentifully in the case of *Graptemys* and *Chrysemys* are to be found in any species if enough specimens be examined.

In order to economize space in the tabulation of these abnormalities brevity in the nomenclature of these vestigial scutes must be attained by numbering them. Combining Gadow's figures with my own results, I have good reason to believe that vestigial scutes occur between every two surviving normal scutes and that the first, second and last costals are also found in a vestigial condition. On this basis, then, there were eleven neurals and ten pairs of costals. These, if numbered from anterior to posterior, would give the numbers 1, 3, 5, 7, 9 and 11, to surviving neurals, and numbers 2, 4, 6, 8 and 10 to vestigial or lost neurals; the numbers 3, 5, 7 and 9 to surviving costals, and numbers 1, 2, 4, 6, 8 and 10 to vestigial or lost costals. In the tabulation these numbers will be used without further explanation. Furthermore, the sex, length and breadth of carapace, brief descriptions of both scutes and bony plates, will be given in separate columns. The significance of the tabulation of conditions of bony plates will be seen later when the subject of correlation between scute and plate abnormalities is discussed. The specimens are numbered and arranged in the order of abnormality, the specimens with largest number of extra scutes coming first, and those with less than the normal number of scutes last. Extra neurals will be listed before extra costals and the latter before extra marginals.

Two kinds of abnormality may be distinguished: symmetrical and asymmetrical. The former are less common and are important in that they furnish clearer cases and thus throw light on the latter. Under the head of symmetrical abnormalities may be mentioned extra neurals in the median line or nearly so; extra costals in pairs symmetrically placed; extra paired marginals. The great majority of abnormalities are asymmetrical, consisting of: extra neurals crowded to one side or the other but usually showing clearly enough the position they would normally occupy; unpaired costals or marginals. In the case of asymmetrical neurals it is sometimes difficult to distinguish the supernumerary scute from the normal scute, on account of the large size of the former and the fact that crowding has forced the two scutes to lie approximately side by side. There are usually correlated points of asymmetry that may be of assistance in deciding the point, but occasionally I have been compelled to trust to my judgment and may possibly have erred. Gadow would probably consider the type in which the normal and supernumerary scutes lie side by side as evidence of the original paired character of the neural row. Were it not for transitional conditions this view might be tenable.

Occasionally it becomes difficult to determine which of five costals is the supernumerary scute, but a reference to the mar-

GRAPTEMYS GEOGRAPHICA.

No.	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities	Plate Abnormalities.
1	20	?	60	54	Complete transverse row of 6 large scutes in middle region. Difficult to diagnose. Double 6 neural (large). L 6 costal (large). R 4 costal (large). Paired marginals.	Plates not fully formed.
2	6	F	188	144	Median 2 neural (large). Paired 1 costals (large). Extra R marginal (large).	No. 1 procaudal fused with 8 neural. Extra R marginal.
3	35	?	44	42	R 8 and 10 neurals (large). R 10 costal (medium). L 10 costal (small).	Plates not fully formed.
4	4	F	122	97	3 neural partially divided. Probably indicates fusion of 3 and 4 neural. Paired 1 costals (large). R marginal (large).	R Marginal.
5	5	F	200	145	R 8 and 10 neurals (large). R 8 costals (large).	9 neural. Double extra procaudal. R costal (medium). L costal (small).
6	3	F	189	139	R 10 neural (large). R 10 costal (large). R marginal (medium).	9 neural. Double extra procaudal. Paired 9 costals (large). R marginal.
7	13	M	98	71	R 8 and 10 neurals (large). R 8 costal (large).	Normal.
8	14	M	84	70	L 10 neural (large). 9 neural, partly divided, and probably represents 8 and 9 neurals fused. L 10 costal (medium).	Normal.
9	9	F	98	81	L 8 neural (large). 10 and 11 neurals fused. L costal (medium).	Normal.
10	36	?	52	47	R 8 and 10 neurals (large). Lacks a R marginal.	Bones not formed.
11	34	(embryo)	21	18	L 8 and 10 neurals (large).	Bones not formed.
12	37	"	25	21	R 10 neural (large). R 10 costal (medium).	Bones not formed.
13	8	F	174	136	R 10 neural (large). R 10 costal (large).	Extra procaudal.
14	10	M	113	76	L 10 neural (medium). L 10 costal (medium).	Normal.
15	12	M	98	70	R 10 neural (medium). R 10 costal (medium).	9 neural. Paired 9 costals.

GRAPTEMYS GEOGRAPHICA.—*Continued.*

No.	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities.	Plate Abnormalities.
16	40	?	57	51	L 10 neural (medium). L 10 costal (medium).	Bones not formed.
17	Same as 10	M	87	73	L 10 neural (medium). L 10 costal (medium).	Normal.
18	7	F	170	138	6 and 7 neurals partially fused. 9 and 11 neurals completely fused. L 6 costal (medium).	Only photographic record retained.
19	56	(embryo)	12	10	R 8 neural (large). R marginal.	Ribs very abnormal.
20	17	M	89	69	Paired 10 costals (small).	Normal.
21	See 17	F	160	118	Paired 10 costals (medium).	Normal.
22	See 17	?	61	54	Paired 10 costals (medium).	Bones not formed.
23	See 17	F	209	169	Paired 10 costals (medium).	Normal.
24	26	?	51	45	Paired 1 costal (medium).	Bones not formed.
25	38	(embryo)	23	19	Median 8 neural (large).	Bones not formed.
26	39	(embryo)	19	16	L 8 neural (large).	Bones not formed.
27	23	F	192	154	Median 10 neural (medium).	Normal.
28	15	M	83	67	L 10 neural (medium).	Normal.
29	11	M	109	82	L 6 neural (large).	Normal.
30	22	M	75	63	Paired extra marginals (small).	Paired extra marginals (small).
31	16	M	78	67	R 10 costal (large).	Normal.
32	See 16	M	82	70	R 10 costal (large).	Normal.
33	See 16	F	72	62	R 10 costal (medium).	Bones not formed.
34	See 16	?	52	47	R 10 costal (medium).	Bones not formed.
35	See 16	?	63	56	R 10 costal (medium).	Bones not formed.
36	21	F	66	57	L 10 costal (medium).	Bones not formed.
37	See 21	?	57	51	L 10 costal (medium).	Bones not formed.
38	See 21	?	29	26	L 10 costal (medium).	Bones not formed.
39	See 21	?	60	54	L 10 costal (medium).	Bones not formed.
40	See 21	F	73	63	L 10 costal (medium).	Bones not formed.
41	See 21	F	99	82	L 10 costal (small).	Normal
42	See 27	?	47	42	L marginal lacking.	Bones not formed.
43	27	?	58	52	L marginal lacking.	Bones not formed.
44	19	F	72	64	R marginal lacking.	R marginal lacking.

GRAPTEMYS GEOGRAPHICA.—*Concluded.*

No.	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities.	Plate Abnormalities
45	See 19	?	58	53	R marginal lacking.	Bones not formed.
46	18	F	77	65	Paired marginals lacking.	Paired marginals lacking.
47		(embryo)			Paired marginals lacking and costals not fully differentiated.	Bones not formed.

CHRYSEMYS MARGINATA.

No	Fig.	Sex.	Length in mm.	Breadth in mm.	Scute Abnormalities.	Plate Abnormalities
48	31	F	130	93	R 8 and 10 costals (large). R 10 costal (large).	Extra procaudals.
49	32	F	73	61	L 8 and 10 neurals (large).	Normal.
50	30	M	104	80	Paired 10 costals (large).	Normal.
51	25	?	52	47	Paired 4 costals (medium).	Bones not fully formed.
52	24	?	57	50	L 4 or R 6 neural.	Bones not fully formed.
53	29	F	84	67	R 10 neural (large).	Normal.
54	28	M	97	66	L 1 costal (medium).	Normal.
55	41	F	73	63	L 8 costal (medium).	Normal.

ginals will usually settle the point, as the normal condition has a very characteristic arrangement of these two sets of elements.

Another source of difficulty arises from the complete or incomplete fusion of adjacent scutes. Fusion is due to the inhibition of the process of division into epidermal areas at a rather late embryonic stage. In some cases the fused scutes show their separate identity, after a year or two of growth, by a separation of their growth rings. Coker has called attention to several such cases in connection with *Malaclemmys centrata* and I have observed the same phenomenon in the marginals of *Graptemys* on several occasions. Usually, however, the indications are clear enough to enable one to recognize the individual elements in a fused scute. It seems reasonable in the present discussion to consider the number of scute primordia involved in a fusion and to give them the full rank of independent scutes.

As in the previous tabulation, the arbitrary terms, large, small and medium, are used. L or R in connection with neurals will indicate that the extra scute is crowded to left or right. The same letters indicate the side on which extra costals and marginals occur.

The following isolated abnormalities have come to hand and may be listed :

Large specimen of *Terrapene carolina* has a R 10 costal (medium).

Medium-sized shell of *Cyclemys dentata* has paired 6 costals (medium). See Fig. 42.

Two medium-sized specimens of *Chelydra serpentina* have L 10 costal (large).

Large *Aromochelys odorata* has R 10 costal (medium).

A reference to the literature enables me to list a considerable number of similiar abnormalities. The names used in the references will be retained.

1. *Ptychemys elegans*, Agassiz, L., Contributions to the Natural History of the U. S., Plate I, Fig. 13, showing : L 4 neural, paired 4 costals, paired extra marginals. (Figured in this paper as Fig. 33.)
2. *Chelopas insculptus*, Parker, G. H., paired marginals lacking.
3. Same : R 8 and 10 neurals, R 10 costal, R marginals lacking.

IN CATALOG OF SHIELD REPTILES IN THE BRITISH MUSEUM.

4. *Emys vermiculata* (Tab. XIII.), 24 neural, R 8 and 10 neural.
5. *Emys singuinulenta* (Tab. XV.), R 6 neural, R 6 costal, L 4, 6 and 10 costals.
6. *Cyclemys dentata* (Tab. XIX.), median 4 neural, R 6 neural, L 8 neural.
7. *Chelodina oblonga* (Tab. XXIV.), R 8 neural.

IN HISTORIA TESTUDINUM, SCHOEPFF, J. B., 1792.

8. *Testudo cinera* (Tab. III.), Fig. 2, paired 1 costals.
9. *Testudo areolata* (Thunberg), Tab. XXIII., median 8 neural, L 6 costal.
10. *Testudo planiceps*, XXVII., L 4 costal.

Discussion.

A scute, whether normal or supernumerary, is a separate and definite entity, resulting from a definite embryonic primordium.

The fact that supernumerary scutes have been found between all of the normal scutes as well as at both ends of the costal series must have some significance. If one assumes that these supernumerary scutes represent the atavistic recurrence of scutes that have been lost in the course of phylogeny, it is possible that the following tabulation will throw some light on the sequence of loss.

Neurals.		*Costals.*	
No. of Scute.	Numbers of Recurrences.	No of Scute.	Numbers of Recurrences
2	1	1	6
4	2 (one doubtful)	2	0
6	5 (one doubtful)	4	3
8	11 (one doubtful)	6	4 (one doubtful)
10	16 (one doubtful)	8	4 (one doubtful)
		10	35

It will be seen that the most frequent recurrences are at the posterior end of the carapace, and that, with the exception of the first costal, the frequency of recurrence diminishes as we proceed anteriorly. What significance attaches to this fact? It seems quite probable that the most frequent recurrences represent the most recent losses and the rarest recurrences the most ancient losses. This rule held good for the suppression of rows of scutes and should apply here also.

On this basis then we can at least say that the succession of suppression was in general antero-posterior, that the earliest losses occurred at the anterior end of the carapace and the most recent losses at the posterior end. One might go further and say that in the neural series the order of suppression was probably 2, 4, 6, 8 and 10. The antero-posterior order of loss is not so clear in the case of the costals, as no. 1 costal recurs more frequently than any other except no. 10. This means a modification of the regular mode of progression. In the costal series it is probable that the antero-posterior succession of losses was interfered with by the rounding-in of the marginals both anteriorly and posteriorly. This rounding-in would necessarily begin about medially and proceed in two directions, hence the second supernumerary scute would be put under pressure before the first and the eighth before the tenth. The antero-posterior tendency, however, would bring about the suppression of anterior scutes, as a whole, before posterior scutes.

Evidences are not wanting that scutes may be suppressed and the method of suppression seems clear. In a specimen of *Cyclemys dentata*, listed as no. 58 and figured on Plate III., Fig. 42, the paired sixth costals are being encroached upon by the seventh costals. The anterior growing margins of the latter have pushed in under the posterior edges of the former in such a way as to severely cut into their growth centers. The dotted line shows the amount of encroachment. Several specimens of *Graptemys* show the same phenomena, and the scutes encroached upon are always the supernumerary ones. This may be looked upon as a recurrence of an ancestral condition and we may infer that the loss of certain scutes has been brought about through the encroachment, more and more severe with succeeding generations, of more vigorous upon less vigorous scutes, resulting in the final complete suppression of the latter. We must also suppose that the rudiments of the lost scutes lie dormant in the embryonic tissues and occasionally for some reason reappear more or less completely. Those that have been suppressed for the longest time would naturally reappear least often and *vice versa*. On this basis, then, we may safely say that the order of loss is orthogenetic if by this we simply mean onward development.

Applying the same methods to Gadow's figures I find a very general agreement, although I am unable to agree with the author's interpretations. The vestigial scutes that occur in Gadow's figures are: neurals 2, 8 and 10; costals 1, 2, 4, 6, 8 and 10. No. 2 costal was not found in my specimens, but is so clearly seen in Gadow's Fig. 1 that I have introduced it into my system. It is possible that No. 2 costal was the most ancient loss and hardly likely to recur in specialized types such as *Graptemys* and *Chrysemys*, since it occurred only once in Gadow's specimens of *Thalassochelys*. It will be seen that Gadow finds no vestiges of neurals 4 and 6. An examination of his figures will show that *Thalassochelys* has attained a high degree of fixity in the anterior portions of the mid-neural series, while all other regions are still in a decidedly variable condition. Hence we are unlikely to find vestigial scutes in this region unless a much larger number of specimens is examined.

Carapace abnormalities have been pictured by authors for over a century and I have on my lists fourteen species, belonging to widely diverse groups, that show the same general abnormalities.

These scattering cases could scarcely be used in determining the order of loss of scutes, but are of importance in that they show that certain abnormalities that are comparatively rare in *Graptemys* and *Chrysemys* occur with a fair degree of frequency in other forms. For example: neurals 4 and 6, and costals 1, 4 and 6 occur from 2 to 4 times in these specimens. The prevalence of abnormalities of this sort over such a wide range of forms strengthens my idea of the universality of the process of scute reduction in *Chelonia*. I have no doubt that such abnormalities will be found in any species if enough forms are examined.

In Gadow's diagrams illustrating the progressive reduction of epidermal scutes (p. 217) it will be seen that the order of reduction differs from the one I have proposed in two points; in the first place he indicates that no. 10 neural is suppressed before no. 8, but this is not borne out by his own figures. Figs. 4, 6, 7, 14, 20, 26, show no. 10 persisting after the total suppression of no. 8, Fig. 26 being especially convincing. Figs. 8, 9, 10, on the other hand, show no. 8 persisting after the suppression of no. 10. The balance is decidedly in favor of the earlier suppression of no. 8, yet there must have been some individual variation in this matter. My own figures show that no. 8 recurs twelve times as compared with seventeen times for no. 10. In my own specimens there are eight cases in which nos. 8 and 10 neurals recur together, nine cases of no. 10 recurring alone, and only four of no. 8 recurring alone. It would seem then that these two scutes were undergoing a process of suppression at about the same time, but that no. 8 was in most cases the first to disappear.

In the second place it seems clear that no. 1 costal persisted longer than no. 10 in *Thalassochelys*, but that the opposite was the case in all the forms in my collection can scarcely be doubted. no. 10 recurs thirty-six times and in many species, while no. 1 recurs only six times.

Some rather remarkable conclusions are expressed in Gadow's paper and should be discussed in this place.

1. He makes the following statement: "Abnormalities are 4 to 7 times as common in new-born as in mature specimens, hence scute reduction must take place during the lifetime of the individual." I have not had the opportunity of putting this matter to a test in the case of *Thalassochelys*, but the examination of several complete nests of *Graptemys* has brought to light the following facts. Two nests containing respectively thirteen and fourteen embryos showed no abnormalities. One nest containing fourteen just-hatched young showed one slight abnormality, a vestigial no. 10 costal. A fourth nest in which twelve eggs came to maturity contained five decidedly abnormal specimens, listed as nos. 11, 19, 25, 26 and 48. This means that barely 10 per cent. of the embryos of four broods are abnormal, while out of 476 specimens of *Graptemys* 48 were abnormal in the carapace scutes, a little over 10 per cent. A large proportion of Gadow's new-born specimens came from one nest, the whole brood of which was abnormal. The others were taken in small sets from various collections, and I believe that such specimens had been preserved because of their abnormalities. A survey of my tabulations will show that abnormalities are no more common in one size than in another. Finally, Coker, in a very recent preliminary paper, delivered before the American Society of Zoölogists in Philadelphia, December, 1904, claims that observations on embryos of *Thalassochelys* gave no support to the theory of Gadow.

2. Gadow considers that certain specimens (Figs. 6 and 24) show evidences that the neural row was originally a double one. That this was the case seems very unlikely from an examination of such primitive conditions as are seen in the tail of *Chelydra* and in the neural keel of *Dermochelys*. The appearances seen in Figs. 6 and 24 may be due to the crowding of linear members of the row until they come to lie side by side. Indications of an approximation to this condition are not uncommon in the specimens which I have had to deal with.

4. *Supernumerary Scutes on the Plastron.*

The *plastron* has, as a rule, reached a higher degree of fixity in the matter of numbers and arrangement of scutes than has the carapace, but that this portion of the chelonian armor has not always had so fixed a character may be seen in the high state of variability of *Aromochelys odorata*, which is almost as marked as that seen on the carapace of *Thalassochelys*. In *Aromochelys* the number of plastron scutes varies from 14 to 9 and all intermediate conditions are readily found. Fig. 52 shows the largest number of scutes seen in the specimens of my collection. In this case there is a well-developed extra pair of scutes between the abdominals and femorals. Fig. 53 shows the commonest condition in which there are the usual five pairs of plates and the gulars are partially fused. Fig. 54 shows the extreme of reduction in which the pectorals have been lost either through crowding or fusion, and the gulars have fused into a single median element.

As in the case of the carapace, we find in several species that have attained a high degree of fixity in the plastron, marked traces of supernumerary scutes. Fig. 49 shows the plastron of a small specimen of *Chelydra* that has an extra pair of scutes between femorals and anals. Fig. 50 shows another specimen of *Chelydra* with a vestigial scute on the right side between humeral and abdominal. Fig. 51 shows a specimen of *Chrysemys* with a well-marked supernumerary scute on the left side between abdominal and femoral. In Figs. 49, 50, 51 and 52 we have supernumerary scutes in four places out of a possible five. As yet I have been unable to find supernumerary scutes between gulars and humerals.

Losses seem to have taken place in two ways: by fusion and by crowding out. Some curious examples of the latter might be mentioned. In *Chelydra* the abdominals have been forced to the sides, but have been retained to bridge the gap between the small plastron and the margin of the much larger carapace. In other cases the pectorals have played a similar rôle. Van Lidth de Jeude describes a specimen of *Testudo ephippium* (Gthr.), in which the pectorals have been crowded to the two sides like the abdominals of *Chelydra*. Other specimens of the same species, according to Rothschild, have the same abnormality to a greater or less degree. The Catalogue of Shield Reptiles in the British Museum shows a specimen of *Monouria fusca* (Tab. III.), in which the pectorals are crowded to the margin of the plastron and have become small and triangular. The same volume shows a specimen of *Sternotherus Derbianus* (Tab. XXII.), in which the pectorals seem to have a tendency to be suppressed or crowded to one side.

On the whole it seems evident that an orderly suppression of alternate scutes has taken place in the plastron as well as in the carapace.

5. *Correlated Abnormalities in the Scutes and Bony Plates.*

The next question that comes up for discussion is whether or not there is any correlation between scutes and bony plates. It has long been noticed by morphologists that there is a certain definiteness about the relative positions and sizes of scutes and plates. This may be described in brief as a definite overlapping of bony sutures by scutes. In the marginal series (see Fig. 1) this is seen in its simplest form — every bony suture being covered by a scute. In the neural and costal series one scute as a rule covers one whole plate and half of two adjoining plates. This arrangement is modified in the anterior and posterior regions. In the former the nuchal plate is partially overlapped by six scutes, viz.: nuchal, first pair of marginals, first pair of costals (normally involving only small corners of the plate), and the first neural. The first costal scutes cover the first and half of the second costal plates as well as the inner edges of first, second, third and fourth marginal plates. The last neural covers normally parts of eight plates, viz.: the two procaudals and the anterior margin of the pygal, about half of the eighth neural and eighth pair of costals, and the anterior margins of the eleventh marginals. Only in the middle portions of the carapace is any definiteness of arrangement seen, yet there is a marked fixity of relations even in the most specialized regions. Gadow bases his reduction series upon an arbitrary connection between these structures, according to which there was originally a scute for each vertebra and rib. He gives no reason for assuming a vital connection between these structures, but simply implies one. In an earlier portion of the present paper it has been shown that there is no ontogenetic connection between the scutes and plates, the former being laid down before the latter have begun to form, while the latter appear comparatively late in development as mere outgrowths of the ribs and neural spines.

If, however, there be any essential connection between these scutes and plates, we would expect to find scute irregularities and abnormalities associated with plate irregularities and abnormalties and *vice versa.*

G. H. Parker ('99) expresses himself at some length on this point in a paper entitied "Correlated Abnormalities in the Scutes and Bony Plates of the Sculptured Tortoise." He describes in detail two abnormal specimens and on this slender basis reaches some rather general conclusions.

Specimen no. 1 has extra eighth and tenth neurals and a small right tenth costal. No plate abnormalities are found in the neurals or costals, but one right marginal plate and a corresponding scute are lacking. Parker designates these conditions as : (*a*) Scute abnormalities unassociated with plate abnormalities, (*b*) scute abnormalities associated with plate abnormalities.

Specimen no. 2 has normal neural and costal scutes, but lacks an entire horizontal row of plates consisting of a neural, a pair of costals and a pair of marginals. The lack of marginal plates is associated with the lack of a corresponding pair of scutes. These conditions are designated as : (*a*) Plate abnormalities unassociated with scute abnormalities, and (*b*) scute abnormalities correlated with plate abnormalities. Parker finds the second specimen shorter than the average normal specimen of the same sex in the proportion of 1.298 to 1.313. This is due to the loss of a vertebra and pair of ribs.

In both cases it will be seen that the only real correlation occurs in the marginals and that here the correlation is perfect. Another correlation not mentioned by the author is seen in the second specimen, where there is a reduction of marginals in sympathy with the reduction in neurals and costals. This correlation is, I believe, of a different sort from that seen in the marginal plates and scutes, in that a common cause has brought out the same general effect in both sets of structures. In both cases of correlation in the marginals Parker concludes that the abnormality is in the anterior portion of the carapace, but an examination of his figures fails to convince me that he has any criterion for thus locating the point of suppression or recurrence of scutes, for a loss anywhere in the marginals would necessitate a general readjustment with reference to the costals. Yet the author concludes that correlated abnormalities are likely to occur only in the anterior portion of the carapace.

To aid this hypothesis he adds a second one, based on a paper by Harrison ('98), in which it is shown that in the frog larva the ectoderm is proliferated chiefly at the anterior end and the mesoderm chiefly at the posterior end. This would cause the ectoderm to slide back over the mesoderm. Parker concludes that the ectodermal structures in the carapace have migrated away from their mesodermal connections so that the posterior scutes are far from their original plates, while in the anterior part of the carapace the scutes are over the same plates that they originally covered.

This ingenious hypothesis loses its force when, after the examination of large numbers of abnormalities, it is found that nearly all true correlations of plates and scutes occur at the posterior end of the carapace.

A reference to the tabulation of abnormalities will bring to light the following facts:

1. Specimens 2, 4, 6, 30, 45, 47, 61 and 62 show very precise correlation of abnormalities in the marginals. All abnormalities in the marginal scutes are correlated with similar abnormalities in the plates.

2. Specimens 5, 6 and 15 have abnormalities of both plates and scutes of the costal series, but in no case are these strictly correlated. In all three specimens the extra costal plates are paired while the extra costal scutes occur on the right side only. These specimens are abnormally long and the undue length may be the common cause of both extra plates and scutes.

3. No. 9 neural plate recurs in the same three specimens (5, 6 and 15) and is associated in each case with one or more extra neural scutes (Nos. 8 or 10). Examination shows that these extra neural plates are irregular structures, are not associated with the neural processes of vertebræ, and hence are to be con-

sidered in the same category as the procaudals. On this account I am strongly of the opinion that they have had a dermal origin like the procaudals.

4. Specimens 5, 6, 9, 13 and 49 have extra procaudal, either single or double, and in each case this plate abnormality is associated with the recurrence of an extra scute in the posterior part of the neural row. The procaudals are certainly of dermal origin and were probably the bony cores of the last neural scutes before the crowding-out process began. Consequently the reappearance of an extra plate and extra scute in this region may with justice be considered as a case of correlation.

5. Specimens 7, 8, 14, 17, 20, 21, 23, 27, 29, 31, 33, 41, 50, 51 and 58 have various kinds of scute abnormalities and perfectly normal plates.

Summarizing, we find that the only invariable correlations are in the marginals which represent scutes with their bony supports (the plates) only slightly separated from one another; that correlations between extra procaudals (also true dermal plates) occur in five cases and no case of plate abnormality in this region is without a scute abnormality; that correlations between an extra neural of small size and irregular shape occur in every case; that extra paired costal plates are associated with asymmetrical extra costal scutes; and that very frequently scute abnormalities appear without any corresponding plate abnormality.

It would appear then that abnormalities are never truly correlated except in regions where dermal plates persist and never in the anterior part of the carapace, because the plates of that region, except the nuchal, have given way to the periosteal plates of the neural and costal series. The nuchal plate still possesses its scute in a reduced condition, so we would not expect to find any abnormality in that region.

Only in the regions of dermal plates do we find any interdependence of plates and scutes, because only here, according to our theory, is there any genetic connection between dermal and epidermal structures.

That we find no true correlations between the plates and scutes of the neural and costal series is just what we would expect from our knowledge of their ontogenetic independence. In the most irregular specimens, having the most grotesque scute displace-ments and additions in the mid-neural and costal regions, there is not a single case of sympathetic plate abnormality.

Another strong case in favor of the entire independence of these structures is furnished by examples of vertebral distortions. A paper by Wandolleck (1904) entitled "A Hump-Backed Tortoise," describes a specimen of *Testudo Græca* in which the neural and costal plates were in utter confusion, due to lateral curvature of the spine. Yet the number and arrangement of the scutes was perfectly normal. Another case in point is that of a deformed embryo in my possession that has ten ribs on one side and the number of costal scutes is perfectly normal (Fig. 56).

Further evidence for the independence of neural and costal scutes and plates is derived from the fact that certain land tortoises undergo striking modifications of plates in order to form a dome-shaped shell of great strength. The costal plates become, dovetailed one into the other and are decidedly wedge-shaped. This condition is not followed in any respect by the scutes, which retain their typical form and arrangement. A. Bienz ('94) describes this condition in *Dermatemys mavii*, and shows that the form and the arrangement of the plates conform with the most approved architectural principles.

The origin of the bony plates of the plastron has been thoroughly worked out and in each case a plate is to be interpreted as a modification of preëxisting bony structures — clavicle, sternum or abdominal ribs. If such structures as these have been transformed into large, flat, bony plates without any fusion of fascia bones or any outside factors of any sort, why is it necessary to explain the costal and neural plates as other than mere modifications of the preëxisting ribs and neural spines?

6. *Correlation in Scute Abnormalities.*

Two tendencies may be noted in this connection:

1. Scutes of the same horizontal row have a tendency to recur together (see Figs. 3, 5, 6, 7, 8, 9, 10, 12, 13, 14, 20, 31, 33 35, 40). A 10 neural most frequently is accompanied by a 10 costal, etc.

2. In asymmetrical abnormalities the tendency is for supernumerary neurals, costals and marginals to occur on the same side (see Figs. 3, 5, 7, 8, 9, 10, 12, 13, 14, 31, 35, 40).

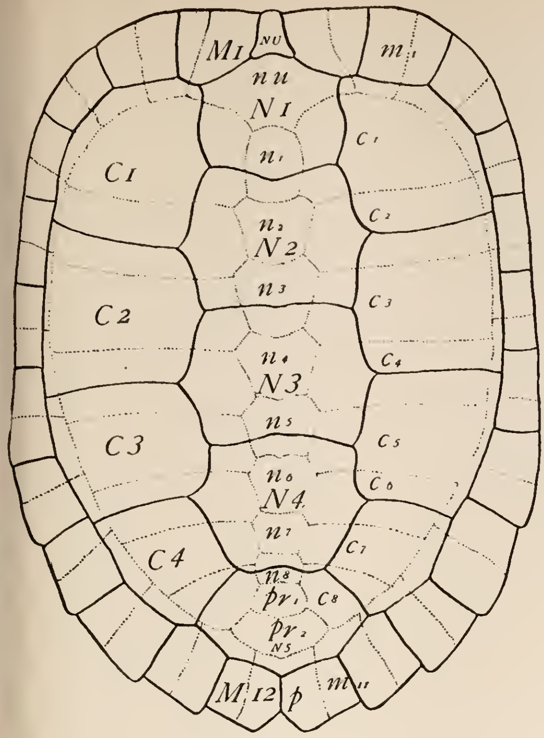


Fig. 1

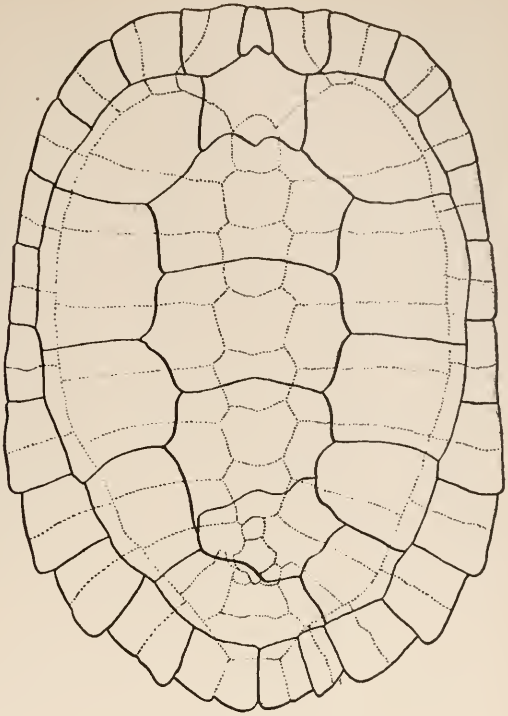


Fig. 3

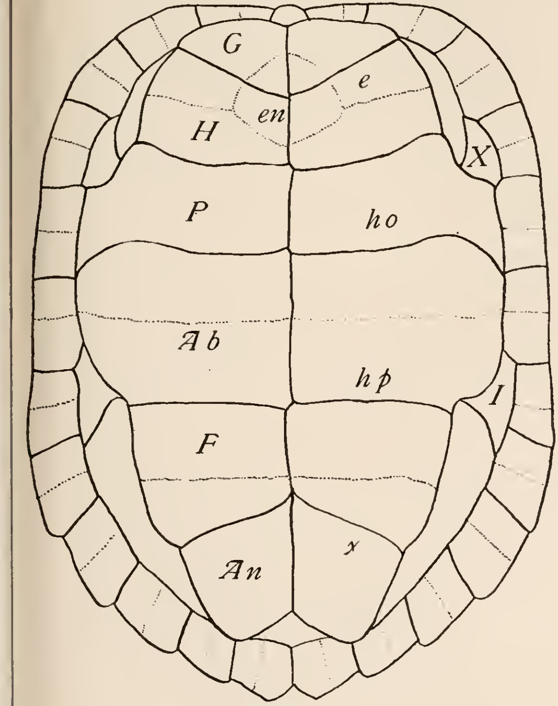


Fig. 2

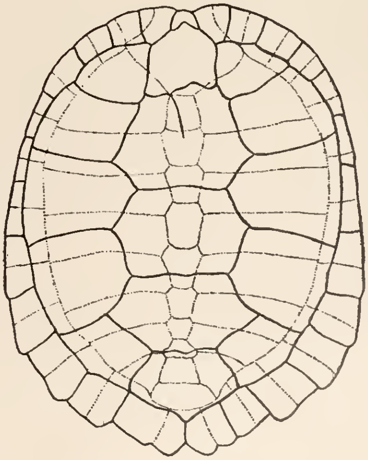


Fig. 4

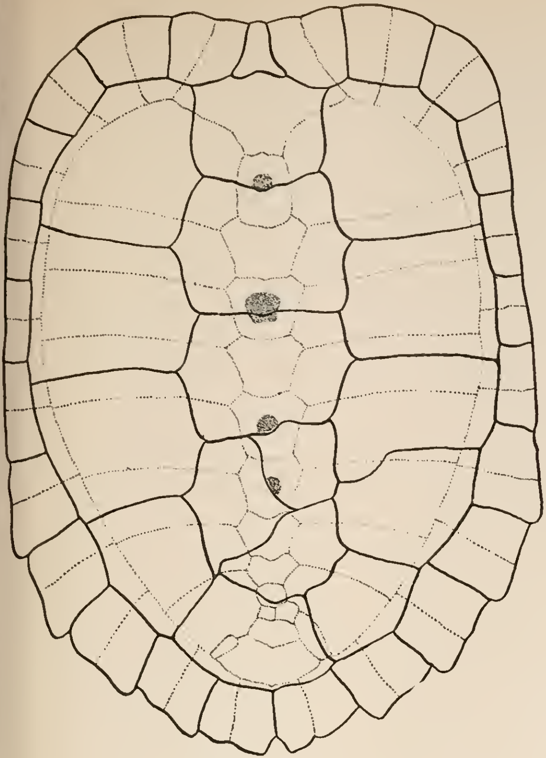


Fig. 5

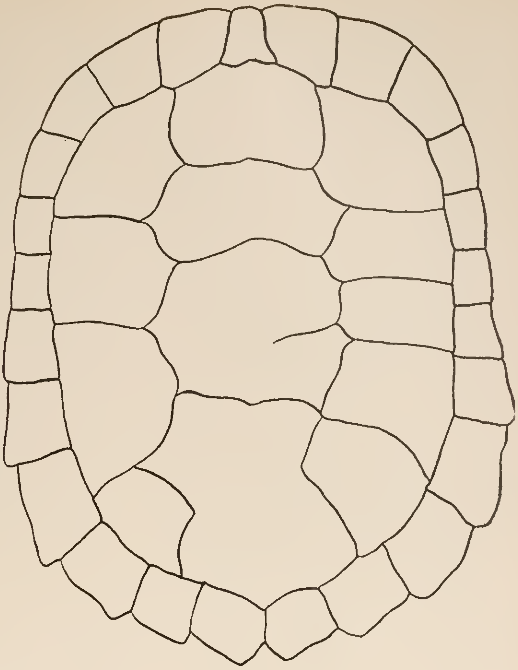
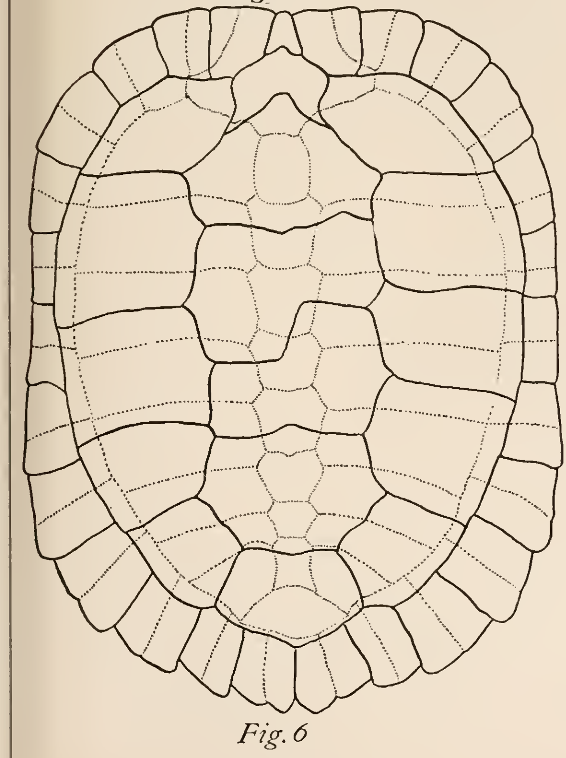


Fig. 7



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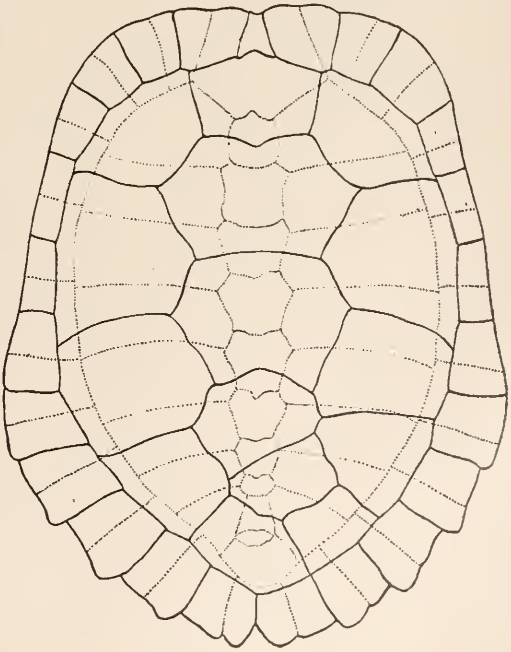

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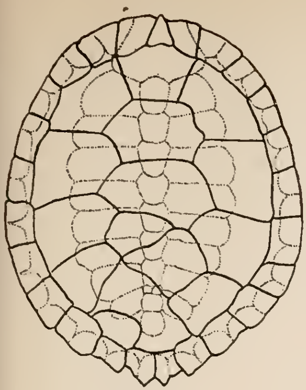
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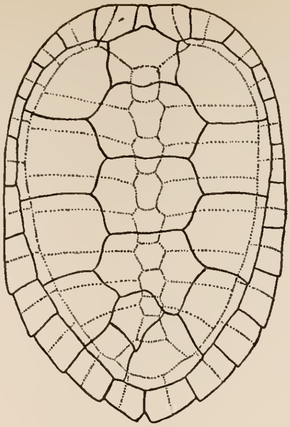
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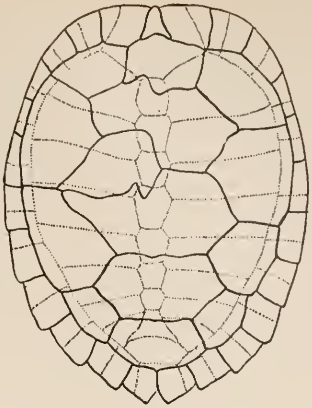
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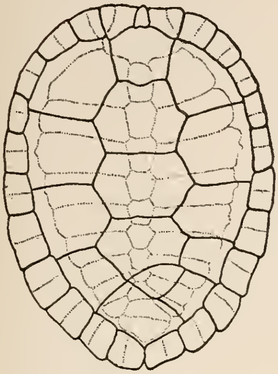
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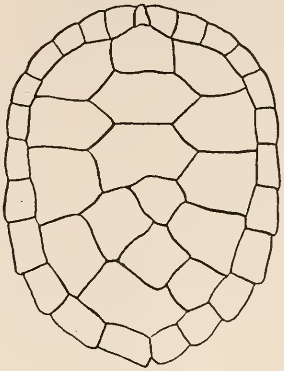
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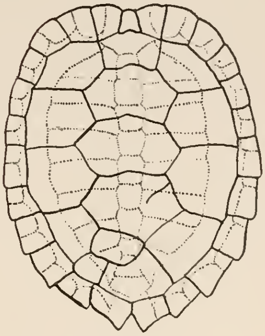
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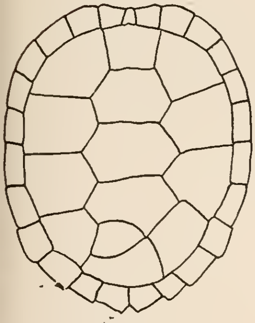
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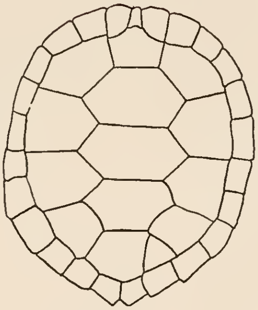
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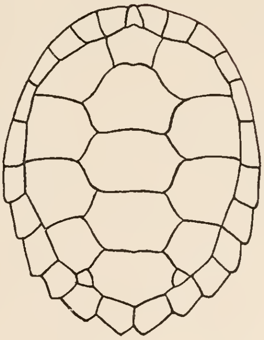
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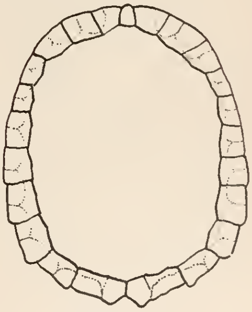
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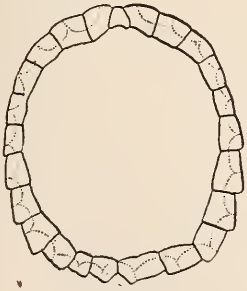
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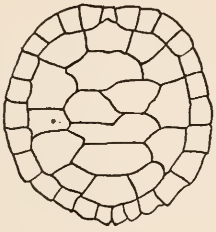
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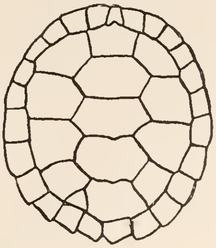
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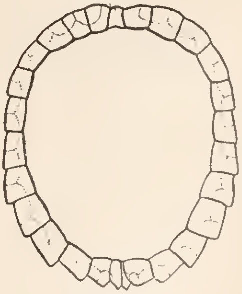
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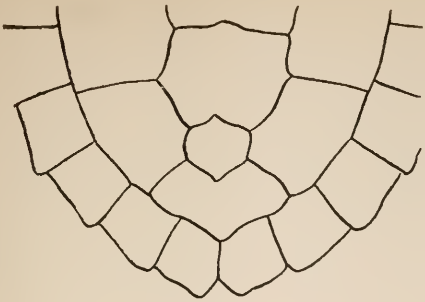

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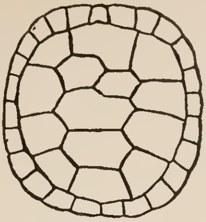

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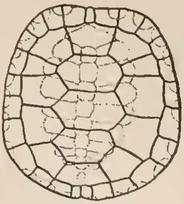

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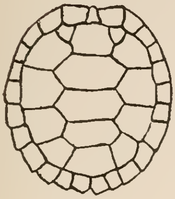

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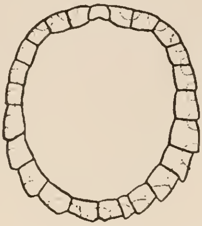

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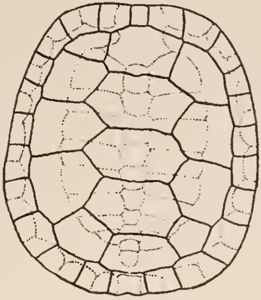

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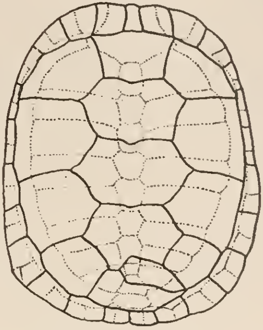

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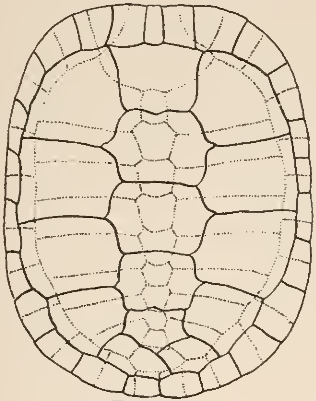

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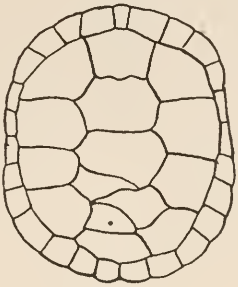

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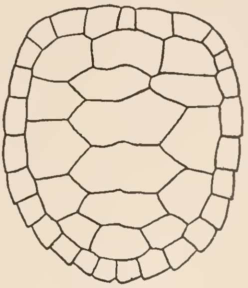

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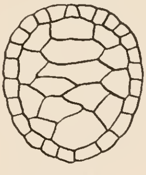

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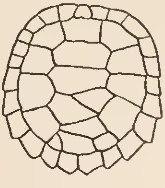

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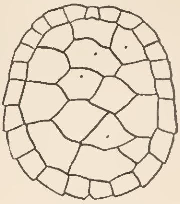

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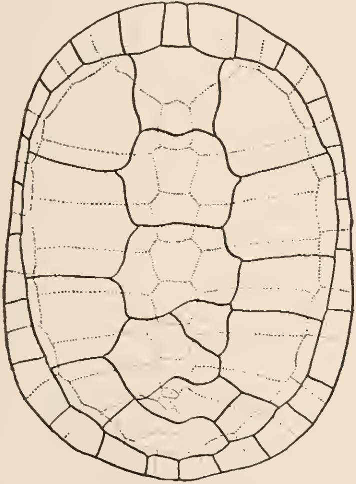

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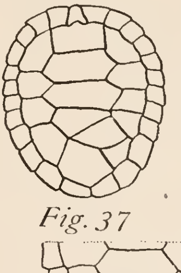

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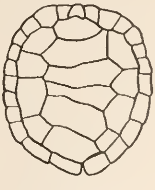

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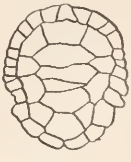

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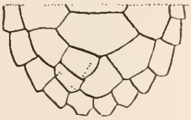

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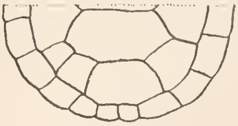

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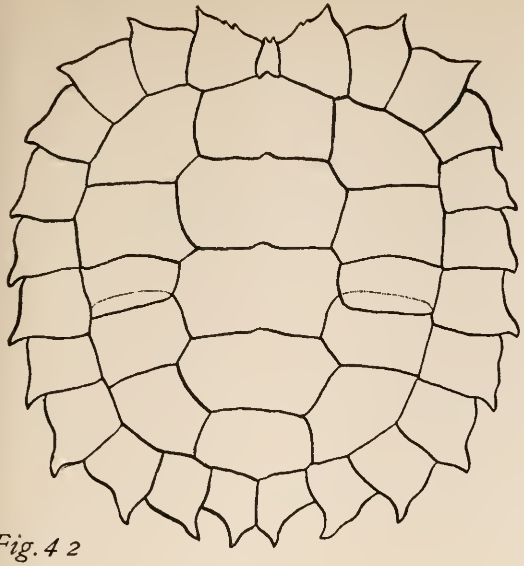
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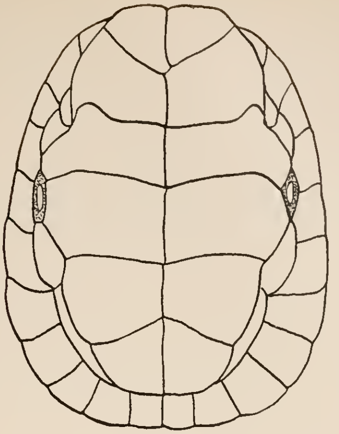
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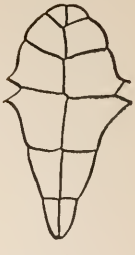
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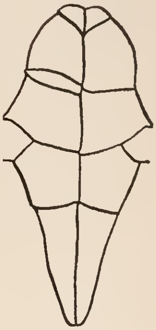
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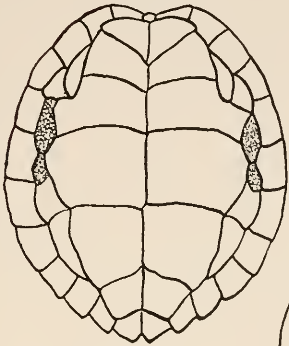
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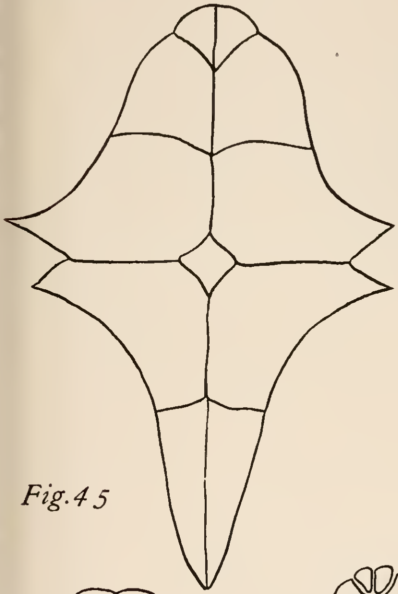
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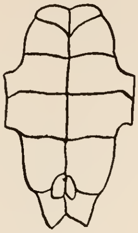
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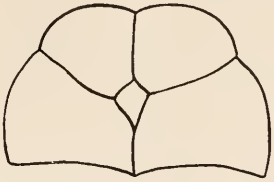
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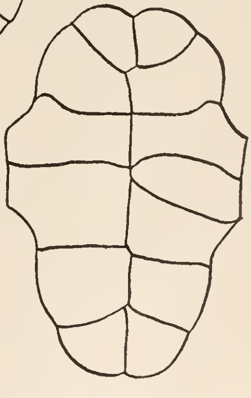
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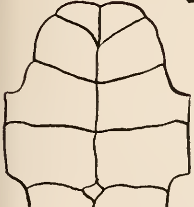
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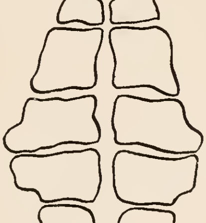
Fig. 52


Fig. 53


Fig. 54

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BIOLOGICAL BULLETIN

THE SIGNIFICANCE OF SCUTE AND PLATE "ABNORMALITIES" IN CHELONIA.

A CONTRIBUTION TO THE EVOLUTIONARY HISTORY OF THE CHELONIAN CARAPACE AND PLASTRON.

H. H. NEWMAN.

CONTENTS.

PART II.

PART II.

IV. THE TAIL-TRUNK OF CHELYDRA SERPENTINA AS A CLOSE APPROXIMATION TO ANCESTRAL CONDITIONS OF CARAPACE AND PLASTRON.

Baur, Hay and others have used as the hypothetical ancestral form an aberrant and perhaps highly specialized Chelonian, *Dermachelys coriacea*, but in the light of the various atavistic recurrences discussed in this paper and in view of the fact that certain definite rows of scutes invariably predominate over others, I have been led to seek elsewhere for primitive conditions.

A closer approximation to the true ancestral conditions is, I believe, to be seen in that portion of the trunk of *Chelydra* that is commonly called the base of the tail. Here if anywhere one would expect to find primitive conditions. The Chelydridæ are generally acknowledged to be our most generalized chelonians, and it is natural to look for primitive characters in this family, especially in the less specialized regions of the body.

A careful study of the portion of the body posterior to the carapace, which for convenience may be called the "tail-trunk," is fruitful of suggestions. A preparation of the bony structures of this region was made from a very large specimen (Fig. 58) measuring nearly three feet from snout to tip of tail, having a carapace sixteen inches long and a tail-trunk fifteen inches long. The preparation shows the following structures:

There are 33 vertebræ ranging in size from very large bones of nearly a cubic inch displacement to very minute ossicles at the tip of the tail. The first five vertebræ are beneath the carapace and have their dorsal processes in close contact with a longitudinal bony ridge that traverses the last three plates of the neural row of the carapace. These five vertebræ have definite flattened ribs that project laterally about at right angles to the axis of the vertebral column and remind one of the flattened ribs in the carapace of *Dermochelys*. The first and second of these ribs are very large and articulate by means of enlarged heads with the proximal ends of the ileum. These specialized vertebræ and ribs form the sacrum (Fig. 58, 1 and 2).

Surmounting the dorsal processes of the eighth to the fifteenth vertebra is a row of six large bony tubercles (*t.* 1 to 6) that have no reference to individual vertebræ. The dorsal processes of the latter are not the centers of ossification for the tubercles and there is no articulation or fusion between the two series of structures. Posterior to the sixth bony tubercle are fourteen tubercles (*y.* 1–14), with either membraneous cores or no cores at all, ranging in size from structures almost as large as the sixth bony tubercle to extremely small scales with dorsal ridges. Anterior to the first bony tubercle are three small soft tubercles (*x*) occurring at intervals of about half an inch, and anterior to these we find the two procaudal (*pr.* 1 and 2) and the single pygal plate (*p*) of the carapace, overlying the first four vertebræ. We have then the following heterogeneous series of structures: two procaudals, one pygal, three small soft tubercles, six large tubercles with bony cores and finally a graduated series of 14 tubercles merging into ridged scales. It seems reasonable to suppose that all these structures of the dorsal row, whether scutes with bony cores, scutes without bony cores, or bony plates that have been separated from their original scute coverings, are essentially homogeneous, and that the differences seen in the different regions are secondary or perhaps tertiary modifications. It seems probable that the scutes at the posterior end of the row represent the most primitive condition, which, through a process of continuous variation have become more tubercular in form and have acquired bony cores by the gradual ossification of membraneous tissue. The three soft tubercles surmounting the fifth to seventh vertebræ probably represent a reduced condition in adaptation to the fact that the base of the tail requires flexibility and must be swung from side to side or partially withdrawn under the carapace. The presence of such large prominences as the bony tubercles would seriously interfere with the mobility of the tail. The procaudal plates seem to have been the last of the neural tubercles to have been flattened out to form the dermal carapace. The last neural scute is probably the original chitinous sheath of one of the procaudals, doubtless the second one. The scute of the first procaudal I believe to have been crowded out in the process of scute reduction that will be discussed later.

Over the 33 vertebræ there occur 27 structures of homogeneous origin, that may be designated scutes. This number is sufficiently at variance with the number of vertebræ to preclude the possibility that they have had a segmental arrangement. Moreover, their irregular arrangement and the dermal origin of the bony cores make it certain that they are independent structures, in opposition to views expressed by Gadow and others.

An examination of the entire tail-trunk of another large specimen revealed the rather striking fact that the number of principal rows of tubercles and large scutes in this region is identical with that of the carapace and plastron, and that smaller, less regular rows of tubercles and scales represent the principal lost rows. A section of the tail-trunk was slit down the median ventral line and flattened out for convenience in drawing, and from this the somewhat diagrammatic Fig. 55 was constructed. I was able with certainty to homologize seven principal rows, three dorsal, two lateral and two ventral, and have named them after their homologues in the carapace and plastron, neurals (*N*), costals (*C*), marginals (*M*), and plastrals (*P*). Smaller and less regular rows of tubercles and scales are homologized with the secondary and lost rows as follows : inframarginals (*IM*), supramarginals (*SM*), interplastrals (*IP*), and neuro-costals (*NC*), a row not occurring in any modern species. In the following more detailed description the above names will be used for the rows concerned.

The costals are large scutes with a marked tendency toward dorsi-ventral flattening. Their apices are directed posteriorly and the growing point is thus situated near the posterior margin of the scute, a fact that causes the scute to grow anteriorly and laterally and very little posteriorly. The marginals are as large as the costals and are flattened so as to expose two surfaces, a dorsal and a ventral. The apex is directed away from the axis of the body and the growth of the scute is principally inward and slightly forward. The plastrals are paired, large, flat and nearly rectangular. They suggest by their appearance the plastron scutes. Their growth is inward and forward, as is that of their homologues in the plastron.

The two secondary dorsal rows, supramarginals and neuro-costals, consist anteriorly of small tubercles that fade out posteriorly into small irregular flat scales. The inframarginals are not tubercular but are rather large and fairly regular flat scutes. The interplastrals are diamond-shaped scutes forming a discontinuous row and occupying the angles made by four plastrals. This row is sometimes entirely absent on the tail-trunk of *Chelydra*.

It is clear then that in the tail and tail-trunk of *Chelydra* we have seven principal rows of scutes and between each pair of principal rows a secondary row, less regular and far less prominent. This would give a total of fourteen rows, of which seven are primary and seven secondary. Supposing that the carapace was originally continuous with the tail-trunk, we must imagine a gradual suppression of the secondary rows by the primary ones, until in the most highly specialized condition, seen in the terrestrial Emydidæ, only the seven primary rows have survived.

The order of loss of the seven secondary rows may be conjectured from an examination of the directions of encroachment of the principal rows upon the secondary rows. On this basis one might give the order of loss as follows: first, neuro-costals, which on account of inward encroachment of the costals and the outward encroachment of the neurals, would receive the severest pressure; second, the supramarginals located between the inwardly-encroaching marginals and the outwardly-encroaching costals; third, the interplastrals, between the two inwardly-encroaching plastrals, but occasionally able to escape pressure by occupying angles between four plastrals; fourth and last, the inframarginals, which were subject to pressure only on one side — that of the marginals — and were in addition required in more primitive forms with small plastrons to help bridge the space between the carapace and plastron. With the great increase in the size of the plastron which has taken place in higher forms, the space allotted to inframarginals became more and more contracted until they were crowded out entirely.

Does this order of loss correspond to any facts in nature? The same conclusion as to order of loss, I believe would be reached if we based our results on the prevalence, scarcity or absence of these rows as normal structures in existing species. No trace of neuro-costal scutes are found. Only one species, *Macrochelys temmincki*, possesses supramarginals. Interplastrals occur as isolated median ventral scutes in several families. Inframarginals occur normally in all the more primitive families and as axillaries and inguinals in all but the most specialized terrestrial forms.

This condition as derived from the tail-trunk of *Chelydra* differs rather radically from the ancestral condition derived from *Dermochelys*, in which there are twelve rows of equal rank. I am inclined to believe that *Dermochelys* is an extremely aberrant type with only a most distant connection with the phylogenetic line of *Chelonia*. The *twelve* keels of *Dermochelys* are comparable, I believe, to the *seven* keels of modern forms, and the irregular rows of plates and scutes between the keels are comparable to the secondary rows of scutes seen in the tail-trunk of *Chelydra* and represented in the carapace and plastron by inframarginals, supramarginals, interplastrals and neuro-caudals.

V. Further Evidence of the Former Existence of a Dermal Carapace in Chelonia, as Derived from Specimens of Graptemys.

The position just taken rests on the assumption that the carapace and plastron were at one time continuous with that portion of the trunk just posterior to them and that the carapace and plastron have undergone a gradual process of specialization that has caused them to depart widely from ancestral conditions. The tail-trunk would then preserve to a greater or less extent its original character.

On this assumption, then, there once existed a complete row of dermal tubercular ossicles overlying the vertebræ. That certain ancient forms did actually possess these ossicles was shown by Hay in the case of *Toxochelys serrifer*, but the question arises whether or not we have sufficient evidence that *Toxochelys* represents the ancestral condition of our modern forms.

For a long time I looked for definite traces, other than the keels, of such ossicles as are seen in *Toxochelys*, but met with no success until I had nearly completed the present paper. Then by merest chance I stumbled on the evidence needed to clinch the argument.

I had kept alive a few specimens of *Graptemys* in a small aquarium, but one by one they sickened and died, with one exception. Their death was doubtless due to the fact that this species is highly specialized in its diet, feeding exclusively on a species of viviparous gastropod that is abundant in Lake Maxinkuckee. They never learn to use other food, and, in lack of their special diet, starve themselves to death.

The surviving specimen was a nearly adult female that had been kept on account of its many peculiarities. After eleven months of captivity it was killed and examined for plate abnormalities. This examination revealed the presence of several small, loose, ossicles that were inlaid, as it were, in the bone of the neural plates and were situated beneath the keels of the second, third, fourth and fifth neural scales, *i. e.*, exactly in the positions occupied by the ossicles found in *Toxochelys*. The largest of these ossicles (see Fig. 5) was situated beneath the keel of the third scute and extended partially under the anterior margin of the fourth scute. The fifth scute is a supernumerary neural (No. 8) and hence the occurrence of an ossicle at its keel shows that it belongs in the neural row in spite of the fact that it is crowded to one side. All of the ossicles are imbedded or inlaid in the centers of certain of the neural plates. If they were merely the loosened centers of these plates, we would surely expect to find them on all the plates instead of just those which lie beneath the keels of the scutes. In what respect do these ossicles, then, differ from those seen in *Toxochelys serrifer* by Hay? Merely in this, that they are much reduced in size, so that each is confined to one neural plate, extending back so as to overlap the anterior portion of another plate.

The specimen is an oddity in many respects. It is unusually long in the carapace; possesses fifteen plates in the neural row instead of the normal number, twelve (three of these probably representing supernumerary procaudals); two extra costal plates or ribs, one quite vestigial; two supernumerary scutes of large size; one supernumerary costal scute of large size on the right side; and two well-developed supernumerary inframarginals on each side. Other minor peculiarities might be noted, but they do not concern the carapace or plastron. All of the anomalies mentioned may be viewed as of atavistic character, and it should not be surprising to find that the curious specimen shows an even more significant atavistic recurrence than any other specimen thus far examined, namely, a reversion to the condition seen in *Toxochelys*. That the genus *Graptemys* originally possessed a median dorsal keel composed of prominent bony tubercles covered with chitinous sheaths (scutes) is rendered extremely probable when we examine the young, especially that of *G. pseudogeographica*, a specimen of which is pictured in Agassiz' Contributions to the Natural History of the United States, Vol. II., Plate II., Figs. 11 and 12. This specimen, which may perhaps be an extreme type, although Agassiz does not suggest that such is the case, shows a series of three very remarkable dorsal tubercles on the second, third and fourth scutes. These tubercles furnish a close approximation in general form to those seen on the tail of *Chelydra*. It will be noted that these tubercles occur exactly in the places where I have found the vestigial ossicles in a specimen of *Graptemys geographica*, and that the most prominent tubercle is that on the third scute, a fact that is of interest in view of the larger size of the vestigial ossicle on that scute.

The adult of *Graptemys pseudogeographica* retains decided traces of these tubercles throughout life and their location is marked by dark blots of pigment that in later life form the only prominent color-markings of the animal. The discovery of vestigial ossicles in *Graptemys geographica* led me to investigate a very large female specimen of *Graptemys pseudogeographica*, that has been in confinement, and, like its relative, starving for many months. Immediately beneath the dark blots of pigment on the neural scutes there are on this specimen thin, scale-like discs of bone with a looser and less dense texture than the underlying bony plates. I have been able to examine but one adult specimen of *Graptemys pseudogeographica*, but this one appears to be perfectly normal in every other respect. It is to be noted that the vestigial plates in the last-mentioned specimen occupy exactly similar positions, with respect to scutes and plates, as do the vestigial ossicles in the anomalous specimen of *Graptemys geographica*, described and pictured above (Fig. 5). The probable explanation of both these conditions is that long-continued starvation has brought about a resorption of the portions that united these ossicles with the underlying neurals. That bone is resorbed either by normal processes or as the result of pathological conditions I have observed in several cases where holes have been eaten entirely through the bone of the carapace, as the result of starvation. Only a thin cap covering the top of the tubercle is of dermal origin, the main portion of the prominence being merely an outgrowth of the periosteum of the neural process. Examinations of developmental stages have revealed no discontinuity between the cap and the rest of the keel, but the microscope reveals the difference in histological structure between the cap and the underlying bony plate.

That tubercular ossicles existed over the neural processes of other ancient reptiles is shown by the fossil *Stegosaurus*. Here the dermal processes are very large and prominent and are much fewer in number than the neural processes that underlie them. This points to the entire independence of dermal bones and the vertebræ, and hence to the non-segmental character of the dermal plates.

VI. The Color Pattern of Chelonia as Confirmatory Evidence for the Former Existence of a Dermal Armor.

Evidence is not lacking that points to an original striped condition of the chelonian carapace. The neck and tail of most tortoises show characteristic stripes which on careful examination may be analyzed into rows of scales with similar coloration. When the scales are large enough, it will be seen that each has a center of pigmentation coinciding with its center of growth. Now the coloration of the carapace and plastron is nothing more than a series of scales or scutes, each with its pigmental center. The striped effect is lost through the great increase in the size of the scute and the consequent separation of the centers of pigmentation.

The pigmentation of scutes is typically concentric in character, whether the pattern consist, as in *Chelydra* and *Aromochelys*, of radiating bands of pigment having their center located at the center of scute growth, or concentric rings as in *Graptemys*, or lastly, of a light area occupying the center of growth, and all the rest solidly colored, as in *Clemnys guttatus*.

Frequently a great complexity of marking arises through the secondary complications of primary markings, but these conditions are seen in a simplified condition in the developmental stages.

It strikes one very forcibly that there is an intimate relation between scute growth and pigment distribution. The two processes have a common center and go hand in hand. The *areola*, or egg-plate (see Fig. 57, dotted lines) forms a convenient locus for measuring both processes. This location corresponds, curiously enough, with the keel of the scute and hence with the center of dermal ossification.

An examination of embryos of *Chelydra* shows that the coloration consists of dark patches of melanin pigment at the tip of the tubercular processes of the keels. The marginals are marked with small black spots at the posterior edge of each scute, sometimes running back over the anterior margin of the next scute. Specimens of *Chelydra* a year or two old have a radiating pattern with the center of pigment proliferation at the keel. In older specimens a solid coloration obscures everything.

Older embryos of *Graptemys* have, as the first indication of pigment, one dark spot at the median posterior margin of each scute, *i. e.*, at the keel (see Fig. 56). Later on two or more other spots of a similar appearance are produced in very definite positions on the various scutes. These secondary spots never become quite so prominent as the primary ones, but continue to develop like the latter into concentric ocellated markings (Fig. 57). The rings that constitute these ocellated spots are formed by repeated splitting of the innermost ring of pigment, in brief are proliferated from a center of pigment deposit. After three or four cencentric rings have been laid down, a well-marked unpigmented band appears on the periphery of the whole spot. It is this light band that produces the most pronounced secondary complexity, for it sends out processes to neighboring spots and forms the characteristic reticulated pattern that has given the name " map-turtle " to the species. In the marginals the pattern is not so complex, since no secondary spots appear. The concentric rings of the primary spot, however, often take on fantastic shapes that all but obscure the original unity of the coloration. It is very interesting to note that the spots in the marginal series are found as a rule half on one scute and half on the next, so that the light peripheral band that separates adjacent spots frequently coincides with the sutures of the dermal plates beneath. The spots then occupy the growth centers of the plates and no longer hold any close relationship with the scutes. *Pigmentation seems to be intimately connected with dermal ossification.* The scutes must have grown away from their original positions, thus overlapping the sutures of the plates, and lending strength to the general structure. The direction of movement has been forward, as is found by examination of the first pair of marginal scutes that run over upon the nuchal plate. The original cause of the forward movement was, I believe, the increase in size of the nuchal plate which must have pushed back the marginal plates. The scutes would of course occupy their original positions or would continue to crowd the nuchal scute into a still smaller space.

That the primary ocellated spots denote centers of dermal ossification will, I believe, be admitted. What then is the significance of secondary ocellated spots, which have an exactly similar appearance and method of development? They must, I believe, represent centers of dermal ossification and hence the location of scutes that formerly occupied the position now occupied by the spots. Evidences that serve to increase the probability of this conclusion may be adduced :

1. Whenever supernumerary scutes recur each has at its growth center an ocellated spot.

2. These supernumerary scutes come in at places where normal specimens have definitely placed secondary ocellated spots.

3. There are never any secondary ocellated markings on the marginal scutes, which agrees with our idea that the marginal rows contain nearly their original number of scutes and hence we would not expect to see traces of lost scutes in these rows.

4. The light bands that form the reticulated pattern of adult *Graptemys* have often the exact shapes of existing dermal plates. This is particularly the case in the procaudal region.

5. Considering the light bands as original scute boundaries, we can count ten costal scute areas in *Graptemys* or *Trachemys*.

6. On the neural scutes of *Graptemys* there are several much smaller ocellated spots that lie near the outer edges of the scute. These spots, I believe, are the vestiges of the small scutes that I have earlier designated as neuro-costals, and that were the earliest rows of scutes to be crowded out (Fig. 57). Four or more well-developed spots occur close to the marginals on the costals and occupy positions similar to the supramarginal scutes of *Macrochelys temmincki*.

At the angles made by adjacent neurals and costals occur ocellated spots that have the appearance of having been squeezed out between two scutes. These I believe to be the vestiges of the lost neurals and costals (see Fig. 57). When the lost scutes recur they have these spots at their growth centers.

In the bridge region of *Graptemys* and *Chrysemys* a confused series of dark colorations appears that seems to have no reference to any existing structures. But when the inframarginals recur, we find that these apparently meaningless markings fall into place as the spots of this lost row of scutes.

On the plastron scutes we find in most species a spot of pigment for each member, but confusing secondary complexity often obscures the real pattern. In *Graptemys* some specimens

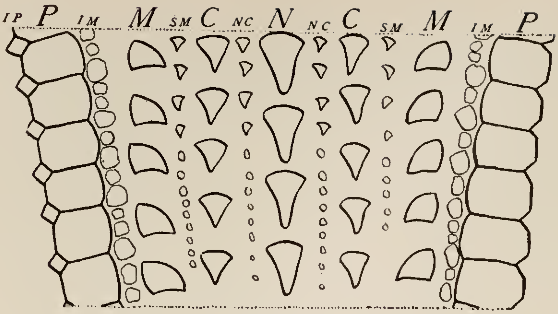

Fig. 55

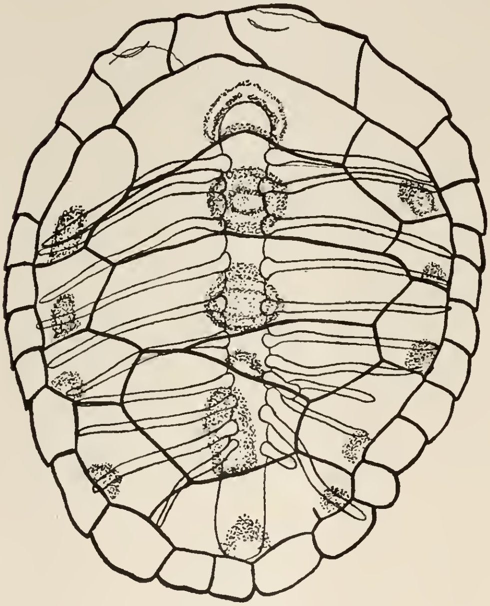

Fig. 56

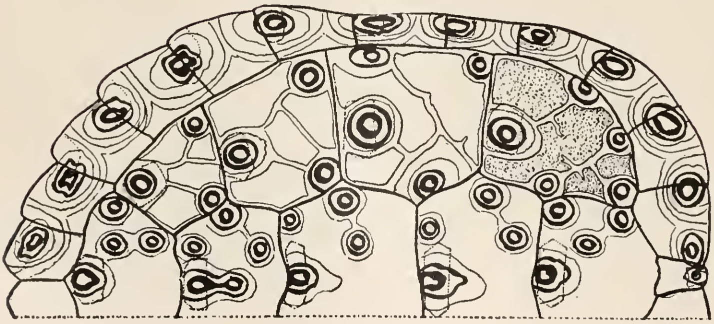

Fig. 57

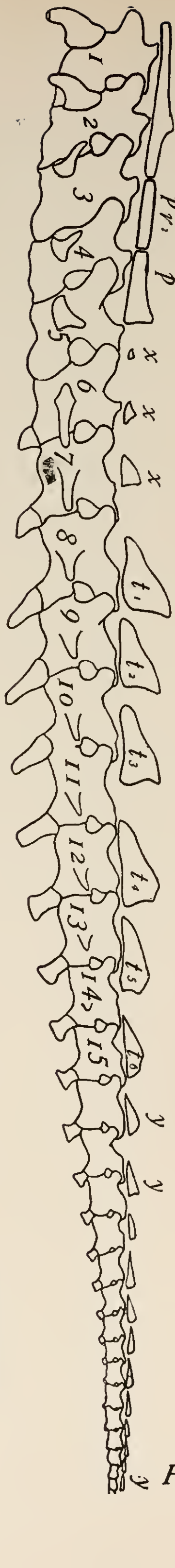

Fig. 58

show a fairly complete set of ocellated spots, but others show almost colorless scutes. Adult specimens seldom show any sign of pigmentation on the plastron. The markings then are largely juvenile in character and are subject to very great individual variation that tends toward the total obliteration of the original color pattern. An examination of a number of specimens, however, shows that each of the plastron scutes may have its ocellated spot.

The most confusing markings of all are those that occupy the median portion of the plastron in juvenile specimens. A remarkable secondary complexity of pattern has arisen in this region that would be almost impossible of solution were it not for the close series of stages leading up from the simplest conditions. The simplest form of marking consists of small diamond-shaped patches of pigment at the inner angles of the plastron scutes. These spread along the margins of the scutes, form bands by splitting, and finally produce the complex lyriform pattern that one finds quite frequently. The position of the simplest marking is identical with that of the hypothetical interplastral scutes and probably once constituted the color-marking of this row of scutes.

All of the carapace and plastron markings have thus been accounted for as the growth centers of existing or lost scutes. This has been done in a species with a highly intricate color pattern and could be applied successfully, I believe, to any other species.

It should be mentioned that the color pattern of *Graptemys* reaches its highest development in specimens of the first year. This is the time when protective coloration is a necessity, as the carapace is not sufficiently ossified to furnish a protection. Old specimens retain scarcely a trace of the original pattern, only a very faint reticulation being visible.

It might be suggested that the ocellated spots of the Trionychidæ are vestiges of scutes long since lost. The general number and arrangement of these spots tends to bear out this suggestion.

SUMMARY.

Palæontological and embryological evidence is at variance as to the origin and character of the neural and costal plates, but observations point strongly to a periosteal origin of these structures, which means that they are in no sense dermal or the descendants of the original dermal carapace.

The testimony of comparative anatomy leads to the belief that the nuchal, procaudal, pygal and marginal plates are the remnants of a once more or less complete dermal carapace and that these plates formed the cores of scutes that must have had a more or less tubercular form. The keels of existing scutes represent these tubercules. The testimony of the tail-trunk of *Chelydra* indicates that there were originally seven primary rows of such scutes and that less prominent rows of scutes occupied the interspaces. These less prominent rows were gradually suppressed, first on the carapace and then on the plastron, beginning in the middle and proceeding laterally. Thus the first loss was the neuro-costal rows, second the supra-marginals, third the interplastrals, and fourth the inframarginals, which to-day persists normally in many primitive forms.

Accompanying the suppression of rows occurred a reduction in the number of scutes in the primary rows, and this reduction took a general antero-posterior direction. At the same time the rapid secondary growth of the neural spines and ribs caused the suppression of the corresponding dermal plates, leaving only the nuchal, procaudals, pygal and marginals in places where the internal skeletal portions failed to extend. Traces of the dermal armor in the mid-neural region have been found, however, in *Toxochelys* and *Graptemys.*

No correlation of abnormalities is to be expected in the neural and costal regions, since the scutes and plates of this region are entirely independent in origin, but in the marginal series, where the plates and scutes retain nearly their original connections, the correlation is perfect. In the procaudal region we find frequent correlations, but, that the correlation is not a necessary one, is shown by numerous uncorrelated abnormalities.

A study of the color-markings of *Graptemys* and *Chelydra* lends confirmation to all the above theories of the chelonian carapace and plastron, and at the same time serves to rationalize the patterns themselves.

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NOTE ON THE INFLUENCE OF SURFACE-EVAPORATION UPON THE DISTRIBUTION OF INFUSORIA.

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During the course of my experiments on the chemotaxis of *Paramœcium* and *Colpodium*[1] I was struck by the fact that in certain media the infusoria showed a remarkable attraction to the edge of the fluid under the cover-glass — these media were *N*/25 methyl alcohol, *N*/50 $CaCl_2$, *n*/5,000 NaOH and *N*/5,000 KOH. This is contrary to the usual behavior of the organisms, in other saline media, in sugar solutions and in the culture medium they avoid the edges of the film of water under the cover-glass and, if undisturbed, form a cluster in the middle of the film, or, if the cover-glass be supported at one end, a little away from the center, towards the supported end.[2]

Jensen attributed this to the increase in concentration at the edges of the film due to evaporation. Since infusoria appear to be very generally attracted by solutions of lower concentration they tend to congregate in the more dilute part of the film, that is, near the center. In considering the apparent contradiction to this rule displayed by the infusoria when immersed in the above-mentioned media it struck me that in all these cases we had the converse of Jensen's experiment — for these media tend to become more *dilute* at the edge of the film. Thus methyl alcohol evaporates more rapidly than water, so that a solution of methyl alcohol becomes more dilute as it stands exposed to the atmosphere — while solutions of highly hygroscopic substances $CaCl_2$, NaOH and KOH absorb water-vapor from the atmosphere and so also tend to become more dilute at the surface. In order to see whether this was the case I tried the effect of adding

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[1] *Journal of Biological Chemistry*, January, 1906.

[2] Jensen, *Pflüger's Archiv. für ges. physiol.*, Vol. 53 (1893), p. 428.

different reagents to the culture-medium, in which the infusoria were suspended, upon their distribution under the cover-glass.

When a substance which evaporates more rapidly than water is dissolved in a watery medium containing salts in solution two contrary effects take place on evaporation — the solution becomes more dilute in respect to the more rapidly evaporating substance owing to its evaporation and more concentrated in respect to the salts owing to the evaporation of water. When the decrease in osmotic pressure due to the evaporation of the more rapidly evaporating substance is greater than the increase in osmotic pressure due to the increased concentration of the salts — then the total result is a decrease in the osmotic pressure. But if the initial concentration of the more rapidly evaporating substance be less than that necessary to bring about the above result then the increase in concentration of the salts will proceed more rapidly than the decrease in concentration of the volatile substance and the total result will be an increase in osmotic pressure at the surface of the fluid. Thus it was to be expected that while under the ordinary conditions of my experiments on *Chemotaxis* — when to a very small amount of culture a comparatively large amount of solution was added — methyl alcohol at a concentration of $N/25$ caused a dispersion of the infusoria to the edge of the cover-glass, when I added 1 c.c. of a solution of methyl alcohol to 5 c.c. of culture it was found necessary to bring the final concentration of the methyl alcohol up to $\frac{5}{6}N$ in order to get the first indication of a tendency to seek the edge — since in this latter case the salts were correspondingly less dilute. The solution of the volatile or of the water-absorbing substance in the culture medium having been made, drops of the mixture were placed on a slide under a cover-glass which was slightly raised at one end.

The following are the experimental results:

Methyl Alcohol. — 1 c.c. of a $5N$ solution of CH_3OH was added to 5 c.c. of the culture-medium in which numerous colpodia and paramœcia were suspended. Result at first uniform distribution of the infusoria under the cover-glass, in a few minutes, however, they began to congregate at the edges — especially at the edges farthest away from the supported end. In a short time all parts of the film, except the edges, were free from infusoria, while dense clusters had been formed along the edges farthest from the supported end. When a 6*N* solution of methyl alcohol was used instead of a 5*N* solution the effect was more marked and appeared more quickly.

Ethyl Alcohol. — 1 c.c. of a 5*N* solution of C_2H_5OH was added to 5 c.c. of culture. The same effect was obtained as with methyl alcohol, but it was slightly less marked and took longer to appear.

Propyl Alcohol. — $\frac{5}{6}N$ C_3H_7OH was too toxic — the organisms being killed too soon to obtain any result — but in $\frac{3}{10}N$, which is about the strongest solution they will stand, there was no attraction to the edges of the film.

Ethyl Acetate. — 1 c.c. of a saturated aqueous solution of ethyl acetate was added to 5 c.c. culture. The organisms were at first uniformly distributed — they then showed marked attraction for the edges of the film, particularly for those edges most remote from the supported end — and in a few minutes all the infusoria were congregated at the edges at the shallower end of the film

Calcium Chloride.—1 c.c. of a $\frac{2}{5}N$ solution of $CaCl_2$ was added to 5 c.c. of the culture—attraction to the edges was observable for a short time. As in the other cases the edges farthest from the supported end became the most densely populated.

Potassium and Sodium Hydroxides.—In my experiments on *Chemotaxis* and under the conditions of those experiments I obtained a marked, though more or less transient, attraction to the edges of the film at a concentration of *N*/5,000.

All the above results hold equally for *Colpodium* and for *Paramœcium.*

In all cases the attraction to the edges finally disappeared — with ethyl acetate the effect passes off in about half an hour.

All these substances which I have found to cause aggregation of the infusoria at the edges of the film are substances the solutions of which tend to become more dilute on exposure to the atmosphere. Methyl alcohol, ethyl alcohol, and ethyl acetate evaporate more rapidly than water while $CaCl_2$, KOH and NaOH are well known absorbents of water-vapor. There is also a general correspondence between the rate at which the volatile substances evaporate and the intensity of the effect which they produce — thus methyl alcohol has a lower boiling-point than ethyl alcohol and it also causes a more marked attraction to the edges of the film.

The fact that in all these cases the attraction to the edges of the film disappears after a certain time may perhaps be due to the volatile substance having become so dilute in the film that the increase in concentration of the salts due to the evaporation of water now takes place more rapidly than the decrease in concentration of the volatile substance. While in the case of water-absorbing substances — they may have become so dilute that the water-absorption due to their presence in the solution now goes on less rapidly than the loss of water due to evaporation. It seems probable therefore that these phenomena of attraction to or repulsion from the edges of the film are in reality special cases of osmotaxis.

I have alluded to the fact, mentioned by Jensen in the paper to which I have referred, that under ordinary circumstances *Paramœcia* under a cover-glass supported at one end tend to collect in the middle of the film but towards the supported end. But when the substances which cause the infusoria to congregate at the edges of the film are added to the culture we obtain precisely the converse effect — the infusoria collect at the edges, *especially at the edges farthest from the supported end.* It thus appears probable that this is also an osmotactic phenomenon connected with surface dilution. One would be inclined to fancy that it was due to surface evaporation having more effect upon the concentration of the small bulk of liquid at the shallower end of the film than upon the greater bulk of liquid at the supported end — but the *ratio* of surface to volume is not greater at the shallower end than at the supported end. Dr. Loeb has suggested to me that it may be due to the fact that diffusion and therefore equalization of concentration is less rapid in capillary spaces than in the bulk of the liquid — that, in fact, the shallow end of the film may act in a manner analogous to Liebreich's "dead space."[1]

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[1] *Zeitschrift für Physikalische Chemie*, Vol. V. (1890), p. 529 and Vol. VIII. (1891), p. 83.

Conclusions.

1. That whereas, under ordinary circumstances, infusoria tend to collect near the center of a film under a cover-glass, when methyl alcohol, ethyl alcohol, ethyl acetate, $CaCl_2$, KOH or NaOH are added in sufficient concentration to the culture-medium, this tendency is reversed and the organisms now gather at the edges of the film.

2. That this lends support to Jensen's explanation of the normal tendency of the infusoria to gather near the center of the film, namely, that it is a special case of osmotaxis brought about by surface-evaporation.

HISTOGENESIS IN INSECT DEVELOPMENT, AND CELL SPECIFICITY.

VERNON L. KELLOGG.

In the development (ontogeny) of insects with complete metamorphosis the imaginal antennæ, mouth-parts, legs and wings are produced from small buds, or histoblasts, in the larval derm. These histoblasts or imaginal buds arise by the shallow or deep invagination (one for each histoblast) of small regions of the larval cellular skin layer including originally, in each case, only comparatively few derm cells. The position of these invaginations, and therefore the participation of derm cells in the future leg or wing development, seems to be determined wholly with reference to the future imaginal organ, and not at all with reference to any difference in degree of differentiation among the cells of the larval derm. The wing-buds arise from the latero-dorsal regions of the meso- and meta-thoracic segments, the leg-buds from the latero-ventral regions of each of the three thoracic segments.

The larval derm is certainly not to be looked on as composed of wholly undifferentiated embryonic cells. These derm cells make up a definite organ, or part, of the larval body, with definitive position and particular functions. All these cells, and no others in the body, secrete[1] chitin ; some of them secrete noxious fluids, ill-smelling, acrid, poisonous. Many of them, perhaps all, secrete moulting fluid at the times of the regular larval moults ; many are specially sensitive, many bear sense-hairs or papillæ.

The invagination and beginning development of small parts of this derm, in a wingless and legless larva of a fly or honeybee or of some other specialized insect, may occur in a very early larval stage (in some cases, indeed, indications of the future histoblasts are apparent in just-hatched larvæ), but in most cases the invagination does not appear until a certain part of the free larval life has been lived. That is, the larval derm has for awhile

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[1] The chitin secreting capacity of the anterior and posterior thirds of the insect alimentary canal is due to their deeply invaginated derm.

subserved only, and has subserved fully, the functions of skin, and its cells have certainly attained whatever differentiation they need for the performance of these functions. It is far and away a long cry back to the embryonic, undifferentiated, the non-specific condition. But apparently any part or region of this derm which may, by its position, be the region or part needed to develop an antenna, a wing, a leg, male clasper, female ovipositor, or a sting, can respond to the need, and by invagination (for protection's sake), rapid growth and proliferation of cells, quick differentiation and arrangement, and final evagination (at the time of the last larval moulting, *i. e.*, pupation) produce the needed organ. This organ may be tubular and segmented, and the segments may be similar (antennæ) ; or dissimilar (leg) ; or it may be a great flattened sac, supported by tubular skeletal ribs and covered by a million and more tiny other striated, pigment-bearing, flattened sacs (the butterfly's wing with its scales) ; or it may be the exquisite mechanism of the bee's sting.

This histogenesis of the imaginal parts of the fly, the bee, and the moth is, to my mind, an extremely suggestive phenomenon when considered in the light of its relation to the theories of cell-specificity or cell-non-specificity. Quite as positively as the more familiar cases of restorative regeneration (legs, eye-lenses, tails and what not of various vertebrates), does this radical histogenesis, common to the ontogeny of all insects with complete metamorphosis, make it impossible to limit the germ-plasm to the germ-cells. It stands strongly opposed to any theory of absolute cell-differentiation or cell-specificity.

Stanford University, Calif.

ONTOGENY OF THE ANNULUS VENTRALIS.

E. A. ANDREWS.

In crayfish of the genus *Cambarus* there is a sperm receptacle in the female which has been known as the *Annulus ventralis* and made use of as a specific character in systematic works.

Nothing has been known of its mode of development beyond the brief mention by Mary Steele[1] who figured the external views of the annulus in specimens of *C. gracilis*, 20, 22, 27.5, 30, 35, 36, 50 and 60 mm. in length. Of these the latter four were probably adults and the former four immature young. These figures show an increasing complexity of external sculpturing and an increase in relative longitudinal diameter; however, as they were made incidentally and without reference to the use or internal structure of the organ they are necessarily insufficient. While the annulus is a necessary reproductive organ in *Cambarus* it is also a new organ in the history of crayfish since it is found only in the most specialized form, *Cambarus*. It seemed, therefore, of interest to find out just how and when the organ develops in the ontogeny of *Cambarus*.

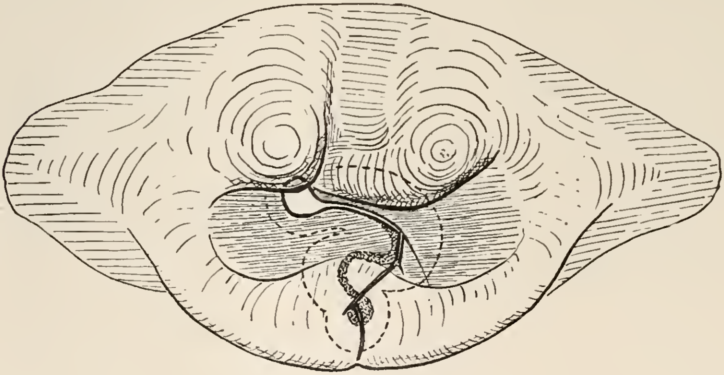

FIG. 1.

The following account of the origin and growth of the annulus refers almost exclusively to *Cambarus affinis* reared in the labo-

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[1] *Univ. of Cin. Bulletin*, X., 1902.

ratory. In large adults, 100 mm. long, the annulus in this species has the appearance outlined in Fig. 1, which is enlarged about twelve diameters. It is a transversely elongated plate, part of the shell, with a central depressed area bounded behind by a cross ridge and in front by two high tubercles or tuberosities. Across the depressed area runs a zigzag line which is in reality a closed suture whence a slit leads inward to a curved tube represented by the thick shaded line. The suture and the curved tube both open out on one side into the depressed area by an orifice partly under one of the tuberosities. The walls of the tube are thick chitinous continuations of the shell, as indicated by the broken lines. Underlying this chitinous mass is the epidermis which forms it and which was found to be folded in as a bent groove. A comparative study of annuli in several species showed that while the external sculpturing is various, the presence of a curved epidermal groove is constant and that, morphologically, this sperm receptacle is a bent epidermal pocket lined by chitin and opening to the exterior by a more or less closed slit.

The position of the annulus, as seen in Fig. 2 which is enlarged one and a half diameters, is on the ventral surface of the thorax between the bases of the fourth pair of legs. The sternal surface behind it is elevated as a rounded knob that may be of importance in discharging the receptacle. Projecting forward towards this are the short pleopods of the first abdominal somite. Anteriorly, on the bases of the third pair of legs, are the large elliptical openings of the oviducts whence the eggs when laid pass back over the annulus.

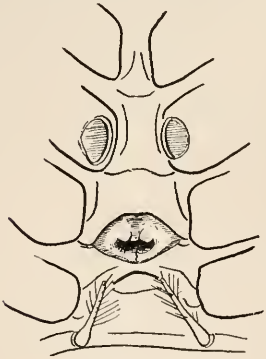

FIG. 2.

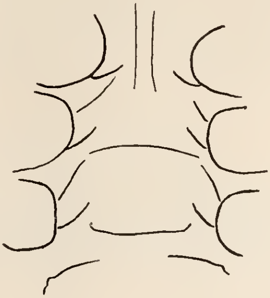

FIG. 3.

In using the annulus as a sperm receptacle the male passes the sperm into the orifice and thence into the posterior part of the tube. The anterior part of the organ, the orifice itself and the following transverse part, which may be called the vestibule, is filled not with sperm but with a cement that protects the sperm from the water. Eventually the sperm issues out through the more posterior part of the suture at the right time to meet the eggs.

When the young *C. affinis* hatches from the egg there is no annulus present. The ventral surface (Fig. 3), multiplied fifty diameters, shows no specialization of the wide level area between the bases of the fourth legs. The sexes are not yet distinguishable and the first abdominal appendages are in all cases but very faintly indicated by slight elevations. Here under a higher power the epidermis was seen to be specialized as a group of nuclei, over which the cuticle was elevated as a slight protuberance in the region that is later to grow out as the first abdominal appendage.

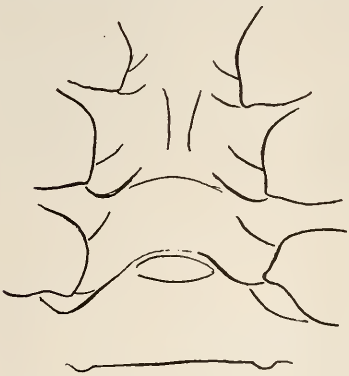

FIG. 4.

After this larva sheds its shell and passes into a second stage the sternal surface is larger (Fig. 4), and the first abdominal appendages are somewhat more protuberant. There is, however, no annulus and no external signs of sexual differences. Between the bases of the fifth legs there is now a transverse elevation of the sternum, which will become the prominent tubercle posterior to the annulus in the adult.

After a second moult the larva in the third stage has a much larger sternal area and for the first time sexual openings and the beginning of the annulus. In the female (Fig. 5), the sternum between the fourth legs is divided by a cross-line into an anterior part articulating with the legs, and a broad posterior region that is, however, markedly short. On the middle of this plate and at its posterior edge is a slight depression or groove destined to become the receptacle for sperm in the adult. In the male the same differentiation of a posterior sternal area is found, but the plate lacks any groove and henceforth remains without any special development. The female is also recognizable by the appearance of the short, curved cuticular ridge on the base of the third leg, each side of the body, which is to be the median edge of the future orifice of the oviduct. From this superficial ridge a cylindrical epithelial tube, the oviduct, leads into the interior of the body. The first abdominal appendages, or pleopods, are blunt papillæ and apparently the same in both sexes, though sometimes in the male they seem more pointed and possibly longer.


Fig. 5.

The annulus is thus a narrow transverse part of the sternum and since its posterior edge projects and overhangs somewhat it might be regarded as a sort of transverse fold. Its surface is entirely flat and simple as compared with the complex adult surface, Fig. 1, except for the slight median depression. Looking at the shell only, Fig. 6 enlarged two hundred diameters, this depression is a wide shallow groove indicated by converging wrinkles in the cuticle on each side the middle line of the animal and extending forward but a slight distance from the posterior edge of the annulus. The posterior edge of the annulus is rounded and protuberant and the groove to some extent extends over this posterior face of the annulus and cannot well be all seen at once from a ventral point of view. While this groove in the shell differs in different specimens it is in no way an artificial result of methods


Fig. 6.

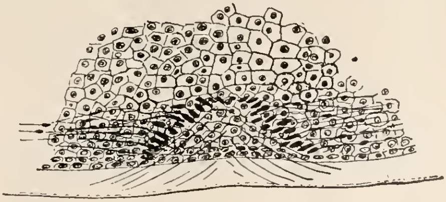
Fig. 7.

of preservation but is based upon a special arrangement of the epidermis, which, Fig. 7 enlarged two hundred diameters, is a single layer of polygonal cells, close under the cuticle. This layer dips down under the groove and on the side of the groove, where seen in profile, the cells are elongated and continued as fine fibers that connect with connective tissue cells and with a membrane separating the epidermis from the large blood sinus just above it. In the figure these cells are represented in black as seen in optical section and also represented as seen in surface view upon another focus. Posteriorly the epidermis had shrunken away from the cuticle, in preparation, and left a wide artificial space. In an actual cross section of this region the groove is found to be just below the posterior part of the ganglion, Fig. 8, that supplies the fifth pair of legs, so that one might argue that the annulus belonged to the last thoracic somite while lying in the penultimate one. As seen in Fig. 8, which is enlarged two hundred diameters, the epidermis cells fit together as polygons only at their outer ends next the shell while their inner ends taper off as fibers which diverge right and left of the central part of the groove. The epidermal cells are thus stretched out laterally and arranged on each side with reference to the superficial median groove that is to become the sperm receptacle.

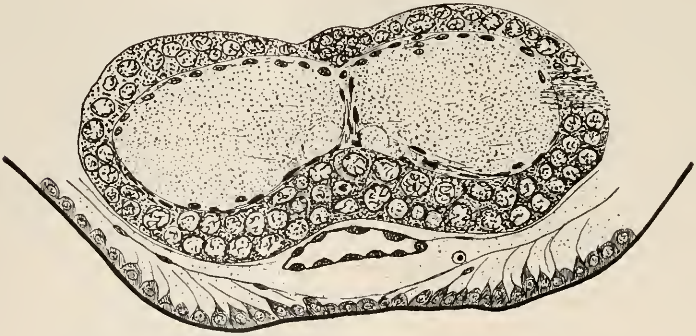

FIG. 8.

Enclosing the epidermal cells is a coagulum of blood partly separated by a membrane from the blood space beneath the nerve cord. In this space lies the large median ventral artery that anterior to this section connects through the nerve cord with the descending artery from the heart, between the fourth and fifth ventral thoracic ganglia.

Young crayfish in the above third stage are about 7–8 mm. long. In the fourth stage they are usually 11 mm. long. By this time the males and females differ noticeably in the lengths of the first pleopods. In the female, Fig. 9, they are still very small but larger than in the previous stage, this figure being enlarged but thirteen, and Fig. 5, fifty diameters. In the male, Fig. 10, on the same scale, the pleopods are long, slender, but simple cylinders pointing toward one another. These two figures also show the increasing longitudinal diameter of the annulus in the female and its simple form in the male, as well as the reproductive openings upon the fifth legs in the male and the third legs in the female.

FIG. 9.

FIG. 10.

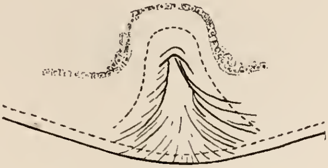

FIG. 11.

In these females 11 mm. long the median groove of the annulus is much more evident than at first, but is still a simple groove as shown in Fig. 11 enlarged 200 diameters. The walls of the folded in and thickening shell that bound the deep and narrow groove are indicated by the broken line. In this preparation the epidermis had shrunken far away from the shell and is indicated in optical section to show its invaginated state where it came under the cuticular groove. The groove seems to grow forward from the posterior face of the annulus and becomes more narrow and closed in anteriorly. At the anterior tip the groove was partly overhung by a cross fold, or tended to burrow under the surface as a short *cul de sac*.

In different females, however, the groove had different lengths and more or less of this covering-over of the front end. In a specimen 10 mm. long the groove was longer and more narrow than the one above figured. In others of 11 mm. the anterior end was crossed by a sharp fold and the *cul de sac* was more pronounced. In some the posterior face of the annulus, which projected and overhung considerably, bore a curved transverse ridge strongly suggesting the posterior rim of the adult annulus, Fig. 1. In a large female of 13 mm. the groove was as in Fig. 11, though a little longer.

The single layer of epidermal cells under the shell was still visible as a series of polygons as in the third stage. While the annulus itself showed no setæ upon it in any stage the transverse ridge of the sternum behind it bore scattered over it some four or five sharp setæ in the third stage, about ten in the fourth and twenty in the fifth.

The fifth stage includes individuals 15–18 mm. long, and in them marked changes had taken place in the annulus, but before describing these we will refer to a few observations made upon the developing annulus in an early stage of another species, *C. Clarkii*, from New Orleans. Here also the young reared in the laboratory had no annulus in the first and second stages, which were about five and six millimeters long, and they also showed no external sexual differences. Young of this species were seen by Hagen,[1] when .3 inch long and still on the abdomen of the female ; in which the females had no pleopods upon the first abdominal somite, but showed the openings of the oviducts, while the males had the first pleopods as little knobs longer than broad and turning inward. Later Faxon[2] stated that the young of this species from under the abdomen of the parent had the general form of the adult when 7 mm. long. Evidently both authors saw the third stage only.

Eggs hatched April 21 reached the third stage June 1, but no observations were made upon the annulus till they had passed into a later stage, June 19, and were 11 mm. long. In this condition the male and female were much like specimens of *C. affinis* of that length, but the first pleopods in the female were much smaller and in the male noticeably shorter than in *C. affinis*.

The groove on the annulus of the female now formed a deep, closed in cavity, Fig. 12, which is enlarged to the same extent as

[1] "Monograph N. A. Astacidæ," Harvard, 1870.

[2] "Revision of the Astacidæ," Harvard, 1885.

Fig. 11. In *C. Clarkii* the groove has closed, leaving a suture, represented by the black line, above an internal cavity, represented in white and this is bounded on the sides and bottom by the infolded cuticle, represented in dotted lines. At the posterior end the groove passes around the edge of the annulus and seems to be still open there. At the front end the groove pushed forward as a blind growth beneath the surface. Thus in *C. Clarkii* the receptacle though probably formed at the same stage and in the same way as in *C. affinis*, yet advances more rapidly, so that in the young 11 mm. long it is comparable, in its closed up condition, to *C. affinis* when 21 mm. long and in the sixth stage.

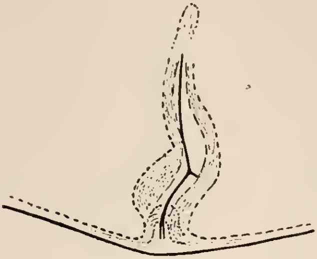

FIG. 12.

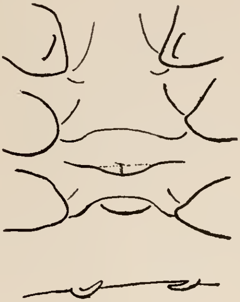

FIG. 13.

Returning to *C. affinis* in the fifth stage, 15–18 mm. long, the general appearance, Fig. 13, enlarged 13 diameters, shows an increase in size of the animal and a great growth of the first pleopods of the female which are now much longer than in the female of the fourth stage and nearly as long as the male pleopods of that stage, Fig. 10.

The receptacle itself is quite diverse in different individuals. In all there is added onto the median groove two elevations or folds which tend to cover over the anterior end of the groove. The groove itself is bent on one side more or less and the overhanging folds are more or less developed. In the specimen shown in Fig. 14 as enlarged 200 diameters the groove bends to one side and passes in under a marked flap or fold that grows over the tip of the groove. This lateral fold may be called the "hood" to distinguish it from the longer fold which passes along the opposite side of the groove and tends to overhang it.

This second fold is slightly developed in this case. In another specimen, Fig. 15, the groove bent very far to one side and the anterior part was concealed under the second fold. We will call this second fold the "transverse" fold, since it ultimately lies more nearly across the median plane. Both folds are oblique, the "hood" and the "transverse" fold being about at right angles where the latter passes under the former. In Fig. 15 not only is much of the groove overhung by the transverse fold, but the entrance to the groove in under that fold is cut off, near the hood, by a short posterior fold that runs parallel to the transverse fold as it emerges from under the hood.

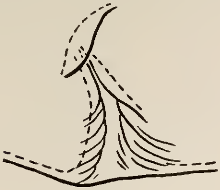

FIG. 14.

FIG. 15.

The cases of unusual bending of the groove to one side suggest the state of things found in the adults of *C. immunis* and *C. Bartoni* where the receptacle is more transversely placed than in *C. affinis*.

The specimen last figured was about to shed and the delicate new shell within the one here figured was more like that figured below for the next stage.

In another case the shell cast off by a larva going into the sixth stage had the groove but slightly bent, Fig. 16, and the transverse fold was less oblique. As there was no fold opposing the transverse fold an object could have passed under its pos-

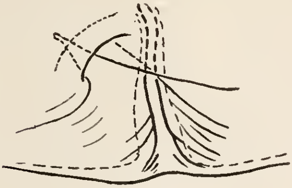

FIG. 16.

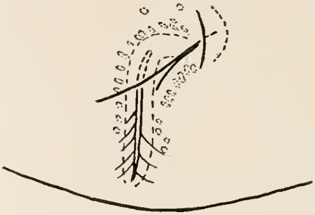

FIG. 17.

terior edge and thence to the anterior part of the groove. The receptacle was now being formed of three different parts: the original median groove, the two oblique folds. The former remains as the posterior part of the adult sperm tube and the latter help to make the orifice and the vestibule, or anterior part of the adult receptacle.

In all these cases the groove bends more or less to one side, and in only one case observed was the bending to the observer's right, which is the left of the animal. In this exceptional animal, Fig. 17, not only does the groove bend to the right, but the hood is on the right and the transverse fold and the posterior opposing fold run at right angles to their usual course. Comparison of Fig. 17 with Fig. 15, shows that they are, in the main, mirror-images of one another. Each reversed, as seen through the paper, would have the symmetry of the other.

This reversal of symmetry in the receptacles of some young is the first visible expression of the peculiar dimorphism of the annulus in the adults of this and other species. While many of the adults have the orifice upon the right side of the median plane others have it upon the left and in all respects these two forms of annulus are mirror-images of one another. Both forms are used as sperm receptacles. Those with the orifice upon the right, Fig. 1, are more common; in one lot of 41 females, 38 had these right-handed annuli.

The characteristic tuberosities of the adult grow out in later larval life and they also are right- and left-handed in the following way: while, usually, as in the above figure, the tuberosity upon the left of the animal sends a ridge under the right tuberosity, in left-handed adult annuli the behavior of the tuberosities is the reverse. In the production of the two symmetrical adult forms there is thus the harmonious development of the groove, folds and tuberosities at different periods and from several areas of the epidermis.

In July the larva may pass into a sixth stage 21 mm. long. On the ventral side, Fig. 18, enlarged thirteen diameters, there is a noticeable increase in the longitudinal diameter of the annulus and in the length of the first pleopods which are now longer than those of the male of stage four, Fig. 10, though closely applied

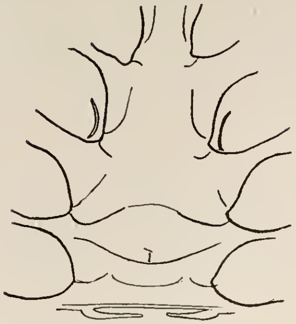

Fig. 18.

along the sternum and, apparently, of no use as yet. The annulus, however, has acquired a rather mature appearance. When enlarged fifty diameters, Fig. 19, it shows toward the center two gentle elevations to represent the future tuberosities and posteriorly a transverse curved rim like that of the adult. The whole plate is still narrow from before back but much less so than in previous stages. The suture of the receptacle is indicated by the zig-zag line and its lateral walls by the broken lines. The former represents: anteriorly on the left the former edge of the hood; posteriorly the closed up groove; and in its middle course the transverse fold.

Enlarged 200 diameters, Figs. 20 and 21, the receptacle presents varieties of development in different individuals. The groove itself may be open posteriorly as in Fig. 20 or quite closed up as in Fig. 21. The anterior part of the groove bends far to one side to end under the hood at the right end of the transverse fold. This part of the groove may be still accessible by passing in under the transverse fold, Fig. 20, or it may be quite shut off by a posterior fold growing along against the posterior edge of the transverse fold as in Fig. 21. Fig. 20 is not much advanced beyond the preceding stage, Figs. 15 and 16, the open groove, the hood and the transverse fold are larger and the invaginated shell is more extensive, as indicated by the broken lines. But in other cases, Fig. 21, the deeply buried groove connects with the surface only by oblique planes that come to the surface as the three

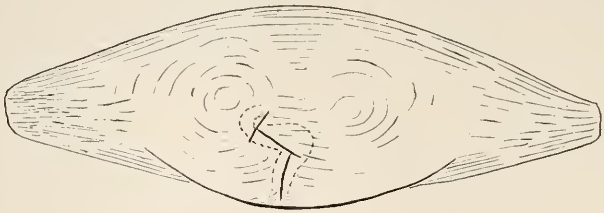

Fig. 19.

successive parts of a zig-zag suture. The inner course of the groove is more sinuous than before in correspondence with the increased bending of the invaginated shell that forms its walls.

The chief features of the adult sperm pocket are thus present except that the anterior orifice seems more closed in some individuals.

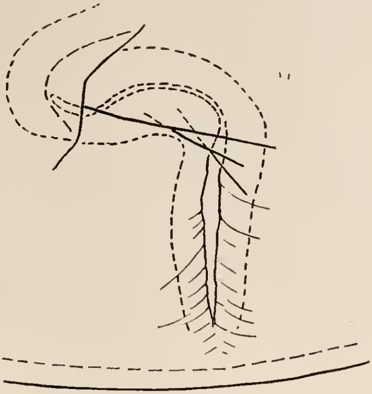

FIG. 20.

Though in some cases it seemed as if the groove itself did not bend to one side to end under the hood, but rather that the transverse fold left a passage from the groove to the hood, the true state of the epidermal groove upon and in which the shell groove is formed was seen when the shell was pulled off. Then, in Fig. 22, enlarged 200 diameters, there is a deep, bent furrow with high sides formed of long epidermal cells, as somewhat diagrammatically represented in this camera lucida sketch of an optical section

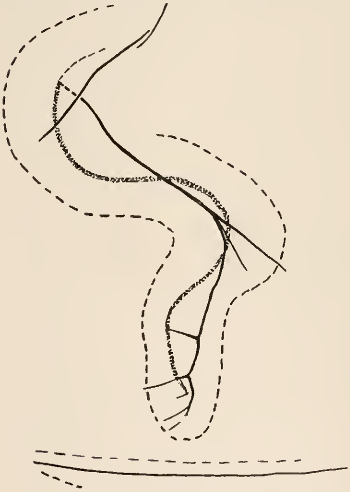

FIG. 21.

FIG. 22.

of the epidermal part of the receptacle. In this specimen the shell was easily removed since a new shell was in process of formation to line the groove. This made the groove more distinct than otherwise is the case. The groove passes forward from the posterior edge of the annulus and then bends to one side much as does the bottom of the shell groove seen as a shaded line in Fig. 21. In each individual, however, the amount of bending seems to be different.

Such a bent groove is very like the simple bent epidermal groove of *C. Clarkii*, which is one of the less specialized species.

While previous experience had shown that larvæ after living eleven days in the above sixth stage might turn into a seventh stage 29 mm. long in the middle of July, no observations were made upon the above larvæ 21 mm. long till October 3, 1904, when they had turned into individuals 25–53 mm. long. Those 25–35 mm. agreed with one another in the development of the annulus and probably represented larvæ that from lack of enough food had remained in the seventh stage while the female, 53 mm. long, belonged to some later stage. In this laboratory it has been found that crayfish of that latter size have their sexual instincts developed in the fall and females 52 and 53 mm. long, being then supplied with sperm by males of the same or even smaller sizes, laid good eggs the following spring when not quite one year old. Thus the above female was probably mature in instincts and in external sexual organs when examined.


FIG. 23.

First, however, taking up the specimens 25–35 mm. long which were all essentially alike except in size, one 35 mm. long enlarged 13 diameters, Fig. 23, presents a noticeable growth of the first pleopods which are now turned forward and sparsely set with hairs as in the adult, and also much increase in the relative importance of the curved ridge bounding the mouth of the oviduct opening on the third leg. As an opaque object the annulus now showed very definite tuberosities separate from the hood and the transverse fold and the tuberosity upon one side of the animal extended over the median line, in carrying out the asymmetry of the adult. The general proportions of the various folds and thickenings of the shell now approximated the adult condition.

The receptacle had not materially changed from the previous stage, Fig. 21, but its invaginated shell walls were greatly thickened and laminated, Fig. 24, enlarged like Fig. 21. It would appear that with the dropping of the bottom of the invaginated groove away from the surface its sides have closed in to form a narrow crevice which comes to the surface as a suture line. Then by the bending of the bottom of the invagination more than its surface suture line these crevices are made into curved oblique planes. The hood and the transverse fold and the posterior opposing fold are intimately associated with the bending of the original groove to one side and then the sinking of that lateral bend away from the surface and diagonally forward.

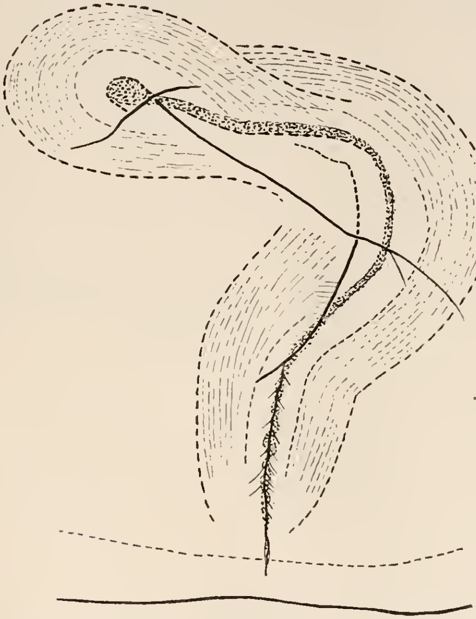

FIG. 24.

The last young to be considered, the larva, 53 mm. in length and sexually mature, has an annulus very much like that of the full-grown adults, but it is still very small, as is seen on comparing Fig. 25 with Fig. 1, both enlarged 12 diameters. Still the tuberosities do not yet overhang the transverse depression enough to conceal the hood and the transverse fold, nor are the transverse depression and the posterior rim as well developed as later. The receptacle itself is much like that in Fig. 24, but its orifice is more perfect, though not yet as patent as in the large specimens, Fig. 1. This young annulus still lacks the posterior enlargement of the sperm tube, indicated in Fig. 1, and the complexity of bending of the tube is less, nevertheless it functions as a sperm receptacle.

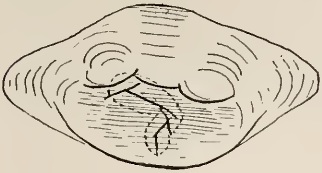

Fig. 25.

Summary.

The specialized sternal plate of the shell of *Cambarus*, which in the adult female bears the sperm-receptacle, is first differentiated in the third larval stage, in *C. affinis*.

The epidermis under this plate grows inward and outward in special areas to form the receptacle. First there is formed an open median groove that then bends to one side, sinks away from the surface and becomes closed. In *C. Clarkii* also one stage shows a like development.

Next definite folds overgrow the anterior end of the groove as it sinks from the surface.

Later elevations and depressions complete the external sculpturing of the annulus.

The anterior part of the groove, with the accompanying folds, form the orifice and vestibule of the sperm receptacle, while the posterior part of the groove forms that part of the receptacle in which the sperm is stored.

A functionally complete annulus is made within five months, but subsequently it becomes more complex. Comparative study of the adults of several species has shown that in *Cambarus* the essential part of the annulus, as a sperm receptacle, is a curved pocket; the above facts indicate that in *Cambarus* in general this pocket arises as an open epidermal groove.

The right or left handed symmetry of different adult annuli is first visible in the fifth larval stage when the median groove has bent to the right or to the left and the accompanying folds have a reversed position in the two cases. Later other outgrowths may also harmonize with the groove and folds to complete the two adult forms; mirror-images of one another.

While the sperm receptacle is a necessary organ in *Cambarus* it is phylogenetically a new one since *Cambarus* is the most specialized genus of crayfish and other genera have no receptacle. In *C. affinis* this new organ is not seen till the individual has reached a third stage after leaving the egg.

Besides beginning late in ontogeny this new sperm receptacle is variable in all its stages of growth. In its early and simple state in the larva it resembles the adult receptacle of a less specialized species, *C. Clarkii.*

In some of its variations of lateral bendings in early larval stages it suggests adult conditions in other species.

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CORRELATION IN THE DEVELOPMENT OF FASCIOLARIA.[1]

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The following pages contain in abstract an account of the embryology of the prosobranch *Fasciolaria tulipa* (var. *distans*), and a discussion of such occurrences in its development as seem to me to be of general interest.

I. The Cannibalism of Fasciolaria Embryos.

The breeding season of *Fasciolaria* at Beaufort, N. C., lasts from about the first of May until the first of July, although egg-cases containing various stages of development were occasionally found in August. The capsules, held together by a basement membrane fastened to oyster shells, conchs or wharf-piling, occur in bunches of 15–30 varying in size from one half to three inches across.

When fresh they are soft and so translucent that the pink or white eggs suspended in their albuminous contents give their color to the whole mass; later, however, the egg-cases become firm and elastic, and obscured by the algæ, polyzoöns, and other organisms which grow over them. Often isolated capsules, or bunches containing only a few are found. These are produced either by females interrupted at laying, or by young females, which usually deposit fewer and smaller capsules than the old ones. The last capsule to be laid in a bunch, whether deposited by an old or

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[1] For the privilege of collecting the material on which this work was done I am indebted to the Hon. George M. Bowers, U. S. Commissioner of Fisheries. The preparation of this paper was begun during my tenure of the Adam T. Bruce fellowship in the Johns Hopkins University, and was finished for the press in the Zoölogical Laboratory of the University of Michigan.

young female, often lacks either the upper or lateral flanges, or both.

The number of eggs in each capsule is much greater than has been supposed, and has an important effect on development. By actual count I found that one capsule contained 2,308 eggs. The highest estimate which I remember to have seen in the literature is from 600 to 800. The ova, densely crowded with pink, brown, or white yolk spherules, which are separated from an unusually large and eccentrically placed germinal vesicle by a zone of clear cytoplasm, vary in diameter from .17 to .25 mm. Even eggs of this size are minute enough to produce the same optical effects that much smaller spheres do, for in making a numerical estimate in a watch crystal one almost invariably supposes fewer to be present than the dish actally contains.

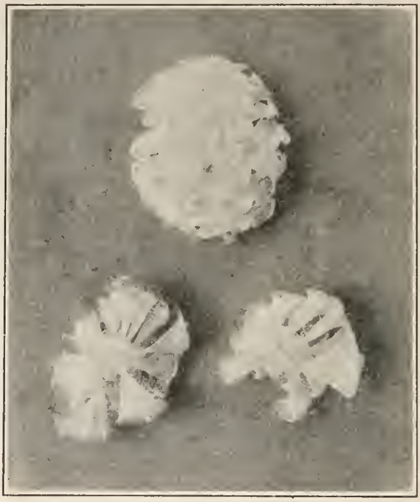

FIG. 1. Egg-cases of *Fasciolaria tulipa* (var. *distans*) one half natural size.

Of all the eggs in fresh capsules only a few are fertilized and develop. The remainder with the germinal vesicle intact are ingested, usually within a week after deposition by the developing embryos. After the eggs have been swallowed several days their germinal vesicles fragment, their pellicles disappear, and their yolk finally is digested.

The fertilized eggs are so few in number and so difficult to find that the only method of determining the average number per capsule was by finding the average number of larvæ in the later stages. The number of fertilized eggs in each capsule cannot have been less than the number of larvæ that come from it. There are reasons moreover, such as the frequency of accidents and the occurrence of dwarfed embryos, easily overlooked, for believing that the number of fertilized eggs exceeds the number of young found in later stages. The contents of 145 advanced capsules showed 6.2 larvæ in each as an average between a maximum of 15 and a minimum of 2. Between these extremes all degrees of fertility occurred.

The stages of development found in a single capsule are as variable as the number of larvæ. Two cell stages may occur in the same case with advanced embryos. It is difficult therefore to form an idea of the rate at which an individual egg develops, particularly as development ceases shortly after the embryos are removed from the capsules. Judging from the great variety of stages found in a single egg-case one may conclude either that not all the eggs are fertilized in the oviduct, but that some are impregnated after the capsules have been deposited, or that for some reason certain ones undergo a longer resting period than others. The importance of these discrepancies at the beginning of development becomes apparent in later stages.

After the early developmental processes have been passed through a larva results so irregular that no two individuals of this age are alike. Fig. 2 omits two eggs which this larva had swallowed, but will serve very well without these to give an idea of the general external appearance of the embryo before the crisis of its larval life has occurred. The larva is represented as viewed from the ventral surface. Anteriorly is the head vesicle (*h.v.*) and posteriorly the body vesicle (*b.v.*). Between the two, under the right external kidney (*e.x.k.*), two of the yolk spheres, derived from the four macromeres of the segmentation period, can be seen. The ectoderm of the body and head vesicles lacks definite cell boundaries, though the indefiniteness is much greater in the anterior than in the posterior region. Each nucleus of the ectoderm stains deeply and is surrounded by vacuoles which decrease in size inversely as their distance from it. Those furthest away from the nucleus are the smallest, and finally only minute scattered granules can be seen where one cell abuts upon another.

The mouth (*mth.*) is nearly perpendicular to the antero-posterior axis of the body. On each side of it are two pear-shaped patches of highly vacuolated ectoderm [which I described ('04) as early stages of the external kidneys, two organs which in *Fasciolaria* reach surprising proportions and probably take a most active part in the excretion of waste products.

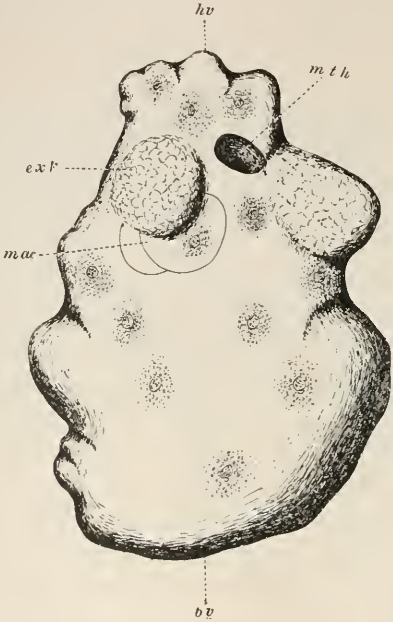

FIG. 2. Pre-cannibal larva of *Fasciolaria*; *b.v.*, body vesicle; *ex.k.*, external kidney; *h.v.*, head vesicle; *mac.*, macromere; *mth.*, mouth. Zeiss *D obj. 2 oc.* Drawn by Mr. Carl Kellner.

The cells of the excretory organs differ from the ordinary ectoderm cells chiefly in the great size of their vacuoles and nuclei. They resemble at this stage the ordinary ectoderm in in the comparative indefiniteness of their boundaries, which curiously enough are perfectly distinct at earlier as well as at later stages of development. This disappearance and subsequent reappearance of cell boundaries is due I believe to changes in the intra-cellular pressure, which varies with the size and number of the vacuoles ('05).

When the larvæ have attained the external condition described, the process of cannibalism begins. The eggs which up to that time are quite uniformly scattered throughout the albumen of the capsules are collected in more or less dense groups by the action of the cilia around the mouths of the embryos. Some hours after the eggs have been collected in the center of each capsule smaller groups, still more densely packed, can be seen, and at the end of two or three days all have been gathered into from two to fifteen spheres which are the cannibals, stuffed almost beyond comprehension. All larvæ which secure sufficient eggs finally have the appearance of the one shown in Fig. 3.

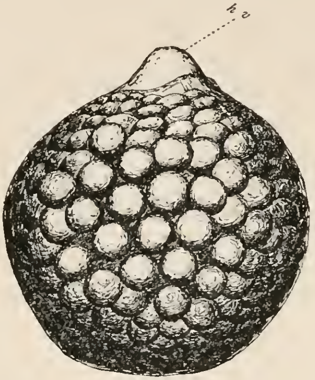

FIG. 3. Fully gorged cannibal larva of *Fasciolaria*. *h.v.*, head vesicle. Enlarged 20 diameters. Drawn by Mr. Carl Kellner.

This embryo is a fully gorged cannibal, so distended with eggs that the body wall is scarcely visible, and the mouth and excretory bodies are quite obscured by the dark background of yolk. All that can be definitely made out is the folded and slightly irregular head vesicle (*h.v.*) which marks the anterior end. Larvæ in this condition are comparatively regular, being in fact nothing more than balls of eggs held together by exceedingly thin transparent membranes. The diameters of fully gorged cannibals vary from 1.16 mm. to 1.90 mm. depending on the actual number of eggs that have been taken in. The average diameter of larvæ in this condition is about 1.48 mm. and one near this average contained by actual count over 300 eggs. Some larvæ of larger size undoubtedly contain more eggs, and some are entirely devoid of them.

What regulates the number of eggs swallowed? As already noted, eggs in all stages of segmentation may occur in the same capsule. Similarly in older capsules larvæ of very different degrees of development may occur together. Of these the most advanced, the first to be ready to swallow, gets most of the eggs, while its tardy mess-mates must take what remains. Those that are very late in reaching the point where they can ingest eggs often get none at all. They remain dwarfed and subsist on the jelly as best they can. Most of these dwarfed larvæ degenerate or are ingested by the cannibals, but occasionally some hatch as "runts" which are normal in all respects except size.

Of the larvæ which develop at a uniform rate those which have the most distensible mouths and the most violent ciliary action in the adoral field get more eggs than those with less distensible mouths and a less active ciliary mechanism. Thus in general, the number of eggs which a given larva secures depends on how early it enters the period of cannibalism and how rapidly and easily it swallows.

Repeated experiments were tried to find out the effect of artificially increasing the food supply of a given larva. One of these experiments gave a very remarkable result.

I was not able to keep the larvæ in a healthy condition outside of the egg cases, nor to satisfactorily reseal a capsule once opened. The problem of artificially increasing the food supply of a given embryo offered some difficulties until I tried the plan of injuring some of the larvæ inside of a capsule. This can be done easily by allowing them to drift into a corner of the egg case, holding them there by pressure on the rest of the capsule and then compressing them with a pair of strong forceps. With care one or two larvæ can be kept out of the corner, and all those which have been coralled can be injured in the manner described.

An injury to the excessively thin body-wall has the same effect as a hole in a bag of grain. The eggs which have been swallowed roll out and leave an empty membrane behind. In this way a thousand eggs, which have been swallowed once, may be set free. In the experiments which I made all but two or three larvæ in each of a large number of capsules were forcibly injured, and their undigested contents offered to the uninjured larvæ. These, although fully distended, in every case began to devour the additional eggs offered them, and in three or four days had mastered twice as many as had before fallen to their respective lots. One experiment, which was particularly successful, consisted in an attempt on my part to ascertain whether one larva would eat all the eggs which had been taken in by its competitors. This was accomplished by first injuring two of these and waiting until the extra number of eggs had been disposed of. Then two of the remaining larvæ were injured and the eggs which they contained — some of which had been swallowed once and others twice — were offered to the three uninjured inhabitants of the capsule. After this second offering of eggs was disposed of, two of the remaining three larvæ, which had grown to more than double their original size, were injured, and their contents offered to the sole survivor. This individual, already excessively gorged, began to ingest the eggs which had been swallowed from one to three times; unfortunately the elasticity of its body was not equal to the undertaking and the larva burst from over-eating before it had finished.

Over-feeding comparable to what happened in these experiments may take place without human interference. It is easy to see how the natural disturbances to which the capsules are subject might be sufficiently violent to burst some of the larvæ whose contents then would be devoured by the survivors. I am convinced that this actually takes place, since the larvae in those capsules which contain only two or three are always much larger than the average. From these observations and experiments I believe that the number of eggs secured depends on promptness and structural aptitude for seizing and swallowing.

II. The External and the Accessory External Kidneys of Fasciolaria.

Development.— The external kidneys, also known as "excretory cells" (Conklin) or "subvelar masses" (Osbore) and very generally called "Urnieren" by German writers, originate early in *Fasciolaria*, and are remarkable not for their size and position on the embryo, but also because they seem to take a very important part in the economy of the larva. Both the presence and the activity of these remarkable bodies are of interest when considered in the light of the feeding habits just described, for these, as might be expected, tax to the utmost the powers of assimilation and excretion.

Without considering the cytological changes which occur in the kidney cells, and which have been described fully in another place ('05) I will mention some of the more important alterations which involve the entire organs. What Conklin ('97) has said of the excretory cells of *Crepidula* applies equally well to those of *Fasciolaria*: "In the early stages these cells form a part of the ectodermic layer, but as the embryo grows older they grow more prominent, and the whole mass is constricted at the base, so that it becomes pear-shaped, the narrow end being attached to the embryo, and the larger end being distal. The surrounding ectoderm cells crowd in at the neck of this constriction and work their way entirely beneath these excretory cells."

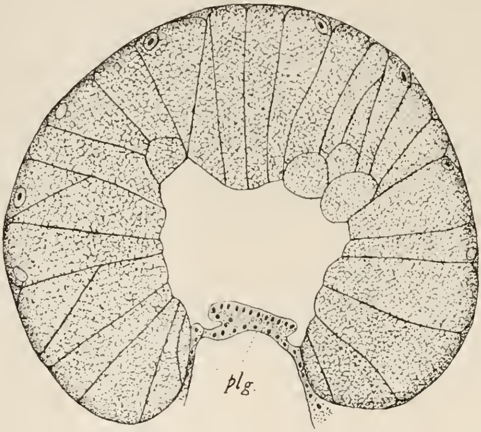

FIG. 4. Vertical section through external kidney of *Fasciolaria* showing connection with unmodified ectoderm and the plug (*plg.*) of undifferentiated cells which later fill the lumen. Leitz $\frac{3 \text{ obj. } 8 \text{ oc.}}{2}$. Drawn by Mr. Carl Kellner.

In *Fasciolaria* three very important changes take place in addition to those already mentioned. The ectoderm cells which "crowd in at the neck of the constriction" do more than this, for some of them coming from opposite sides join to form a plug which projects into the hollow of the rounded excretory organ. (Fig. 4, *plg.*) As the excretory organs at later stages have no lumens whatever, I conclude that these cells become modified secondarily into excretory cells like the primary ones.

The second important change which takes place in the external kidneys involves their position and results in the adoption of that curious relation with the velum which led Osborn ('85) to speak of them as "subvelar masses." When this change begins the embryo as a whole has enlarged greatly (Fig. 5), and has both by growth and by the stretching, due to the ingestion of eggs, become much wider than it was, especially at the base of the head vesicle. This increase in size brings about the removal of the external kidneys from the anterior surface of the larva to the lateral surfaces. Later the great activity in the velar ridge, by which this becomes prominent, as well as the growth of the whole anterior end of the larva, result in lifting the external kidneys upward and carrying them laterally away from the central mass of yolk on which at first they lie directly. Further lateral growth

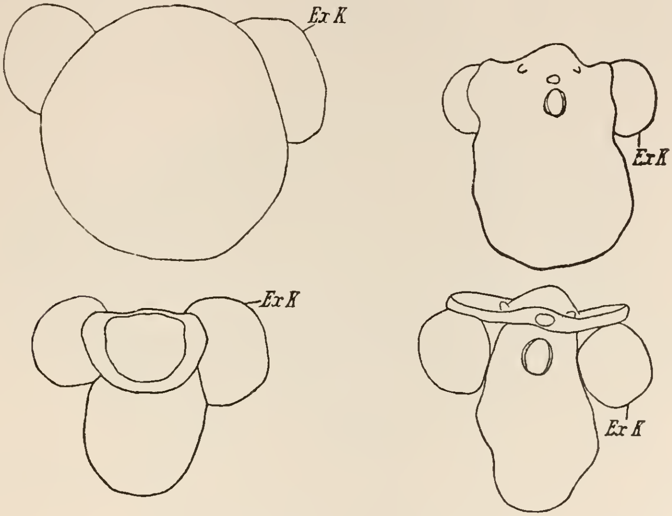

FIG. 5. Four stages in the migration of the external kidneys (*ex.k.*) from the lateral surface of the cannibal to their final subvelar position on the veliger.

of the velum carries the external kidneys outward and still farther away from the embryo, until finally they are far off at the sides of the body and hang down from the under side of the velum (Fig. 5). In this position the organs, which are white, viscid masses of ovate shape, having at the height of functional activity an average width of .7 mm., a length of 1.0 mm., and a depth of .9 mm., remain throughout the life of the veliger until this begins to assume the adult form. When that stage is reached the external kidneys begin to decrease in size, to degenerate, and the nuclei of the cells to disappear. Finally, in most cases, the kidneys are dropped off before the velum is resorbed. There are exceptions to this however, for often a much more intimate relation exists between the velum and the excretory organs hanging down from its under surface. This greater intimacy is well illustrated by Fig. 6, which is a typical section of these cases. In this organ, after the original lumen had been filled by the plug cells, the fully developed velum itself grew into the small pit which remained at the proximal (final anterior) end of the kidney. In this instance the velum was so crowded in the remaining hollow of the external kidney that the velar cavity itself was entirely obliterated by the close approximation of its upper and lower walls. In cases of this kind the velum drops off with the external kidney when the veliger assumes the adult form.

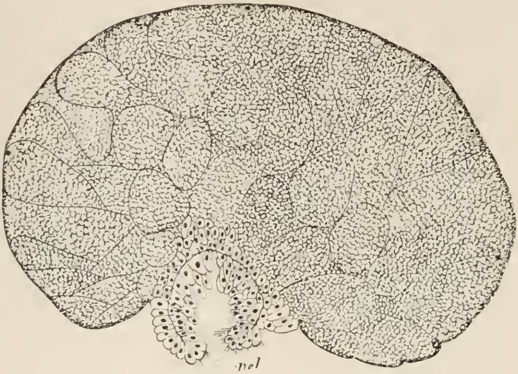

FIG. 6. Vertical section through external kidney showing the intimate relation which it may have with the velum (*vel.*). Leitz $\frac{3\ obj.\ 3\ oc.}{2}$.

This intimate connection between the velum and the kidneys recalls the "ansæ" of *Bithynia tentaculata*, described by Sarasin ('83). In this form the relation between the external kidneys and the velum is still more intimate than in these exceptional cases in *Fasciolaria.* Indeed the velum of *Bithynia* seems to be modified into a functional excretory apparatus, without having renounced its original duties as an organ of locomotion.

Accessory External Kidneys. — One of the most unexpected facts which I have encountered in my study of *Fasciolaria* embryos is that in addition to the large excretory organs no small proportion of larvæ possesses, sometimes in surprising places, accessory multicellular or unicellular external kidneys. In many cases these are found on the underside of the velum just behind the great kidneys; here they may become a third as large as the primary organs. In other instances single cells of the postoral or preoral velar row, and even of the head vesicle become modified into secondary unicellular kidneys. In some

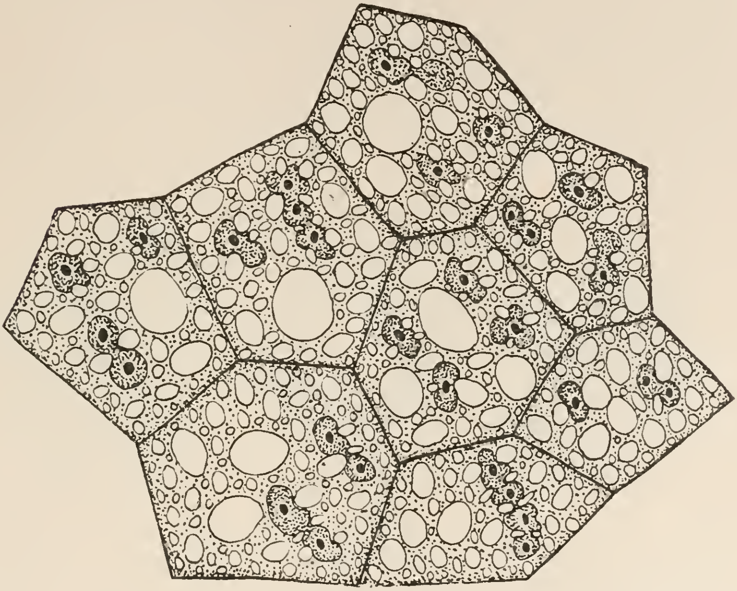

Fig. 7. Surface view of a portion of a functionally active external kidney, showing the thickened cell boundaries, the vacuolated cell contents, the amitotically dividing nuclei, and the nucleoli with their surrounding clear areas. Zeiss *A obj. 4 oc.*

of these accessory organs amitosis was observed, and in all the same extra-nuclear signs of activity which characterize the cells of the primary kidneys.

Function. — Between the two extremes of development which I have described the nuclei of the external kidneys divide amitotically and the cells become polynucleated. The nuclei are large, granular, very irregular in outline, and each one has at least one nucleolus surrounded by a clear area. In addition the cells are characterized by their thick boundaries and the highly vacuolated condition of their contents.

On a former occasion ('04) I showed how the vacuoles in the cells might be traced to the halos surrounding the nucleoli, but the figures intended to illustrate this are unsatisfactory since certain features which I hoped would not appear so unnaturally prominent when reduced to one half are over emphasized. More satisfactory figures will be found in my later paper ('05). The vacuoles which in some cases can be traced directly to the extranucleolar halo, in others to the nucleus from which I have seen them escaping, certainly suggest a high state of metabolic activity.

In the summer of 1903, with a view to determining the nature of the activities in the external kidneys, I removed several hundred, extracted them in chloroform water and asked my father to make a careful analysis of the extract. The details of this analysis have already been published ('05) but as all the decimal points were omitted by a careless printer, I republish the following corrected summary. One liter of the aqueous extract of the external kidneys contained

N as albumen,	.1242
N as free ammonia,	.0099
N as urea or homologues,	.2163
Total N,	.3504

My interpretation of the above analysis was that the external kidneys excreted waste products, but a reconsideration of the evidence, shows that it can become decisive only after corresponding analyses of extracts of other tissues have been made. Regardless of the value of the chemical evidence, which is certainly not negative, I still think that all the morphological results point to the probability that the external kidneys are excretory organs.

With the aid of such evidence as I have been able to obtain, and Osborn's interesting discovery ('04) of amitosis in the endoderm, I think I may formulate a scheme to account for the transfer of materials which probably takes place. Osborn ('04) announced that he had discovered amitosis in the endoderm of the gastrulæ of *Fasciolaria* and associated, very justly, I think, these nuclear phenomena with digestion. I have been able to verify these results, not, however, until I had been thoroughly led astray by the assertion that the nuclear divisions in question occur in the gastrula stage. All my earlier larvæ show no signs either of the "cuboidal endoderm" or of the amitoses of which Osborn speaks. The latter phenomenon I finally discovered in a larva that had a velum, and was therefore well past the period of cannibalism. Fig. 8 is compounded from several sections oblique to the antero-posterior axis of this larva. The œsophagus is cut transversely and the cells which compose it are dorsally, large, polynucleated, and highly granular, with vacuoles at their distal ends. The nuclei appear to have arisen amitotically. Some of these cells have burst and their contents may be seen oozing out. They bear a striking resemblance to the cells of the external kidneys.

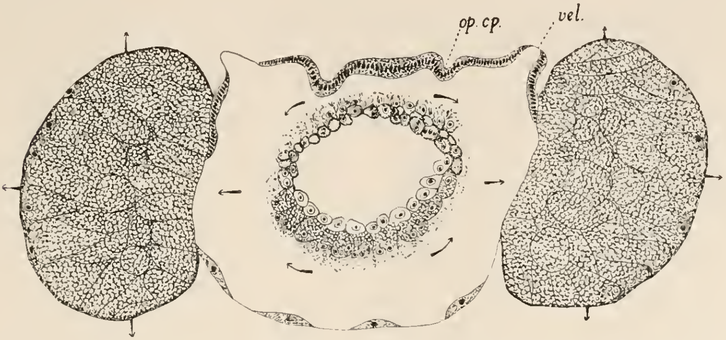

Fig. 8. Composite figure made up from several adjacent sections oblique to the antero-posterior axis. The external kidneys, not yet subvelar in position, are cut horizontally. *Op.cp.*, optic cup. *vel.*, velum. The arrows are intended to indicate the probable paths of waste products reaching the exterior from the digestive tract seen in transverse section, via the external kidneys. Leitz $\frac{3\ obj.\ 3\ oc.}{2}$.

The cells of the lateral and ventral walls of the œsophagus have a very different appearance. These, instead of being long and granular, are very irregular in shape, the cytoplasm being almost invisible, in irregular strands, or crowded closely against the cell boundaries. In the center some of the cells have a large clear space in which the nucleus lies. The nucleoli sometimes occur in the clear vacuoles which are scattered about in the nuclei. Many of the latter are in process of amitotic division.

Around the outer margin of the lateral and ventral walls of the œsophagus many of its bounding cells seem to have broken down, whereas others are clearly engaged in this process. By this means the outline is made extremely irregular, being frayed, and fringed with granules and the fragments of cells. This breaking down of the cells, their general vacuolated appearance, the occurrence of amitosis, and the finely divided state of the chromatin in the nuclei, all indicate that these cells are actively engaged in metabolism, and the simplest supposition is that they are engaged, as Osborn has suggested, in the process of digestion.

That something is leaving these cells is demonstrated by their appearance. I believe therefore that in the course of their activity as digestive cells, they excrete waste products, and that these pass across the "body cavity" into the cavity of the velum, transude through its basal membrane into the external kidneys and through these ultimately to the exterior. The arrows are intended to give a graphic representation of this process.

Homologies.— The earlier writers who considered the functions of the external kindeys of gastropods fall into two general groups, those who affirm and those who deny that these organs are renal organs. The same writers however have been at much greater discord with regard to the homologies. Thus Salensky ('72) homologized the external kidneys of *Calpytrea sinensis* with the primitive kidneys of pulmonates. Fol ('75) did the exact reverse by attempting to homologize the "Urnieren" of pulmonates with the primitive kidneys of prosobranchs. Bobretsky ('77) who describes the "Urnieren" of *Nassa mutabilis*, *Natica*, and *Fusus*, seems to have accepted the homologies advocated by Salensky. Bütschli ('77) in the same year, however, in a paper on *Paludina vivipara* took strong exception to Fol's homology. He pointed out that this supposed homology was between totally different structures, and that while the primitive kidneys of *Paludina* might be homologous with the primitive kidneys of *Lymnæus* and *Planorbis*, the external excretory organs "Urnierenzellen" of these forms could not be homologized with the primitive kidneys of *Paludina.*

Thus the significant fact was brought out that there are two kinds of "Urnieren," the one, since proved to be of mesodermal origin (*Paludina*, *Planorbis*), opening to the exterior by a pore, the others, collections of modified ectoderm cells, situated either on the postoral row of the velum (*Lymnæus*, *Planorbis*), or behind the velum (*Crepidula*, *Fulgur*). The confusion due to the attempts to homologize the external kidneys of prosobranchs with the primitive kidneys of pulmonates, and the converse proposition of tracing an homology between the external kidneys of pulmonates and the primitive or head kidneys of prosobranchs, was probably greatly increased because all these structures were called by some German writers "Urnieren." In this connection it is interesting that Korschelt and Heider ('93) in copying Bobretsky's figures for their text-book changed the labelling so that "Un" is replaced by "Ex."

Rabl ('79) came to the conclusion that the external kidneys of *Planorbis* were a part of the velum, and that they have nothing to do with the "head kidneys." Sarasin ('82) describes a most intimate connection between the velum and the external kidneys in *Bithynia tentaculata*. In fact he described the two under one name, the "Ansæ." In speaking of their homologies he says: "Nach den Erfahrungen von Bobretsky, Bütschli und mir, liegt auf jeder seite der Prosobranchier Embryonen, ein Häufchen grosser Ectodermzellen, das bei *Paludina* und *Bithynia* mit Wimferöffnung nach aussen mündet. Nach Bütschli und Fol finden sich diesselben bei *Planorbis*. Ist dies richtig, so haben die Süsswasser Pulmonaten zwei Organ paare, die als Urniere zu deuten sind ein vorderes und ein hinteres Paar. Hat Rabl recht dass die von Bütschli zuerst gefunden grossen Zellen bei *Planorbis* und *Lymnæus* zum Velum gehören, so sind wahrsheinlich die von Bütschli und mir bei *Paludina* und *Bithynia* gefundenen Organe den hinternen Urnieren der Süsswasser Pulmonaten homolog." Sarasin's position, except that it admits the ectodermal origin of the primitive kidneys in *Paludina*, is in the present state of our knowledge tenable. Erlanger ('92), though denying the excretory function of the "Ansæ" of *Bithynia*, conceded that on morhological grounds these cells might be considered the equivalents of the peculiar velar cells of the pulmonates and of the marine prosobranchs.

Heymons ('93) introduced another source of confusion by attempting to homologize the excretory cells of opisthobranchs with the external kidneys of prosobranchs. This homology seems far-fetched, since the excretory cells of *Umbrella* are situated near the anus of the larva, though they originate much further forward. This difference in position seemed of no importance to Heymons, since, as he says, McMurrich had already shown that the excretory cells may lie at various distances behind the velum. Conklin ('97) says in reply: "this difference in position seems to me, however, to be a very considerable one. In all prosobranchs these cells lie close behind the velum, while in *Umbrella* they are removed from that structure by almost the whole diameter of the embryo. Further, the fact that they sink into the interior in *Umbrella* would indicate that they are different from the excretory cells of prosobranchs."

Conklin ('97) finds that the "external excretory cells" of *Crepidula* have no connection with the velum. This is also true, in a sense, of *Fasciolaria*. In this form the external kidneys originate long before the velum, so that their ultimate connection with that organ is not primitive but secondary, and due to their origin near the place where the velum originates. In this respect they are not fundamentally a part of the velum, any more than the external kidneys of *Crepidula*.

It is obvious from the literature considered so far that the discussion of homologies has involved at least three different kinds of excretory organs. In order to emphasize the differences I shall henceforth call those mesodermal, or possibly ectodermal, structures of pulmonates and prosobranchs which open to the exterior through a pore, primitive kidneys; those modified ectodermal excretory cells which may occur in addition to primitive kidneys, external kidneys; and finally such external organs as Heymons and others have described in opisthobranchs, excretory cells.

Anyone who has seen Stauffacher's ('98) beautiful figures of the primitive kidney of the trochophore of *Cyclas cornea* can never compare similar structures with external kidneys in prosobranchs and pulmonates, or excretory cells in opisthobranchs. Meisenheimer ('98) introduced a complication however, for his very complete study of the development of *Limax maximus* leaves almost no doubt that the primitive kidney of this gastropod is of purely ectodermal origin. The reasons which Stauffacher has advanced that the primitive kidney of *Cyclas* is mesodermal, are as convincing as Meisenheimer's that in *Limax* it is ectodermal, so that it is necessary for the present to subdivide the group of primitive kidneys into ectodermal primitive kidneys and mesodermal primitive kidneys. If the conclusions of Stauffacher and Meisenheimer be indeed correct, then two sets of homologies can be granted; the mesodermal primitive kidneys of prosobranchs, pulmonates and lamellibranchs may be homologous; and the ectodermal primitive kidneys in the same groups may be homologous. No homology however can be granted between a primitive kidney of mesodermal and one of ectodermal origin without doing violence to the whole conception of homologies. Whether a conception which separates organs as much alike in structure and probably in function as the ectodermal and mesodermal primitive kidneys of larval molluscs should be violated, is a question which at present I do not feel able to discuss.

Are the external kidneys homologous with ectodermal primitive kidneys? I believe that there is no more reason to homologize external kidneys with ectodermal primitive than with mesodermal primitive kidneys, for the differences between external kidneys and primitive kidneys, of whatever layer, are the same. That in the one case both organs should originate from the same germ layer is no criterion on which to base homologies, for if it were we should be logically driven to homologize not only all ectodermal structures, but all structures of whatever origin.

In considering the relations of the larval excretory systems of molluscs it seems to me to be of great importance to keep the differences which I have tried to emphasize constantly in mind, but the distinctions once made, the task of recognizing true homologies is by no means a simple one. Possibly all mesodermal primitive kidneys are homologous, and possibly all ectodermal primitive kidneys are, but certainly not all external kidneys. The occurrence of unicellular or multicellular accessory external kidneys in different regions of embryos already so well endowed with excretory organs as *Fasciolaria* shows that the embryological measuring rod which has been so carefully applied to these larval structures of molluscs is less accurate than some of the investigators who have used it for the discovery of alleged detailed relationships.

III. The Origin of the Habit of Cannibalism.

Even though there are differences in the early extra-ovarian histories of the food products, the consumption of eggs and embryos by the developing larvæ of *Fasciolaria* is fundamentally similar to those other cases among gastropods in which certain young are used as food after being broken down, or are preyed upon directly by their competitors. It seems to me justifiable, therefore, to include all these methods of nutrition, based on the consumption of materials derived from the ovary of the mother, but not contained within the eggs from which the consumers come, under one term, cannibalism. The various degrees to which cannibalism is developed in different gastropods have been arranged in series by earlier writers, and though this series is both interesting and instructive it has no phylogenetic significance, and I shall try to show that the phenomenon in *Fasciolaria* can be explained independently.

McMurrich ('85) noticed that some of the "ova" in the capsules of *Crepidula fornicata*, *C. plana* and *C. convexa* break down and are used as food by the survivors, although this process is not so pronounced as in *Purpura floridana*. Koren and Danielssen ('57) described the case of *Buccinum undatum* which is very similar to that of *Fasciolaria* in the disproportion between the number of embryos developing in the capsules and the number of eggs furnished by the female. *Buccinum*, however, differs from *Fasciolaria* because many of the ova, which do not form embryos, divide. Carpenter in the same year ('57) corrected the view of Koren and Danielssen that the embryos originate by conglomeration, by describing the development of *Purpura lapillus* in which from 12 to 30 eggs develop into embryos, whereas each of the remaining 500 or 600 divides without regularity into from 14 to 20 fragments.

Selenka ('72), who confirmed Carpenter's results, did not consider the division of the "sterile" eggs equivalent to the regular segmentation of the fertile ones, not only because of the irregularity of the former process, both as to form and occurrence, but also because he found no nucleus. *Neritina fluviatilis*, according to Blochmann ('81), has capsules in which all the eggs are provided with nuclei that take the usual part of the formation of polar bodies and female pro-nuclei. After these processes no regularity can be detected in the divisions of the sterile ova, the later behavior of which led Blochmann to agree with Bütschli ('77) that these eggs are unfertilized.

Brooks ('77) observed that of the 6 to 20 eggs in the capsules of *Urosalpinx cinerea*, all undergo development normally, though exceptionally some may break down and serve as food for the survivors. This case of exceptional cannibalism furnished McMurrich (*loc. cit.*, p. 408) with "a clue to the manner in which the phenomena seen in *Fasciolaria*, *Purpura lapillus*, etc., have been brought about. An occasional egg in a capsule has from some cause or other broken down, and has been drawn into the digestive cavity of the developing embryos. This process having proved useful is continued, and an arrangement such as I have described above for *Purpura floridana* obtained. From this it is but a step to what occurs in *Buccinum*, *Purpura lapillus* and *Neritina*. In *Fasciolaria* the process is, as far as we know at present, at its culmination."

This series was not conceived of as genetic, for this would have been justified only if it had been previously shown that each of the forms mentioned was evolved from one next lower in the scale of cannibalism. That such has been the history of cannibalistic prosobranchs is not supported by any evidence known to me, and the series is therefore probably a collection of graded parallelisms.

The real clue seems to me to lie in another factor which, as McMurrich himself pointed out, is invariably present where cannibalism has advanced to any great extent. "This is (*loc. cit.*,

p. 409) the non-fertilization of the majority of the ova, whereby it is impossible for them to develop to any great extent, and whereby they naturally break down when they have endeavored to segment. We see this in *Neritina*, *Buccinum* and *Purpura lapillus*. In *Fasciolaria*, as stated above, the process reaches its climax, and in this case the sterile, nutritive ova do not show the least trace of segmentation, nor do they ever show signs of maturation."

This second factor seems to me most important, and in view of recent results on the development of the germ cells of gastropods, places the origin of cannibalism in a new light. In the first place the ingested materials may consist of undivided eggs, or of embryos or of a combination of these classes of constituents. Thus in *Neritina* and *Fasciolaria* the ingested eggs do not divide. In *Buccinum* most of the ingested ova divide, though some do not, and in *Crepidula*, *Purpura* and *Urosalpinx*, the ingested materials are derivatives of either early or late stages of development. The habit of *Fasciolaria*, though coming in the class with that of *Neritina*, also resembles that of *Crepidula*, *Purpura* and *Urosalpinx*, for the abnormal larvæ of the pre-cannibal period, as well as many of the abnormal late ones, including some dwarf larvæ, are ingested together with the unfertilized eggs. That these materials are taken in by the embryos requires no explanation in this connection, for not only are they obviously useful as food, but the structures in virtue of which the larvæ cannot help ingesting all the available substances in their environment were evolved, for locomotion and for swallowing other things, in all probability long before *Fasciolaria* exchanged its pelagic larval life for the safer one within capsules. To explain the origin of the habit of cannibalism it seems to me necessary to answer only two questions : (1) Why do some of the embryos break down ? (2) Why are many of the eggs infertile ? By answering these questions materials to be swallowed are accounted for : the consumers, and the mechanism by which the consumption is brought about, are the results of that portion of the phylogenetic history of *Fasciolaria* which preceded the origin of cannibalism. Given therefore ingestors, and materials to ingest, cannibalism follows as a natural consequence, needing no further explanation.

That some of the embryos break down is due, in *Fasciolaria* at least, to the fact that they have been outstripped in development by their competitors, whose superior strength, traceable to individual differences in the sizes of the fertile eggs, in the time and places of fertilization, in the ability at various stages to withstand the effects of exposure at low tide, or the jostling and even injuries to which the capsules are naturally exposed, probably enables these more fortunate ones to set up such lively currents by means of their cilia that their weak relatives are either whirled about until seriously harmed, or are so crowded into the mass of collected food ova that injury is certain and death probable. I do not believe that it is necessary, therefore, to trace the sterile eggs historically to degenerating embryos, for even if it were easy to see how selection could occur in this particular case, the physical conditions which account for the breaking down of some of the embryos at the present time, were probably always operative and seem to me of themselves to explain the facts sufficiently well.

That many of the eggs are unfertilized is more difficult to understand. This is true of *Fasciolaria*, of *Neritina* (Bütschli, Blochmann), and of *Purpura* (Selenka). In this last form Selenka was not able to discover a nucleus. The infertile eggs of *Fasciolaria* do not react to the influence of chemicals, though in some cases when a few drops of ammonia were added to the water Professor Conklin found that a number of eggs formed protrusions at one end. I have repeatedly tried artificial fertilization, with perfectly fresh eggs, in pure sea water, in a mixture of sea water and the albuminous contents of the capsules, or in the pure albumen. In no case did I succeed in finding more than the usual number of segmenting eggs in such collections, and in no case did the undivided ones seem to have attracted the spermatozoa. Since fertilization normally precedes maturation in the gastropod ovum, I conclude that these eggs are imperfect.

In what does their imperfection consist? This is a question which I cannot answer. It seems to me, however, that the dimorphism of the spermatozoa may point the way to a solution.

Meves ('02) described most remarkable differences between the development of the oligopyrene or worm-shaped spermatozoa and the eupyrene or ordinary hair-shaped spermatozoa of

Paludina. These differences in development first become manifest by the occurrence of two sizes of primary spermatocytes, the larger of which give rise to the oligopyrene spermatozoa, whereas the smaller ones give rise to the eupyrene forms. When the larger primary spermatocytes undergo maturation no reduction takes place, but the normal number of chromosomes (fourteen for *Paludina*) appears at the nuclear plate. These chromosomes are so distributed in the ensuing division that one of the resulting daughter cells (secondary spermatocytes) has four and the other has ten. When the secondary spermatocytes divide to form the spermatids, only one of the chromosomes of the secondary spermatocytes undergoes division, all the others (three for one class of spermatocytes and nine for the other class) degenerate. In this way it happens that the nucleus of the oligopyrene spermatozoa is composed of a single chromosome, whereas that of the functional eupyrene sperms has seven.

Fasciolaria, in company with a large number of other prosobranchs, has two kinds of spermatozoa, and there can be little doubt that these correspond respectively to the oligopyrene and the eupyrene sperms of *Paludina.* What the reason for this dimorphism may be is not clear, but so far as I can see, there is no evidence to show why it might not also occur among eggs, particularly of a form presenting such well marked differentiation of its male sexual elements as *Fasciolaria* does.

That *Fasciolaria* has two kinds of primary oöcytes which differ most remarkably in their reactions with spermatozoa, and consequently in their ultimate fate, is beyond dispute. This difference may possibly be due to an homology between the infertile oöcytes and those primary spermatocytes which give rise in the manner described by Meves to the oligopyrene spermatozoa. Whether further investigations establish this homology or not, the presence of the infertile eggs is the keystone of the conditions that determine cannibalism. The origin of this process therefore is to be sought in those circumstances that determine the formation of the sterile ova. That these ova should be ingested follows from the automatism of the larvæ, based on structures much older than the habit of cannibalism, and not to be explained by it. The persistence of those processes which give rise to the nutritive ova however can be explained by the theory of selection, since this method of feeding the young is useful to the species.

The sterile ova — to whatever cause due — have an important influence on development; indeed, all the facts which I have discussed or mentioned in the preceding pages can be united into a system of correlations, each part of which has antecedents or consequents, or both, traceable to the nutritive ova as the first link in a long chain of events. It is clear that without the sterile eggs cannibalism could not occur. It is equally clear that the larvæ prepare for this process, and that they are profoundly modified as the result of it. Thus, to consider first external characters, the frothy, irregular ectoderm of the precannibal period is well fitted for the stretching caused by the ingestion of the eggs, for these produce a distension so great that unless provision were made for it in advance a far larger number of embryos would be destroyed by it than as a matter of fact succumb.

The immediate results of the ingestion of the eggs are an entire change in the shape of the embryo, and a great increase not only in its size, but also in the size and organs of the young. The external kidneys are most clearly correlated with the cannibalism. In this case also we meet with provision, for the excretory organs appear, and are more highly developed than in any other gastropod embryo known to me, long before their chief need can be felt, and long before they have reached the highest development which they ultimately attain. The early appearance of the external kidneys, which a comparison with other prosobranchs shows to be secondary, brings about a change in their position, for if they developed at the time the velum appears, as they do in *Fulgur* (McMurrich), or after this appears as they do in *Crepidula* (Conklin), they would not be carried outwards by this organ, ultimately to hang down from its underside. The early development of the external kidneys is thus a case of cœnogenesis, and their final location on the embryo an excellent example of a conspicuous result due to a remote influence, for although this ultimate position and activity are connected with the egg-swallowing habit, this connection is indirect, since fully-developed external kidneys occur in dwarfed larvæ devoid of ingested ova. It might be urged from this that there is not even an indirect connection between the cannibalistic habit and the excretory organs, but this is by no means true. It is an accident in the lives of the dwarfed embryos that they fail to secure any eggs, for they prepare for them as much as their more successful competitors do. The fact that the preparations for an event which never comes to pass are elaborate cannot show that this event had no influence on the lives of the anticipators. What it does show is that this influence is not direct, for the habit of preparing for cannibalism has become fixed through selection.

Another correlation of importance is the amitosis in the external kidneys and in the œsophageal endoderm. Here the need for the rapid digestion of great quantities of food material and the excretion of waste products has called forth a process unusual in embryonic cells, but, as I shall try to show in another paper, not pathological. There can be little doubt that through these amitoses other correlative changes are brought about, particularly in the development of the œsophagus, where the gaps made by the degenerating embryonic digestive cells are certainly repaired before the adult stage has been reached. Thus the whole development, from early stages to late, the structure, shape and size of the larvæ and the size and hardiness of the young, and whatever these stand for in their further lives, are affected by cannibalism, the origin of which is traceable, I believe, not to the advantage which accounts for its persistence, but to some as yet unknown cause which determines the existence of the sterile nutritive ova.

Zoölogical Laboratory, University of Michigan,
Ann Arbor, January 4, 1906.

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A CHAMELEON-LIKE CHANGE IN DIEMYCTYLUS.[1]

(Preliminary Report.)

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In connection with some work done upon the heliotropic response in the salamander *Diemyctylus viridescens*, it was noticed that under certain conditions the animals changed color within a few hours in a most remarkable manner. The following paper is a report of the results obtained in a study of some of the conditions affecting this color change.

That light and heat are active stimuli in the case of many animals is a well-known fact. It has also been shown repeatedly that not only do these stimuli affect the movements and orientation of the animals, but may, in some instances at least, bring about changes of color as well.

The literature upon this part of the subject is not extensive, so it is impossible at present to formulate any general rule with respect to which these changes may take place. It may be said, however, that there is a remarkable uniformity in the results obtained by different investigators.

In attempting to explain the pigmentation of the salamander, *Salamandra maculata*, Fischel[2] found that the temperature to which the animals were subjected was an important factor. He observed that the larvæ of the salamander which developed in warm water were of a lighter color than those which developed in cold water. If dark-colored larvæ were placed in warm water they became lighter in color, the degree of the change varying with the age of the larvæ at the beginning of the experiment. The newly-hatched larvæ were found, also, to be much more susceptible to the changes in temperature than the older larvæ. As the larvæ became older the effects of the temperature to which they had been subjected tended to become fixed. If light-colored larvæ were placed in colder water, the converse change was noticed,

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[1] From the Zoölogical Laboratory of Syracuse University.

[2] Fischel, *Arch. f. Mikr. Anat.*, XLVI., pp. 719–748.

and this also tended to become permanent as the age of the animals became greater.

Flemming[1] observed that the conditions of light as well as of temperature make their impress upon the coloration of the salamander. Animals left in dark aquaria become and remain dark, while those in the light, for example, in white porcelain dishes, become light, the temperature of the water being the same in the two cases.

The recent work of Carlton[2] upon the chameleon *Anolis* gives us even more striking evidence of the part played by external conditions in the coloration of animals. He found that the skin of *Anolis* can be made to assume one of two colors, dark brown or pea green. The brown state for animals in confinement is taken on in daylight and is produced by the outward migration of pigment granules from the bodies of the melanopores into the processes and ultimate branches. This outward migration is accomplished in about four minutes. It may be brought about either by mechanical stimulation of the skin or by an act of the nervous system. The brown state is ordinarily maintained by a tonus established by the sympathetic nerves and dependent upon the stimulation of the nervous end organs in the skin by the light. The melanophores of *Anolis* are not directly stimulated by the light. The green state is taken on in the dark, and is produced by the inward migration of the pigment granules of the melanophores whereby the reflecting ochrophore becomes exposed to the light. This inward migration requires about twenty-five minutes. It may be induced by any means which will bring the melanophores into the unstimulated state.

Experiments upon Diemyctylus.

The material upon which these observations were made was the salamander *Diemyctylus viridescens*. Specimens were collected in the fall of the year and had been kept in the laboratory during the winter in glass jars. The water in the jars was frequently changed and the animals were fed upon raw beef at regular intervals. In the spring observations were also made upon specimens just taken from their usual environment with similar results.

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[1] Flemming, *Arch. f. Mikr. Anat.*, XLVIII., pp. 690–692.

[2] Carlton, *Proc. Amer. Acad. Arts and Sci.*, XXXIX., No. 10, pp. 259–276.

When under their normal conditions the salamanders are of a dirty yellow brown color upon the dorsal surface and a lemon yellow upon the ventral surface. Upon both surfaces there are black pigment spots about one half millimeter in diameter. There are also upon the dorsal surface a number of scattered red pigment spots. This description may apply equally well to animals just taken from their native surroundings or which have been kept in the laboratory for a considerable period at the room temperature. Specimens taken at random from the ponds do not vary much in color.

When the animals are exposed to a temperature which is much below that to which they have been accustomed it was found that there resulted a change in the appearance of the skin which would continue as long as the low temperature was maintained. Instead of a dirty yellow, the skin assumed a much darker color, becoming dark brown, dark green or in extreme cases almost black. This change took place in the course of a few hours and remained so long as the animals were subjected to the artificial condition. The amount of change in color which took place as the result of the change in temperature was in a measure a function of the total amount of temperature change. If the change were only a slight one the color response would be correspondingly slight, while if the temperature change were greater the color change would be also more pronounced.

If, on the contrary, the temperature of the water in which the animals were living was raised, it was found that the animals responded to the increased temperature by a lightening of the skin. Under these conditions the yellow in the skin became more pronounced and the darker colors less so. This change also was found to continue so long as the water remained warm. When the water became cold the color of the skin would return after a given interval to what it was at the beginning of the experiment.

Another fact of interest in connection with this color change is that if the temperature of the water be maintained at a constant point and the intensity of the light be changed, the color of the animals will be found to respond in a very definite way. It must be stated, however, that the response in this latter case is less pronounced than that obtained by a change in temperature, and may indeed be obscured by the action on a change in temperature. If the animals were placed in the dark for several hours they all became darker, if in the light for a number of hours they all became lighter. It was further found that the change in color due to a change in light conditions might be inhibited by contrary changes in the temperature conditions. The following table represents various combinations of light and temperature conditions which have been tested and the results obtained in the coloration of the animals.

Temperature.	In Dark.	In Light.
High,	Ordinary,	Very Light.
Low,	Very Dark,	Ordinary.

By high temperatures are meant temperatures of from thirty-five to forty degrees Celsius; by low, temperatures below ten degrees Celsius.

From the table it is readily seen that we are here dealing with two sets of forces which tend to neutralize each other when applied in opposite directions and to augment each other when applied in the same direction.

The manner in which this change is brought about is the same as that described by Carlton for *Anolis*. Small granules of pigment migrate outward from the large pigment cells along the ray-like processes of the cells when the animal is placed in the dark, thus obscuring the yellow pigment which lies in the deeper layers of the skin. When the animal is brought again into the light the pigment granules again migrate into the bodies of the pigment cells, and the lighter yellow pigment of the deeper layers of the skin becomes visible. The operation is a rather slow one and may occupy an hour or more of time.

In certain animals a condition of permanent darkness was established through a section of the optic nerves. So long as the animal had one or both eyes functional the action of the skin was as stated above. When both eyes are made functionless a marked change takes place. Within a short time the skin begins to darken and within two hours has taken on a dark brown, dark green or black color. The appearance of the animals is similar to that produced by the action of darkness and low temperature. At ordinary room temperatures this change is permanent.

But it is a remarkable fact that in this blind condition the animals are even more than normally sensitive to changes in temperature. If the temperature on the water be raised by any considerable amount the color of the animal changes correspondingly, becoming much lighter. On the other hand if the temperature of the water be lowered they again resume the intense dark color which came on as the result of the operation. These changes in color usually occupy a time of not far from two hours.

The question now arose as to how this color change was controlled. It was at first thought that it must be controlled through the central nervous system, but the following experiments seem to throw doubt upon such an explanation.

Section of the Spinal Cord. — It has been shown above that section of the optic nerves brings about a most remarkable result in the coloration of the salamander. Now if the impulses effective in bringing about this change pass to the skin through the brain and spinal cord we should find upon section of the spinal cord that the parts of the skin supplied by nerves arising from the spinal cord below the cut should respond in a different way from those parts of the skin supplied by nerves arising from the spinal cord above the cut. In order to test this possibility a number of salamanders were operated upon in the following way. The spinal cord was completely sectioned at the level of the third or fourth thoracic vertebra while under the influence of ether. At the same time other individuals were subjected to the same anesthetic for the same length of time to assure us that any change in the appearance of the animal was not due merely to the effect of the ether. After the animals had been given opportunity to recover from the shock effects of the operation, no change being found in the color of the skin as the result of the first operation, they were again taken and subjected to section of the optic nerves. Following this operation, usually within a period of not more than two hours there was found that same remarkable darkening of the skin which has already been described. And this darkening of the skin involved the whole dorsal surface of the animal and not merely that part of the skin controlled by nerves arising either above or below the section of the spinal cord. From this it is seen that the nervous impulses must have some other path of communication between the optic centers and the pigment cells of the skin than that furnished by the brain and spinal cord. It might be possible that the skin itself could furnish such a channel of communication, but a more reasonable means would seem to be through the the sympathetic nerves. In this our results agree with those of other observers.

There is also in the vicinity another variety of *Diemyctylus viridescens* known as *miniatus*. This is a land form and is of a vermilion color. It possesses few or none of the black pigment spots and apparently very few pigment cells. A series of experiments was also carried out upon this form but in no case was there any change of color noticed after section of the optic nerves.

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THE GROWTH AND REGENERATION OF THE GILLS IN THE YOUNG NECTURUS.

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During the summer of 1904 the writer made a series of observations and experiments on the growth and regeneration of the gills of the larval *Necturus*. The work was done with the hope of obtaining more information as to the relation which the regenerated gill bears to the normal in its rate of growth, in its size, and particularly in its pattern.

The larvæ were kept in aquaria, from which they were taken at intervals, placed in flat-bottomed watch-glasses above mirrors, and sketches made of the ventral surfaces of the gills. The period of observation on the growth of the normal gills was from the time (12–13 mm) of their first appearance, to the time (18–20 mm.) when four pairs of filaments were present. At this time the gills were cut off and preserved.

These larvæ were then placed in separate aquaria and like the normal were sketched at successive intervals. The period of observation on the regenerating gills was from the time of excision until about the same number of filaments were present as in the normal at the time when they were cut off.

Ten series were started, but only eight were completed, owing to the death of two larvæ. That the normal processes might be as little disturbed as possible but a single gill was removed from each larva. This one was in each case the anterior on the right side of the head. Since the eight experiments gave similar results, but three have been described and illustrated in detail.

The first appearance of the gill bars is to be seen in the 9–10 mm. larva as slight swellings on each of the three gill arches. When the larva has reached a length of 12–13 mm., the first and second gill bars measure about 1.0 mm., while the third measures 0.5 mm.

The first gill filament appears at this time as a slight swelling on the postero-ventral surface of the first bar, midway between its base and apex; soon a second filament appears on the antero-ventral surface opposite the first, as shown in Ser. II., Fig. 19. Often the buds form at different levels, giving rise to unsymmet-

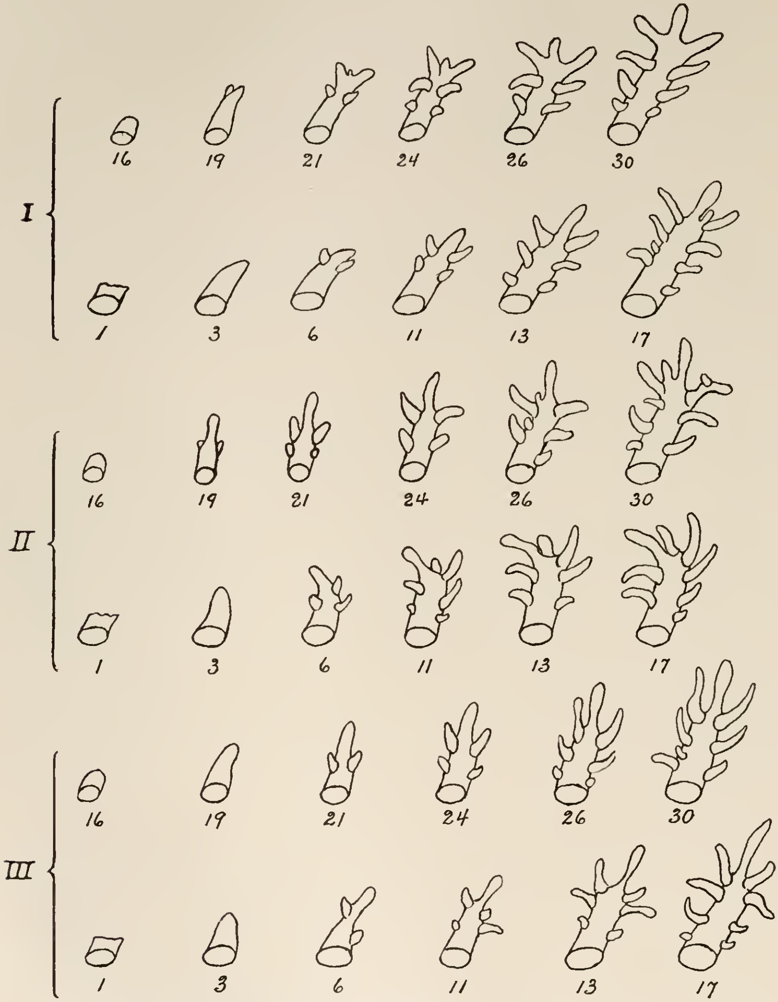

FIG. 1. Each series is bracketed and designated by Roman numerals. The upper row in each series represents the changes in the normal gill. The lower row of each series represents the changes in the regenerating gill of the same larva. The numerals indicate the days of the month: June 16–30 and July 1–17. All the figures are magnified about ten times.

rical patterns. While the lateral buds are elongating the tip of the gill bar becomes drawn out into a median filament, as in Ser. III., Fig. 21. Sometimes the tip of the gill bar forms one of the first pair of filaments, in which case a median bud forms the median or apical filament.

A second pair of filaments forms some 48 to 60 hours later. In most cases these are bilaterally symmetrical, as indicated in Fig. 21. In two out of the eight series the buds did not arise at opposite points, and in these cases more or less irregularity occurred in the succeeding pairs.

The third pair of filaments shows considerable variation both in time and place of origin. In five cases they appeared about 48 hours after the second pair. In the remaining three cases they were not formed until the end of the third day. The filaments of this pair usually arise in such positions that they are bilaterally symmetrical, as shown in Ser. III., Fig. 26. In two series conditions were observed something like that represented in Ser. II., Fig. 26, where only one of the third pair arose in the usual position. In place of the other there arose an intercalated bud on the opposite side.

The fourth pair of filaments may develop as bilaterally symmetrical structures, as in Ser. I., Fig. 30, or may be represented by a single intercalated filament, as in Ser. III., Fig. 30, or again the filaments may show such great irregularity that it is impossible to tell what filaments are to be considered as belonging to the fourth pair, as in Ser. II., Fig. 30.

In the later growth of the gill the filaments not only continue to form in pairs at the base of the gill bar but also to arise irregularly from intercalated buds. In addition the filaments send off lateral branches which form secondary filaments. In the older larvæ (30–40 mm.) the patterns become more and more irregular.

As a general statement one might say that in the earliest filaments much regularity prevails, but in the formation of the later filaments the regularity decreases.

When the gills had reached the stages shown in Fig. 30 they were cut off with spring scissors as near the head as possible and as they regenerated their changes were carefully followed and compared with the successive changes observed in the growth of the normal gills.

In Ser. I. the first pair of buds formed at about the same relative time as in the normal, but instead of conforming to the rather exceptional pattern of the normal they were symmetrically placed, as shown in Fig. 6. The second pair of filaments while symmetrical were not present until 96 hours later. In the normal the second pair arose 72 hours after the first pair. The third pair appeared some 48 hours after the second and in both time and place of origin conformed to the normal. The condition about 132 hours later is shown by Fig. 17. Comparing this figure with Fig. 30 it will be noted that but a single filament is present to represent the fourth pair of the normal. Again there are present two intercalated buds while the normal shows none.

In Ser. II. the filaments appear later than in the normal and instead of arising as a pair there are three buds present (Fig. 6). As the later stages show, the first pair is made up by the tip of the median and the first lateral filament. About 96 hours later, as shown in Fig. 11, three more buds are present. Two of these form a new pair at the base of the gill bar, while the third arises between the tip of the gill bar and the first lateral filament. When compared with the normal of this stage it is obvious at a glance that there is no conformity. No other changes occur which merit detailed description. If the stage shown in Fig. 17 be compared with that of the normal shown in Fig. 30 it will be readily observed that the regenerated gill is quite unlike the normal, but on the whole it presents a more regular pattern than the normal.

In Ser. III. the first pair of filaments appeared at about the same relative time as in the normal but at widely separated points, as shown in Fig. 6. The second pair formed some 96 hours later and, alternating with those of the first, gave rise to the pattern shown in Fig. 11. The third pair, as shown in Fig. 13, appeared 72 hours after the second as symmetrically placed structures. The fourth pair were likewise symmetrically placed.

It may be remarked that the remaining five series showed, with slight variations, the same results as described above. In the entire eight series not a single case was observed in which the pattern of the growth changes in the normal gill were repeated in the regenerating gill.

If we compare the rate of growth in the regenerating gill with the rate of the normal gill we find that the stage reached in normal growth in sixteen days is reached in regeneration in about eighteen days.

That the pattern of the growing gill is not retraced by the regenerating has been established beyond question in these experiments. In the regeneration of the foot of the young *Necturus* the course of normal development is pretty accurately repeated and the same is known to be true of many other forms.

Previous experiments by others seem to indicate that if wide variations occur in normal growth we should expect to find like wide variations in regeneration.

One pattern of gill is as efficient for respiration as another provided it posesses the same number of filaments. The same is true of the regenerated gill. The normal pattern of the foot is duplicated in regeneration because the type evolved is that best adapted to the needs of the animal.

If these considerations be well founded we are led to regard physiological efficiency as the important factor in the regeneration of the gills.

In other words we may conclude that functional and structural regeneration may run parallel or they may follow widely diverging lines.

OBSERVATIONS ON THE CHANGES IN THE HYPODERMIS AND CUTICULA OF COLEOPTERA DURING ECDYSIS.

W. L. TOWER.

The internal changes which occur during the periodic removal and redevelopment of the chitinous portion of the integument of insects are little understood and but few observations have been made thereon. In this paper are given observations and conclusions concerning some of the changes found in the integument of *Leptinotarsa decimlineata* and *Chrysobothris femorata*. These two beetle larvæ are good examples of two types of larvæ, the first living freely exposed upon their food plants and are typical examples of those insect larvæ which pass their lives upon plants in exposed places and must go through ecdysis exposed, and the second of larvæ that live in burrows or cells, protected from external interference to a great degree. Corresponding to the difference in their habitats they show differences in the internal changes accompanying ecdysis.

The life of an insect larva is made up of a series of instars or stages, each of which represents a very precise cycle of development and physiological activities. These cycles of changes are of interest and serve to give a good basis for the orientation of the changes which I am about to describe as accompanying ecdysis. The changes within a single cycle are given in the tabulated form on page 177.

Each larval stage properly begins with the period of growth following the reconstruction period of the preceding ecdysis and ends with the end of the next reconstruction period. Looked at from the exterior the stages extend from ecdysis to ecdysis, but as shown in the above table the period of exuviation is the middle one of the short and rapidly passed over periods in which the process of ecdysis is begun, achieved and the animal recovers from the effects thereof. The changes with which this paper deals are confined largely to the last three periods in the cycle.

CYCLE OF PERIODS IN ONE LARVAL STAGE.

Period of Growth.	Period of Maximum Nutrition.	Period of Differentiation.	Period of Preparation.	Period of Exuviation.	Period of Reconstruction.
Larva actively feeding.				Larva not feeding	
Feeding voraciously; rapid increase in size and in thickness of integument; intensification of color.	Feeding voraciously; integument reaches its maximum thickness; abdominal region distended to its full capacity; surface smooth; fat body growing rapidly.	Feeding less actively; abdomen much distended; fat body greatly increased; marked growth of imaginal organs and rapid differentiation of gland cells and leucocytes.	Feeding but little; stops feeding; restless; seeks sheltered place; rapid development of exuvial glands; of zymogens in the hypodemal cells; hypodermis with basement membrane drawn away.	Resting quietly; contractions begin, become rhythmical; dissolving of secondary cuticula; formation of exuvial fluid; rupture of exuvial sutures; detachment of the muscles; formation of new cuticula; removal of old cuticula.	Larva weak; soft; extended; quiet; cuticula hardens; color develops; secondary cuticula begins to form; glands subside; hypodermis becomes condensed into thin layer; larva begins to move about and begins feeding.

The Exuvial Glands.— The existence of an exuvial fluid in insects was first clearly demonstrated by Newport, but its origin remained obscure and was attributed to diverse sources. It was first shown to be in part at least, due to glandular activity by Gonin in *Pieris brassicæ*. Gonin found, especially upon the pronotum, a considerable number of large unicellar glands which were in his preparations definitely seen to be extruding their contents between the old and new cuticula to form a part at least of the exuvial fluid. Similar unicellular glands are found in other insects, especially at pupation, in free living forms such as most of the larvæ of butterflies and moths and of many leaf feeding beetle larvæ. In *L. decimlineata* there are found from 50 to 225 of these glands upon the pronotum in the last larval stage, and their sole function seems to be to develop an enormous amount of the exuvial fluid. The glands are found all through the life of the animal and upon all parts of the body, but in fewer numbers than upon the pronotum at pupation. Some idea of the abundance of these glands may be gained from the section shown in Fig. 1, a section from the pronotum of *L. decimlineata* at pupation when these unicellular glands are closely crowded and are all ejecting their contents into the space between the old and new cuticula (c^1, c^2, c^3).

These glands arise in the embryo and in later larval stages through the modification of the hypodermal cells. In Fig. 2 are shown five stages in the development of these glands.

The first stage in the development of one of these glands in larval life is usually found in the latter portion of the period of maximum nutrition and shows the nucleus greatly enlarged, the chromatin greatly increased in amount and scattered, with the cytoplasm seen (Fig. 2). In the period of differentiation thisd cell grows very rapidly projecting inward below the hypodermis, but retaining a delicate connection with the cuticula (Fig. 2, *B*). The cell remains in this condition through the following ecdysis and appears after ecdysis unchanged, excepting that the gland now has a delicate duct leading to the surface, this being developed as the result of the glands not forming chitin at this point of attachment to the outer surface of the hypodermis (Fig. 2, *C*), During the last larval stage the character of these glands, especially in the periods of differentiation and preparation, change rapidly (Fig. 2, *D* and *E*), becoming first larger, the nucleus and chromatin growing immensely in size and quantity, the cytoplasm remaining dense and slightly vacuolated. At the begin-

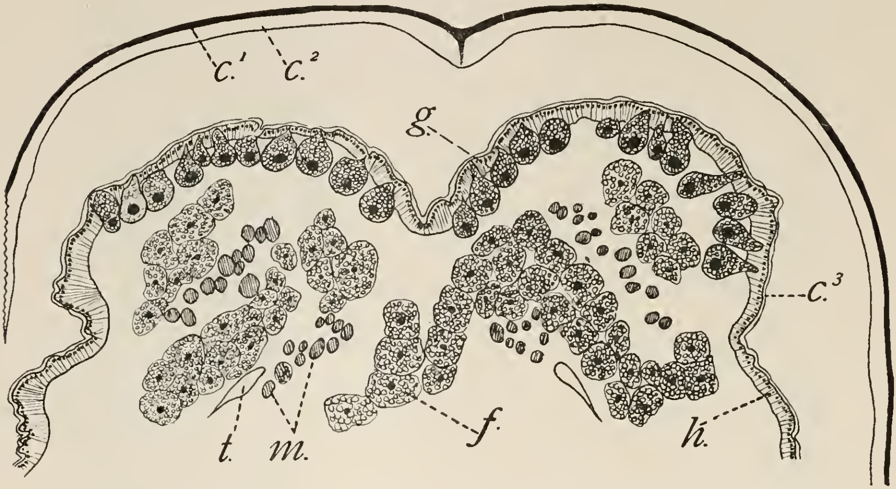

FIG. 1. *L. decimlineata.* Section of pronotum of larva about to pupate, in early stage of exuviation, showing large number of exuvial glands, *g*. *C′*, cuticula of larva being removed; C^3, developing new cuticula; *H*, hypodermis; *f*, fat body; *m*, muscles; *t*, trachea.

ning of the period of preparation there is a rapid change, chromatolosis of the nucleus, a marked shrivelling and decrease in size. and the cytoplasm becoming entirely vacuolated (Fig. 2, *E*). As ecdysis begins and the old cuticula separates from the hypodermis the contents of these glands are forced out between the old cutic-

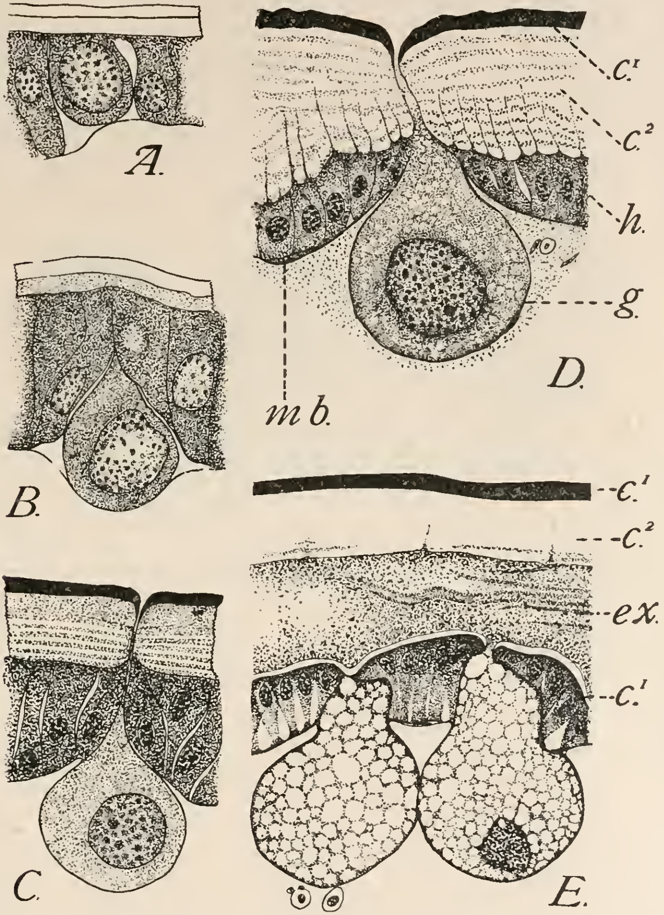

FIG. 2. *L. decimlineata.* Five stages in the development of the exuvial glands. *A*, young stage showing rapid growth of nucleus in hypodermal cell; *B*, later stage showing cell much enlarged and projecting below the hypodermis, taken just before second ecdysis; *C*, third stage just after second ecdysis, showing the cell as a fully developed unicellular gland with outlet to surface; *D* and *E* show the great increase in size of these glands preceding pupation (at *E* is shown glands extruding their contents to form part of the exuvial fluid); c', primary cuticula; c^2, secondary cuticula; *h*, hypodermis; *mb*, basement membrane.

ula and the new to help form the exuvial fluid. Eventually nearly all of the contents of the glands are extruded, leaving them small and shrunken as shown in Fig. 3, *A*.

After ecdysis and especially after pupation the degeneration of these glands is rapid, as shown in Fig. 3, the cell speedily returning to a normal hypodermal cell in size and ultimately it breaks down completely.

As far as I can discover these glands are exactly like those found by Gonin in *Pieris brassicæ*, and I have observed the same structure in *Pieris rapæ* and *protodice*, and *Clisiocampa americana* among the Lepidoptera and they are widespread in the Chrysomelidæ and Coccinnellidæ, especially in the tropical species. Nowhere have I found these unicellular glands in larvæ that live in burrows, or in the soil or in cells, but only in larvæ living freely exposed upon plants where there exists the greatest liability to rapid desiccation. In *L. decimlineata*, *multitæniata* and their tropical allies these glands developed in the embryo in small numbers over the entire body surface and are active at each ecdysis. As the larva grows, however, the number of these glands increase until at the time of pupation there are very many of them scattered over the body, but they are most numerous upon the pronotum. During the pupal period nearly all degenerate rapidly and but few are functional at the final transformation.

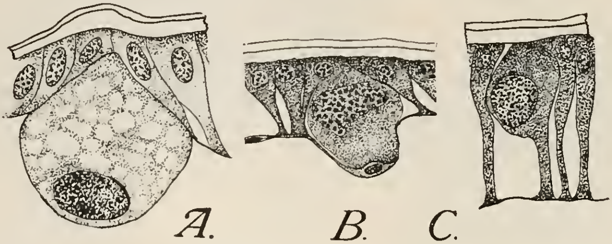

FIG. 3. *L. decimlineata*. Three stages in the degeneration of the exuvial glands. *A*, gland immediately after ecdysis; *B*, stage of gland during the period of reconstruction; *C*, gland nearly reduced to normal hypodermal cell in period of growth.

These glands and their increase in number is, I believe, an adaptation in these freely exposed larvæ, to enable them to pass with the least mortality through such critical periods of their life as ecdysis and pupation. I can see no reason why this adaptation would not be one of direct selective value and be greatly developed by selection, because those individuals with an abundant supply of exuvial fluid would have a far better chance of passing safely these critical periods than those with a lesser supply of the exuvial fluid.

Changes in the Integument. — In the integument the preparatory changes preceding ecdysis begin before the larvæ cease feeding and consist largely in the withdrawal of the protoplasmic processes of the hypodermal cells from the pore canals in the secondary cuticula (Fig. 2, *D*) and the gradual change in the shape of the hypodermal cells whereby they become greatly elongated and their outer ends and the basement membrane separated by two, three or four times the usual distance. A section (Fig. 4) shows this condition in which the hypodermis is in the form of a flattened epithelium.

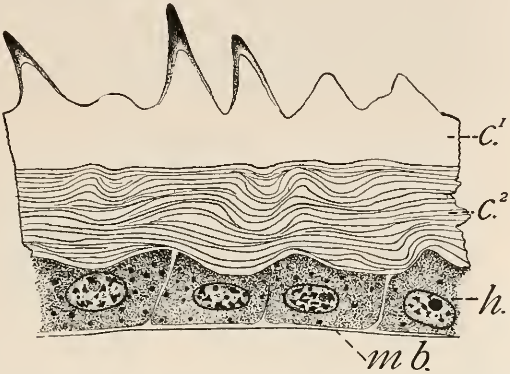

FIG. 4. *C. femorata.* Section in latter end of period of differentiation of last instar, from the mesothorax.

The changes from the condition shown in Fig. 4 go on slowly until at the beginning of the first contractions all the protoplasmic processes have been withdrawn and the hypodermis is much thickened, due to the drawing away of the basement membrane. With the first contractions the old cuticula and hypodermal cells separate over almost the entire body surface and only the muscle attachments remain to hold the old cuticula to the animal.

At the time when the contractions begin or slightly before, a thin layer of exuvial fluid is found, especially in the anterior parts of the body between the hypodermis and cuticula, and the inner surface of the cuticula appears rough and corroded. This corrosion of the inner side of the secondary cuticula continues until it is often almost entirely removed, as shown in Fig. 5.

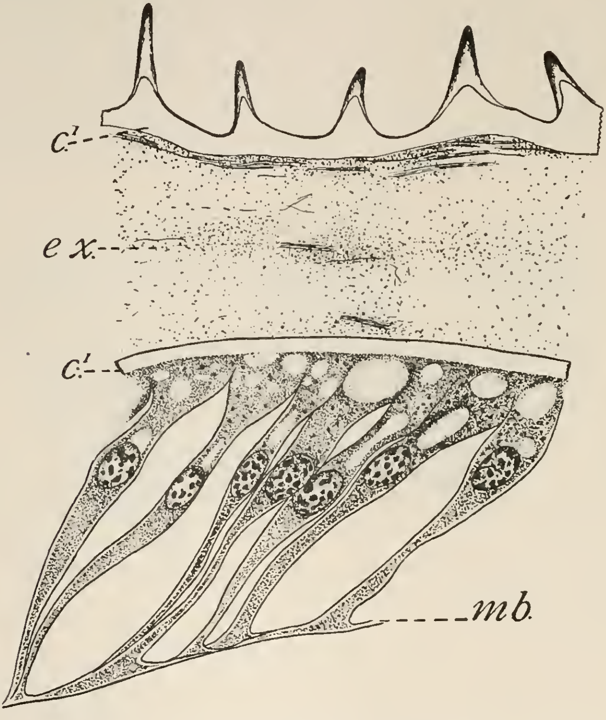

FIG. 5. *C. femorata.* Section of the integument from same location as the section from which Fig. 4 was taken, showing decrease in thickness of the secondary cuticula and great extension of the basal ends of the hypodermal cells to form a relatively thick but open layer of hypodermis.

This dissolving of the secondary cuticula is a most constant phenomenon in ecdysis and has been found in all the insects that I have examined, but in varying degrees. The same disintegration of this layer is shown in Fig. 6 of *L. decimlineata.*

This origin of part of the exuvial fluid in *L. decimlineata* and of all of it in *C. femorata* has not, I believe, been heretofore suspected but we can at once see the great utility of this process and especially the advantage gained in having the cuticula thinned and softened by the dissolving action which is evidently going on. As far as my experience goes this solution of the cuticula varies greatly in different preparations, being almost but never entirely absent in some and exceedingly active in others. I have not determined absolutely what brings about this dissolution of the secondary cuticula, but it is probably due to enzyme action. Attempts have been made to isolate these enzymes but thus far without any marked success. I do find, however, in the hypodermal cells in the late part of the period of differentiation and the early part of the period of preparation, granules which react and stain exactly like zymogen granules and which are derived from the nucleus by chromatolosis and which disappear after the action of dissolution of the old cuticula has begun. These may not, however, have anything to do with the disintegration of the old cuticula. It is clear, however, that the action upon the cuticula is chemical as there is not the slightest indication of phagocytes or other organic elements being present. The most logical supposition is that the hypodermal cells secrete a substance that dissolves the old secondary cuticula and thus thins and softens the integument as well as supplying a considerable part of the exuvial fluid, thereby greatly facilitating ecdysis.

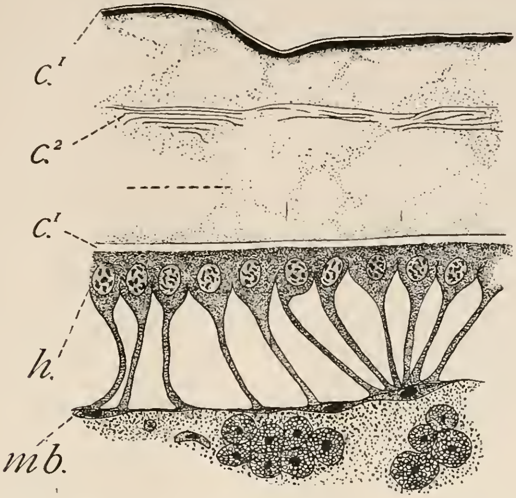

FIG. 6. *L. decimlineata.* Section of pronotum in early part of the period of ecdysis showing greatly drawn out hypodermal cells and almost entirely dissolved secondary cuticula. Fragments of cuticula are floating in the exuvial fluid (C^2).

These changes which occur in the cuticula involve only the secondary cuticula and are the same irrespective of what the habitat of the larva may be, as shown in the figures given and result in all in the thinning and weakening of the old integument. All these changes take place in the latter part of the period of preparation and the first third of the following stage.

After the separation of the cuticula and hypodermis the new primary cuticula begins to form at once. It appears first as a thin delicate lamella spread evenly over the entire outer surface of the hypodermis and grows rapidly in thickness until finally just before ecdysis takes place it reaches its final thickness. It is developed as a delicate structureless membrane secreted by the hypodermal cells and there appears at no time evidence in favor of the oft-repeated statement that the cuticula is the hardened outer ends of the hypodermal cells. After ecdysis this primary cuticula hardens rapidly and develops its coloration through enzyme action precisely as in the adult beetle, a process which I have described elsewhere.

As soon as ecdysis is over the deposition of the secondary cuticula begins. This layer is, as is well known (Vossler, Tower) a carbohydrate allied to tunicin and is deposited in alternating layers through the periods of reconstruction and growth when it attains its maximum thickness. It is everywhere penetrated by delicate pore canals which are the fine canals occupied by the protoplasmic processes of the hypodermal cells which do not become detached from the primary cuticula until just before ecdysis when they are withdrawn. With poor killing and preservation, however, they are all withdrawn and the canals appear empty, but are not so in life.

The hypodermis also goes through a regular cycle of changes in the shape of the cells during each of the cycles. These changes are first an increase in the number of cells in the growth period, and second changes in shape and arrangement so as to give the body wall the greatest rigidity and strength during the period immediately following ecdysis before the new cuticula hardens. In Fig. 7 I have given a series of stages in semi-diagrammatic form showing the change in the shape and arrangement of the cells. In Fig. 7, *A*, in the growth and differentiation periods the cells are hexagonal flattened epithelial and of themselves would be a relatively weak layer if left alone. In the period of preparation, however, the inner ends elongate, the basement membrane draws away and the cells come to form a much thicker layer, until during the period of ecdysis they present the condition shown in Fig. 7, *D*, which arrangement, even though the thick cuticle be absent, gives a far greater rigidity to the body wall than the arrangement seen in Fig. 7, *A*. After ecdysis is over the cells gradually assume their epithelial character. This series of change represented in Fig. 7 evidently is for no other purpose than that of making the integument as rigid and strong as possible during ecdysis. The arrangement developed is one which in a mechanical way is the best possible under the conditions. In fact the arrangement of the basement membrane, the long drawn out hypodermal cells and the developing cuticle is exactly the system used by engineers in large bridge work or in the building of large spans or girders.

The change in the integument are all in the direction of strength-

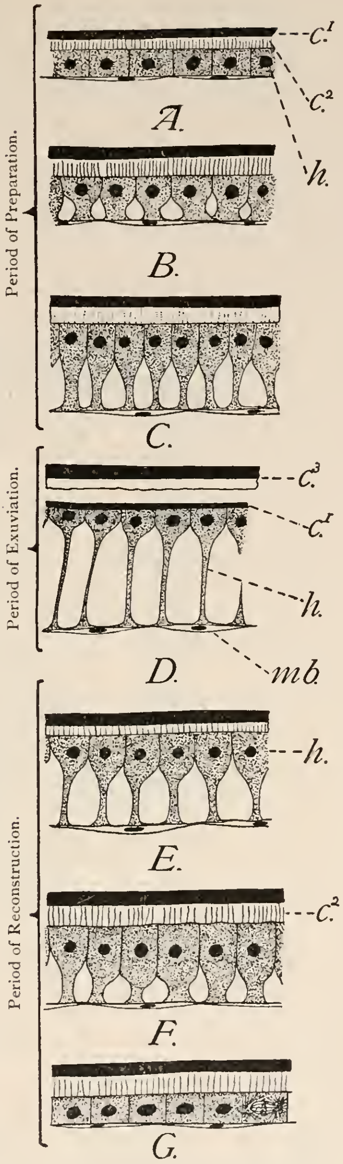

FIG. 7. Diagrammatic representation of stages passed through by the hypodermis before, during and after ecdysis, to show changes in the form of cells. c', primary cuticula; c^2, second cuticula; c^3, old larval cuticula being removed; *h*, hypodermis; *mb*, basement membrane. All figures are from *preparations* of *L. decimlineata*.

ening it to enable it to resist the strain and pressure it must undergo during and after ecdysis, and by solvent secretions to weaken the old cuticula and decrease the resistance offered by it to its removal and to provide from the material to be cast away a part of the lubricating fluid, to facilitate the withdrawal of the body from its old cuticula covering.

The Exuvial Sutures. — It is commonly stated that the rupture of the integument during ecdysis is caused by the pressure of the hæmolymph during the contractions. It is probably true that the pressure of the hæmolymph and the violence of the contrac-

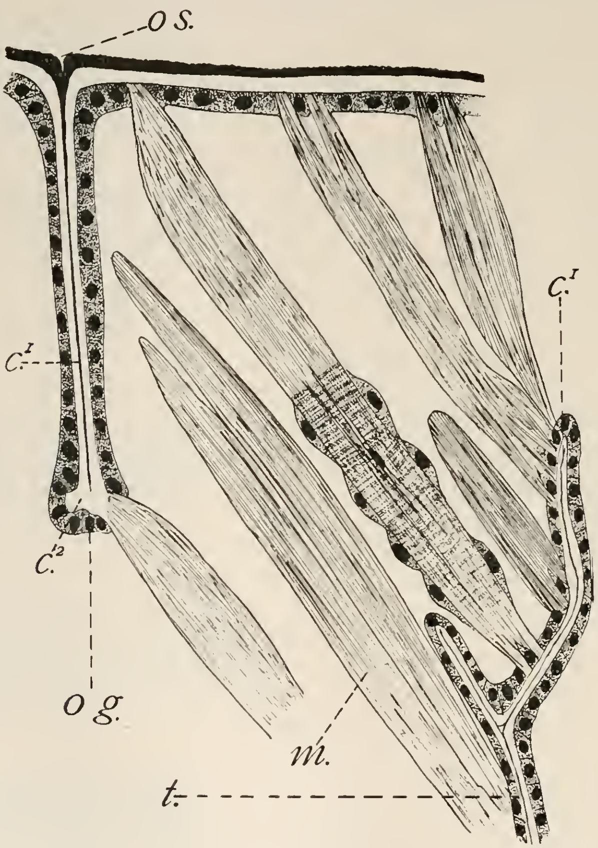

FIG. 8. *L. decimlineata.* Section of the head in the region of the occiput. Last larval instar showing the line of weakness formed in the mid-dorsal line by the inflexed body wall. *os*, occipital suture; *t*, crura of tentorium; *og*, occipital fold.

tion aid in this process, but that the rupture should occur so regularly in the mid-dorsal line is remarkable, if this be the only cause of the rupture. In the insects that I have examined there always exists in the mid-dorsal line a special device to aid in the splitting of the old cuticula at ecdysis.

This apparatus in *L. decimlineata* consists of a well-marked laterally flattened invagination extending caudalward from the region of the occipital suture, where it extends deeply into the head (Fig. 8).

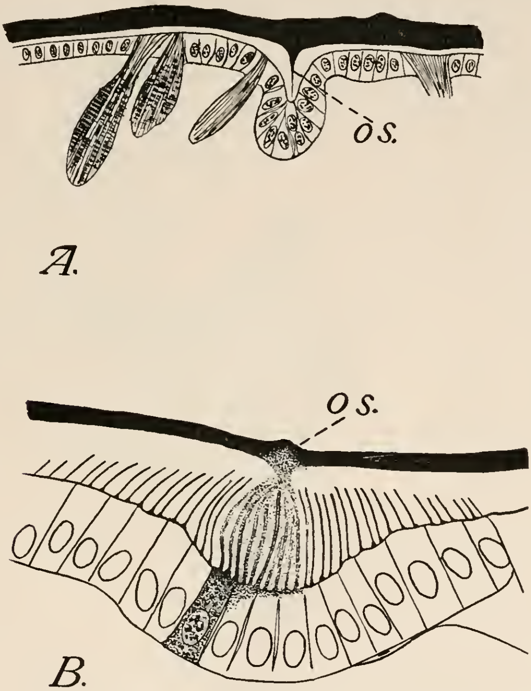

FIG. 9. *L. decimlineata.* Section through mesothorax. (*A*) and second abdominal segment; (*B*) to show continuation of the modifications of median dorsal line of body to permit of rupture at ecdysis.

This line of weakness in the mid-dorsal line is also the last point where the ectoderm closes in in development and is in *decimlineata* turned inward to form a median dorsal fold from the anterior edge of the occiput to the first abdominal segment and in this median dorsal furrow the chitin is easily broken during ecdysis. When the secondary cuticula represented in white in the figures is nearly dissolved away the muscles are still attached and a slight contraction of the muscles shown in Fig. 8 would suffice to rupture the cuticula in the median line. And that is exactly what occurs. That is, the rupture of the integument is not due to pressure but to the pull of muscles after the cuticula has become softened. The rupture occurs always in the same place because there is there a weak spot in the cuticula and special provision for the rupture thereof.

The same sort of structures are found in *C. femorata*, *P. rapæ*, *C. americana* and I suspect in most insects and it is perfectly clear in all of those examined that the rupture of the integument is as described above and in all it occurs at this same place.

Detachment of the Muscles. — The detachment of the muscles does not begin or progress far until after the formation of the exuvial suture and is then accomplished by a few violent contractions aided no doubt by the corrosion of the tendinous portion of the muscle exposed beyond the surface of the hypodermis.

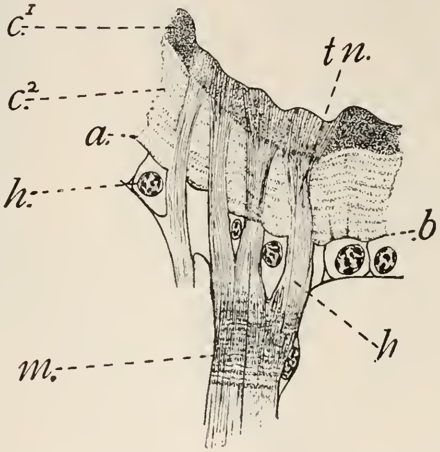

FIG. 10. *C. femorata*. Section of the integument showing the relations of the muscles to the cuticula and the method of the insertion of the muscles. When ecdysis occurs the muscles break from the cuticula along the line *AB*.

The normal insertion of a muscle in insects to the cuticula is shown in Fig. 10 of *C. femorata*, where the modified tendinous ends are seen to go to the very outer surface of the cuticula. This seems to be general for insects, almost identical conditions being shown by Holmgren in Lepidoptera.

When the muscle ruptures the break always occurs along the line *AB* (Fig. 10) level with the surface of the hypodermis, and the long drawn out tendinous ends of each muscle are again incorporated into the new cuticula as it is formed, cuticula being developed between the tendons by specialized hypodermal cells and the tendinous ends themselves becoming chitinous.

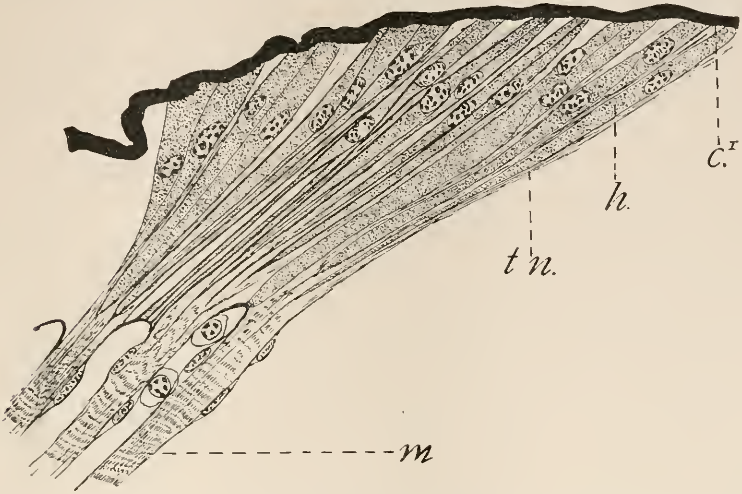

FIG. 11. *C. femorata.* Section to show the relation of the muscle fibers immediately after separation from the old cuticula and after the new cuticula has been partly developed.

These muscle changes, as far as they are related to the hypodermis and cuticula behave exactly in Coleoptera as Holmgren has described for the Lepidoptera and the same behavior probably is true for insects in general.

Cuticula Structures Removed and Replaced at Ecdysis. — Considerable diversity of opinion prevails as to what and how much of the chitin is removed at ecdysis in insects. I find in *L. decimlineata* that ecdysis takes place in the following structures:

1. *Body Wall.* — Cuticula entirely removed, including the chitinous covering of all hairs, spines and scales, sensory pits and sense organs, including chitinous lenses of both simple and compound eyes.

2. *Alimentary Canal.* — Entire cuticula lining of both fore and hind gut, including all cuticular structures in the mouth and the linings of the ducts of all the bucal glands.

3. *Tracheal System.* — All tracheal lining of the main tubes and the smaller trachea, but not of the tracheoles. It is possible that the lining of these latter is not chitinous, or is very soft and does not harden and therefore does not need to be removed.

4. *Internal Supporting Structures.* — Tentorium, apodemes and the lining of all ducts and glands, including the reproductive organs or such as exist.

Conclusion. — The observations and figures which I have given show that ecdysis is a more important and far-reaching process in the development of insects than might at first be supposed. Inadequate as are the observations herein recorded, they show changes comparable to other periodic phenomena in animals, in the existence of the periods of preparation, active ecdysis, reconstruction, and quiescence. We cannot of course conclude from this that ecdysis is in any way related to other periodic phenomena in animals, but rather that there exists in all a similarity, due in all probability to the existence of deep-seated laws of growth which control all of these phenomena.

All Arthropods and especially insects cannot grow or further develop without the process of ecdysis, owing to the hard resistant non-extensible chitinous outer portion of the integument. Among insects ecdysis bears a direct relation as far as the specialization of the process is concerned, to the degree of specialization of any particular groups. Thus, in the lower forms like the *Campodea*, according to Grassi, there is a single ecdysis, but this is fragmentary. The integument is shed in small pieces, while in the Colembola the chitin is shed in bits throughout life. A species of *Peripatus* found in the rain forests of Vera Cruz, Mexico, when about one fourth of an inch long did not shed its cuticula at all during its growth. From these primitive conditions of ecdysis found in the most generalized of tracheates and resembling possibly in this function their supposed annelid ancestors, there exist all degrees of specialization up to the most highly developed condition found in Coleoptera and Lepidoptera.

The specialization of ecdysis which goes hand in hand with the specialization of the integument consists essentially, as I have shown, in the development of a mechanical strengthening of the body wall during ecdysis by the rearrangement of the hypodermal cells in a very precise bridge-like structure; the arrangement for softening and dissolving a part of the cast chitinous covering, thus decreasing resistance; and finally the provision of a sufficient fluid lubricator to enable the animal to slip out of its old covering with the least danger of rupturing the body wall, or distortion of the appendages. In these three provisions are features of great utility which present many striking adaptations to the habits and habitats of individual species.

That ecdysis is a crucial period in the life of insect larvæ is the generally accepted belief, but how crucial is quite an open question. During several years in which I have reared larvæ of *Leptinotarsa* for the study of their evolution I have found that ecdysis is one of the phases on insect breeding needing most careful attention. This is especially true in experiments wherein there are used greatly changed conditions of existence, and the failure of many experiments can be attributed directly to improper care and surroundings during ecdysis. Even under normal conditions each recurring cycle acts to eliminate from one to eight per cent. of the larvæ and the average per cent. of individuals in my experiments with *L. decimlineata*, which cover eleven years, that were killed during ecdysis alone, is 13.69 per cent. Often the percentage runs far higher, frequently lower, but even 13 per cent. of a population is a huge death rate to be directly due to one particular process. The mortality from ecdysis occurs largely as the result of failure to rupture the integument, due primarily to its not becoming softened owing to the failure of the cuticula solvents to develop. The larvæ dying from this cause in insect breeding are often passed over as belonging to the death rate of a certain stage, while in reality it is largely the elimination due directly to ecdysis. Comparatively few larvæ die during ecdysis, but about from 1 to 1.5 per cent. become deformed and die in the following stage. Apparently none are killed during the reconstruction stage, excepting by their enemies. If we knew the whole history of say *L. decimlineata*, I think we should be very near to the truth if we hold that in any given population 15 per cent. are on the average eliminated by the process of ecdysis and the accidents which may occur along with it.

Ecdysis is, in fact, extremely important in the physiological activities of insects, each recurring period being accompanied by great disturbances in all parts and functions of the body besides those described in this paper. Muscles, digestive apparatus and excretory system are deeply influenced by the process, and in some forms at least are the seat of developmental dangers occurring also in rhythmic cycles.

As far as my experience and observations go ecdysis is a peculiar process which periodically reduces various organs and parts of organs to a condition strongly resembling an undifferentiated embryonic condition from which are again built up old or new characters by exceedingly rapid reconstructive processes, each passing through in its development, stages seen in the development of the same or similar earlier larval characters. This repetition of stages in the development of colors or other characters is solely due to the recurrent rhythmic processes necessary to further growth and development. Ecdysis is a process of intense importance in the life of the individual and generation and one upon which natural selection can work most effectively. This preliminary paper suffices to call attention to the importance and deep-seated nature of ecdysis in insect economy, the general features of the internal changes accompanying this process, some of its most striking anatomical changes and its direct selective value in the evolution of insects.

Hull Zoölogical Laboratory,
The University of Chicago,
February 6, 1906.

Vol. X. *April, 1906.* *No. 5*

BIOLOGICAL BULLETIN

TWO TRANSITIONAL STAGES IN THE DEVELOPMENT OF CYCLOPS SIGNATUS, VAR. CORONATUS.

A Preliminary Note.

ESTHER F. BYRNES.

While studying some of the inhabitants of a pool of spring water at Cold Spring Harbor, Long Island, during the months of July and August, I was perplexed by the constant occurrence of two comparatively large *Cyclops* that I was unable to identify as belonging to any known species.

The prevalent adult forms in the pool were *C. signatus* var. *tenuicornis* (*annulicornis*) and var. *coronatus* and *C. serrulatus*. That the forms in question bore no relation to *C. serrulatus* was quite evident. In certain characteristics they agreed with *C. signatus* but for some time I hesitated to associate them with *C. signatus* because of constant correlations in structure, and because the larger of the two forms was occasionally found to be sexually mature.

The smaller of the two cyclops, Plate VII., combined the following characteristics: the antennæ usually contained ten segments, but occasionally nine segments were present; the rami of the swimming feet were two-jointed, with an armature that indicated immaturity as follows:

First Foot.		Second Foot.	
Outer Ramus.	Inner Ramus	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	3 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine,	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta.
3 inner setæ,	5 inner setæ,	4 inner setæ,	5 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	Inner Ramus.	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	3 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta.
4 inner setæ,	4 inner setæ,	4 inner setæ,	3 inner setæ.

The rudimentary fifth foot was two-jointed, having an almost square basal segment bearing an immature seta on its outer distal angle, and a slightly elongated distal segment bearing three hairs.

The larger cyclops, Plate VIII., combined the following characteristics : the antennæ contained eleven segments ; the rami of the swimming feet were three-jointed with an adult armature, as follows :

First Foot.		Second Foot.	
Outer Ramus.	Inner Ramus.	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	3 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine,	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta.
3 inner setæ,	3 inner setæ,	4 inner setæ,	3 inner setæ.

Third Foot.		Fourth Foot.	
Outer Ramus.	Inner Ramus	Outer Ramus.	Inner Ramus.
3 outer spines,	1 outer seta,	2 outer spines,	1 outer seta.
1 apical spine,	1 apical spine,	1 apical spine,	1 apical spine.
1 apical seta,	1 apical seta,	1 apical seta,	1 apical seta?
4 inner setæ,	3 inner setæ,	4 inner setæ,	2 inner setæ.

The rudimentary fifth foot was two-jointed with an almost square basal segment, bearing an immature seta externally, and a distal segment, longer than broad, armed with two immature spines and a median seta.

The study of a large number of these forms showed that both forms were always found together ; that they always accompanied *C. signatus*, var. *coronatus*, having seventeen antennal segments ; that in the absence of strong color markings and hairs they gave evidence of being young ; and that they were, as a rule, sexually immature, although the larger cyclops occasionally contained ova. I became convinced of their larval character, as well as of their relationship to *C. signatus*, and determined to test the correctness of the conclusion by breeding experiments. Accordingly, a few fertile females with appended embryos, were isolated in a carefully prepared aquarium. At the end of two months this aquarium was examined for the desired forms, when numerous individuals in both stages were found to be present. Later, one cyclops with eleven antennal segments was found with numerous large ova in the ovaries.

The rearing of the young from the adults proves conclusively that the forms in question are transitional stages in the life history of a form with seventeen segments in the antennæ. The experiments show also that *C. signatus* may become sexually mature in the larval state.

That this sexually mature young was a true larva, and not a form in which the growth of only the antennæ had been retarded, is indicated by the relatively small size of the individual when compared with the adults, by the incomplete number of segments seen in the antennæ, and by the transparency of the skin and the general absence of hairs and spines.

In the formation of the antennal segments there is a constant sequence. The nine-jointed antenna becomes transformed into the ten-jointed antenna by the division of the third segment from the base into two small ones, and the eleven-jointed condition arises from the ten-jointed, by the formation of a short second segment, the permanent second segment of the seventeen-jointed antenna. Occasionally the antennæ contain twelve segments, but intermediate conditions between the eleven and the seventeen-jointed stages are not frequent. In *Signatus*, prior to the completion of segmentation in the antennæ, two greatly elongated segments (the seventh and eighth in the eleven-jointed stage) break up simultaneously into the required number of small segments, four and three respectively, while the third segment breaks into two, thus providing seventeen segments, the number in the adult.

In a former paper, " Heterogeny and Variation in Some of the Copepoda of Long Island," I was of opinion that " some facts point to the probability that the Cold Spring Harbor forms . . . are morphologically undeveloped." I am now convinced that those cyclops combining nine, ten and eleven joints in the antennæ, with a two-jointed fifth foot, having a distal segment armed with three undeveloped hairs, are transitional stages in the life history of *C. signatus*, although the variety is less easily determined.

The study of various larval stages of *C. signatus* shows the rudimentary fifth foot to be the most characteristic organ by which the species can be easily identified even in very early stages. Whether the hairs on the fifth foot are setose or spiny depends in large measure on the age of the individual, and is of little importance.

There are two generally recognized varieties of the species *C. signatus*, namely, var. *coronatus* (*C. fuscus* of Jurine) and var. *tenuicornis* or *annulicornis*, called *C. albidus* by Professor Marsh.

Both of these forms occur side by side and are regarded by some authors, Herrick and Brady, as transitional stages of one and the same form, while other authors regard them as distinct varieties.

I do not wish at this time to express an opinion on the relationship of *C. coronatus* and *C. tenuicornis*, but the correlations indicated in this paper for *C. signatus*, var. *coronatus*, can also be observed in the forms described as *C. signatus*, var. *tenuicornis*, in which the hairs on the inner margins of the stylets are lacking, as well as the serrations in the hyaline plate in the sixteenth and seventeenth antennal segments ; and in which the second segment of the antennule, as well as the basal segment of the rudimentary fifth foot, are relatively long, while in *coronatus* they are conspicuously short.

The armature of the fourth pair of swimming feet, often shown in keys to the *Cyclops*, is by itself of little value as a means of identification, since several distinct species agree in the armature of this appendage, although they have no agreement in any of the other swimming feet, nor in the larger outlines of the body. It is to be regretted that fuller details of the *Cyclops* are not given as a means of securing a rapid acquaintance with common forms, and with their later transitional stages, which occur in great abundance side by side with the adults, and, like the adults, are sometimes found with eggs.

Cold Spring Harbor,
Long Island, N. Y., July, 1905.

PLATE VII.

C. signatus, coronatus.

Leitz camera drawings. Reduced one half.

FIG. 1. An outline showing the proportions of the thorax, abdomen, and antennæ when there are but ten segments in the antennæ. 3 × 3.

The abdomen has but three undifferentiated segments, to the last of which are attached the characteristic caudal stylets.

The antennæ reach to the posterior edge of the second thoracic segment, and contain ten segments each. Only the right antennule is represented.

FIG. 2. The external ramus of the fourth swimming feet. All the rami of the swimming feet are two-jointed. The proximal seta of the inner margin is conspicuously separated from the remaining setæ, which become the inner armature of the terminal segment after the formation of the third joint. 3 × 7.

FIG. 3. The rudimentary fifth foot is characteristic of the species *C. signatus*. The hairs are neither setose nor spiny. 3 × 7.

The internal fringe of hairs on the caudal stylets, the short, broad, basal segment of the fifth foot, and the short second joint of the antennule indicate that this is the larval form of *C. signatus coronatus*.

FIG. 4. An antenna with nine segments.

FIG. 5. An antenna showing the third segment dividing to form the tenth segment.

FIG. 6. An antenna with ten segments fully formed.

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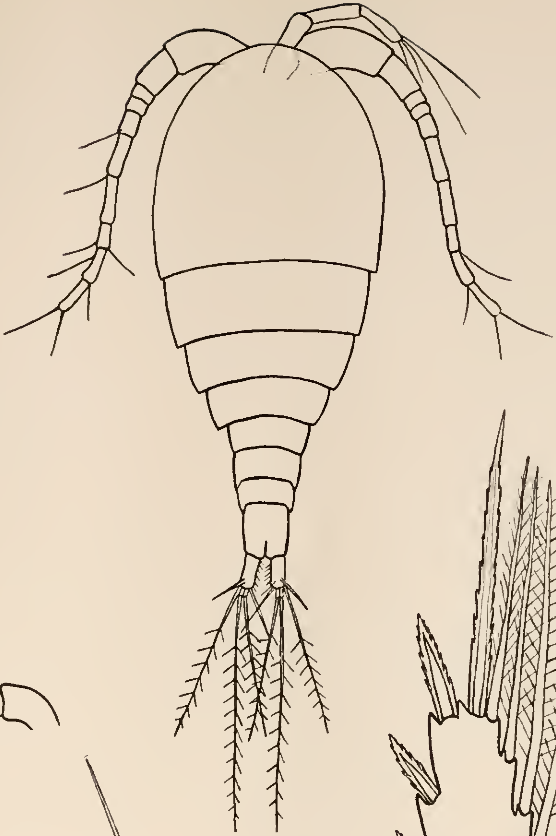

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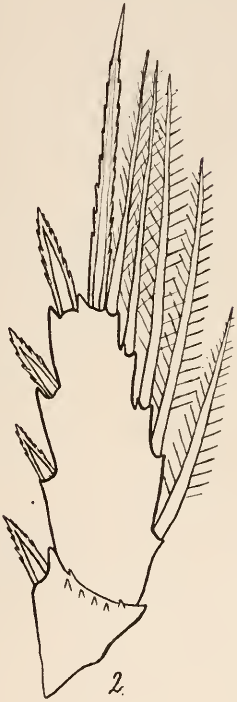

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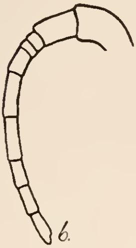

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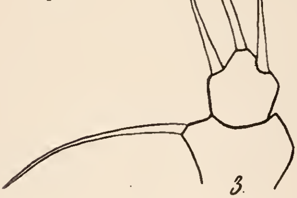

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Plate VIII.

C. signatus, coronatus.

Leitz camera drawings. Reduced one half.

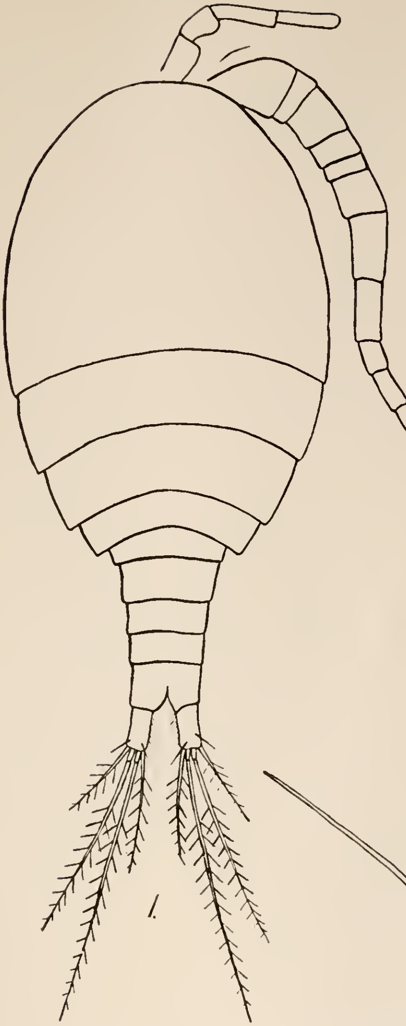
Fig. 1. An outline showing the proportions of the thorax, abdomen and antennæ when the antennæ contain eleven segments. 3 × 3.

The abdomen has acquired four segments in which there is still little differentiation. The caudal stylets remain unchanged.

The antennæ have acquired a new segment, the small second segment from the base of the antenna, making eleven in all. Only the right antenna is represented.

Fig. 2. The external ramus of the fourth swimming feet. All the rami of the swimming feet are three-jointed, and in their armature, have reached the condition of the adult. 3 × 7.

Fig. 3. The rudimentary fifth foot remains practically unchanged. 3 × 7.



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THE MALE AND FEMALE EGGS OF PHYLLOXERANS OF THE HICKORIES.

T. H. MORGAN.

The predetermination of sex in the egg has been demonstrated only where small male and large female eggs occur, and it is a very striking fact, so far overlooked I believe, that in these cases the difference in the male and female eggs is connected with the development of degenerate males. In *Dinophilus apatris* the male is smaller than the female, is degenerate, and the sexual organs appear to be precociously developed. In *Hydatina senta* the male is smaller than the female and degenerate. In the *Phylloxera* of the grape and of the hickories the male is small, wingless, without digestive tract, and the sexual organs, as I shall show for one species at least, develop very early, so that the spermatozoa are fully formed before the male leaves the egg.

In *Dionphilus* it is believed that both the male and the female eggs are fertilized, but in *Hydatina* and in the phylloxerans the male and the female eggs are not fertilized. Sex in these cases is, therefore, determined independently of fertilization and preexists in the egg. In cases of this sort it would obviously be of great interest to discover what conditions determine that some eggs become males and others females. Is the difference, for example, connected with a visible difference of the nucleus, or of the cytoplasm? This question I believe I am able to answer, but the deeper-lying problem as to the causes that lead to the difference observable in the egg I have not fathomed.

The material for study was collected from the galls of the hickories in the spring and summer of last year, and inclúded five or six species, of which three only will be mentioned here. The typical life history of these phylloxerans is the following: The fertilized egg, attached to the bark, hatches in the spring producing the stem-mother, who migrates to the young leaves and attaches herself to one spot on the under surface of the leaf, which becomes the center for the formation of a gall within which she becomes enclosed. She lays a large number of eggs inside the gall that are all of one size, and give rise to the individuals of the second generation, which, developing wings in most species, ultimately leave the gall. These migrants contain two kinds of eggs, larger eggs that give rise to females, and smaller eggs that give rise to males. The eggs are deposited upon the bark of the hickory. From them the male and female individuals are hatched. These soon pair and the female lays her single, large, winter egg on the bark of the tree. This egg hatches in the following spring, and produces the stem-mother.

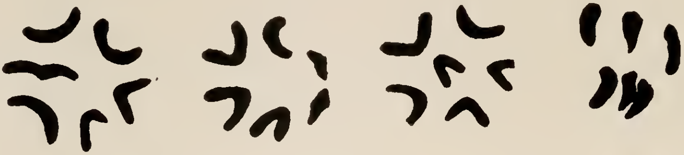

FIG. 1. *Phylloxera globosum.* Polar spindles of male and female eggs. The first figure (to the left) is from a large female egg; the second, from a small male egg; the third and fourth figures are from eggs whose size was not determined.

A few species have a somewhat different cycle, and in one of them the male and the female eggs are laid within the gall itself. Owing to this condition a large number of the eggs in all stages of development can readily be collected. I have been fortunate enough to obtain a species of this sort, viz., *Phylloxera* sp. ? and have obtained an abundance of developing male and female eggs. The life history of a species of this sort according to Pergande is as follows: The stem-mother lays eggs that give rise to a generation of wingless forms corresponding to the winged migrants of other species. These wingless individuals contain large and small eggs which they deposit within the gall. From these eggs the minute males and females emerge. In a few cases a winged individual — a migrant — is found with the wingless individuals. It seems probable that the wingless condition is the secondary one, hence the occasional appearance of winged forms.

The Male and the Female Eggs of the Migrants. — The eggs are mature in the migrant before it leaves the gall, and the polar spindle is present. It is a difficult matter to find the spindle, and the chance is also small of getting one cut parallel to its equatorial plate. Nevertheless, I have found a fair number of such cases in which the number of the chromosomes could be counted with perfect accuracy. In *Phylloxera globosum* there are only six chromosomes in the polar spindle (Fig. 1), which makes it a very favorable object for study. In all eggs in which the chromosomes could be counted the same number was found, and this is true both for male and for female eggs. In this respect there seems to be no difference between the two kinds of eggs.

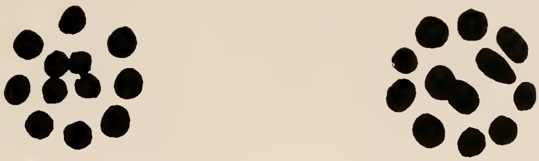

FIG. 2. *Phylloxera* sp.? Left hand figure is a spermatogonial equatorial plate. Right hand figure is from a somatic cell of a male embryo.

In *Phylloxera* sp.? the number of chromosomes in the male and female eggs seems to be twelve (Fig. 2). It is more difficult to count so many chromosomes with accuracy, at least in the eggs that I have so far seen; but as the clearest cases observed showed twelve chromosomes, and as in other eggs eleven, or twelve, or thirteen seemed to be present, and since the number of chromosomes in the spermatocytes is definitely six (Fig. 3), there can be little doubt that the number in the polar spindle is twelve. In *Phylloxera caryæ-globuli* there are twenty-

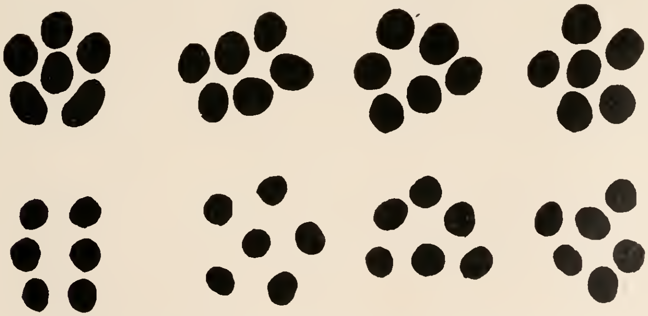

FIG. 3. *Phylloxera* sp.? Spermatocyte divisions. Equatorial plates. Upper row, first spermatocyte divisions; lower row, second divisions.

two chromosomes present of very different sizes, as seen in the figures (Fig. 4).

In passing, it is worth while calling attention to the very different number of chromosomes found in these species of the same genus; species in fact, that are so similar that they can only be distinguished with great difficulty. It does not seem probable in the light of cases such as these that the absolute number of the chromosomes can be a matter of any special significance. If the chromosomes are all composed of the same identical substance it is difficult to account for their constancy in number and sizes in each species. If the chromosomes are different in composition, as the conditions just mentioned would seem to indicate, the differences can scarcely be associated with differences in the structures of the body, since closely similar individuals are produced in one case with six chromosomes and in the other with twenty-two chromosomes. This question is one of the most puzzling problems in the whole range of cytology at the present time, and it would be unwise to draw any conclusion from the meagre facts known to us. I wish only to indicate as a possible view that the chromosomes may be different chemically from each other, and yet at the same time this difference may have no connection with differences in the form of the body.

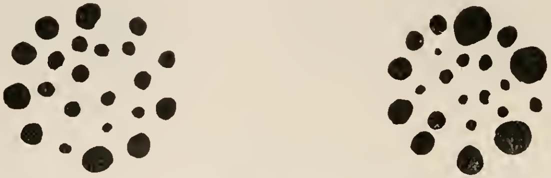

FIG. 4. *Phylloxera caryae-globuli.* Polar spindle-equatorial plate of egg of migrants.

Further History of the Chromosomes. — Starting with the stem-mother the history of the chromosomes through the life cycle is as follows: The number of chromosomes present in the polar spindle of the eggs laid by the stem-mother is the somatic or whole number. The number in the somatic cells of the embryo that develops from this egg is also the whole number. The number in the polar spindle of the male and the female eggs is, as has been stated above, the whole number ; and this is true for the somatic cells of both male and female embryos. In the spermatogonial cells the whole number of chromosomes is present, but in the two following spermatocyte divisions the half, or reduced number, occurs. I have seen the spindle in one winter egg, which seems to have only the half-number of chromosomes. It is evident, therefore, that the complete number of chromosomes is characteristic both for somatic and germinal cells throughout the life cycle, except for the winter egg and the spermatocytes, where the reduced number occurs preparatory to fertilization. After fertilization the number of the chromosomes would be the same as the whole number. Thus it is evident that there is no reduction in the number of the chromosomes in the parthenogenetic egg, but there is in the winter egg. The former is not, the latter is, fertilized. The results for *Phylloxera* are in these regards parallel to those that Stevens has recently obtained in the aphids.

The Cytoplasm of the Male and Female Eggs. — Since no discernible difference was detected between the chromosomes of the small male and the larger female eggs, I examined with some care the cytoplasm of these two kinds of eggs. In the male eggs there is less yolk and the center of the eggs is occupied by a clear mass of cytoplasm, while in the female egg there is more yolk and no central cytoplasm. I have not observed any other differences, and even those just noted can not be made out with certainty for all eggs, but represent the extremes of the series. Double and triple stains gave no differences that I could detect. In the ovaries the eggs showed no differences. In *Dinophilus*, according to Richard Hertwig, the large female eggs are formed by the union of several cells. It would be difficult to detect such a difference if it existed in the case of *Phylloxera*, since the ovarian eggs appear to be fused together at their inner ends, and each egg as it leaves the ovary remains attached by a cord of protoplasm to the fused center of the egg-mass.

There are striking differences in the development of the male and the female embryos, and these I have followed in some detail, but can not present the results at this time. One important difference must however be alluded to, namely, the precocious development of the relatively enormous reproductive organs of the male. The testis is present as a large mass of cells at the time when the blastoderm is first laid down, and the spermatogonial divisions occur at this time. The two spermatocyte divisions occur when the embryonic plate is forming, and before the fibers appear in the nervous system. The size of the mass of spermatocyte cells is very large compared to the rest of the embryo, and it fills the greater part of one half of the egg. The spermatozoa are fully formed at the time when the embryo is developed.

The precocious development of the large testicular mass in the male egg suggests that a preëxisting mass of cytoplasm from which the testis develops may be present in the egg. The central mass of cytoplasm might supply such material, but I have not been able to make out any connection between the two. The result is due rather to the more rapid development of certain embryonic cells than to the presence of any large cytoplasmic mass of preformed material. The question arises whether we are to regard the large eggs that produce the females as doing so simply because they are large, or are they large because they are already female eggs? The latter alternative seems the more probable. In the light of certain recent experimental results, more especially those of Driesch and of Godlewski it seems highly probable that the *early* development of the embryo is due almost entirely to cytoplasmic influences. If this is true also in the case of the eggs of *Phylloxera*, then I think we may safely ascribe the difference in the size of the male and female eggs to the difference in the kinds of cytoplasm that are present when the egg is fully formed, so that the immediate determination of the sex is a cytoplasmic phenomenon. Whether this cytoplasmic difference can be traced to a preëxisting cytoplasmic basis, or to nuclear influence, or to the influence of external conditions is quite unknown, but in the absence of any nuclear differences it seem questionable whether we should assume that such exists.

THE ORGANIZATION AND EARLY DEVELOPMENT OF THE EGG OF CLAVA LEPTOSTYLA AG.

CHAS. W. HARGITT.[1]

A recent paper by Harm ('02), on the development of *Clava squamata* describes several features so unlike conditions which I have found in the American species, that it has seemed worth while to study anew, and in some detail, the entire life history, including also some interesting peculiarities of the organization of the egg not hitherto given any degree of attention among cœlenterates.

The general facts concerning the morphology, habits, etc., of *Clava leptostyla* Ag. were described long ago by Professor Agassiz ('62) with sufficient fulness to obviate the necessity of any considerable details as to these points. Allman ('71) has likewise given a somewhat similar account of *C. squamata*, though lacking in embryological details.

The following account is based upon studies carried on at Woods Holl during some two summers, chiefly upon living material, though for the details of cytology specimens were killed by several standard methods, among which were corrosive-acetic acid, picro-acetic, Carnoy's solution, Petrunkevitch solution, and corrosive formalin. Upon the whole, the first two of the reagents named gave by far the better fixation. As will be noted in another connection, for certain cytological results the picro-acetic solution was exceptionally good.

The most common habitat of the species in this vicinity was found to be the fronds of the common rock-weed, *Fucus nodosus*. Occasionally I have taken it from the piles of docks, or similar situations. It occurs most frequently in shallower harbors, or tide-pools, and is frequently found at low tide quite exposed, except as the moist vegetation may afford a measure of protection, lying thus for hours subject to the desiccating influences of sun and air, and apparently without serious injury. Of course, this

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[1] Contributions from the Zoölogical Laboratory, Syracuse University.

vicissitude is common to many organisms of similar habitat, yet few are so little protected by some skeletal feature as is this wholly naked hydroid. The breeding season at Woods Holl is apparently during June and early July, though colonies may be found in seemingly thriving conditions both earlier and later. Whether *Clava* like *Pennaria*, *Eudendrium* and others, has its phases of decline and recovery I have not been able to determine from actual observation, though the fact that at recurring intervals it appears in the same locations would seem to support this view.

Origin and Growth of the Eggs.

Weismann ('83) was the first to give any direct attention to this problem in *Clava*. According to him the ova arise in the entoderm in close relation to the supporting layer, though suggesting that perhaps the most primitive stages may have an ectodermal origin.

On this point Harm ('02) is quite specific in claiming their origin in the ectoderm, and their later migration into the entoderm, where in the region of the gonophores they become definitely differentiated and complete their development. This author even goes so far as to suggest that they may be distinguished in the planula as primitive germ cells, — "urkeimzellen" (p. 47, Fig. 48).

Of course, I am not prepared to discuss the matter so far as it relates to *C. squamata*, not having seen this species, but so far as it concerns *C. leptostyla* I have no hesitation in saying that eggs probably never arise in the ectoderm, but always in the entoderm of the peduncle of the gonophore, or in that of the polyp very near the base of the gonophore. In thousands of sections studied, both by myself and by several of my students, there has been no exception to this statement of fact. *Clava*, like other hydroids, has its breeding season, during which the germ-cells are extremely abundant, and at other times these cells are either entirely absent or very scarce. Again they do not appear except in direct relation to the forming gonophores or in that immediate region. In fact in *Clava leptostyla* the morphology of the gonophores and their development as dense, bud-like clusters from a single peduncle to which they are attached by narrow pedicels, make it almost certain that the vast majority of all egg-cells arise in the gonophores themselves as, of course, is the case in almost all free gonophores, or medusæ, and as is invariably the case with the spermatozoa.

Therefore, while it may not be impossible that " urkeimzellen " should perhaps exist in undifferentiated stages, still the probability is so extremely remote as to render doubtful to a degree any but the most thoroughly substantiated claims.

It may be stated in passing, that the gonophores of *C. leptostyla* are extremely degenerate, hardly more, indeed, than sporosacs, yet it is possible to distinguish rudiments of medusoid structures. They originate as buds of the hydroid, involving both ectoderm and entoderm, and also supporting layer. Occasionally this lamella seems to partially disintegrate at the terminal portion during the outgrowth of the peduncle. The gonophores remain entirely closed except at the time of rupture by the escaping planula. From the primary peduncle secondary pedicels arise, forming a racemose-like cluster, within each of which from one to four eggs may develop, though the usual number is two or three.

There seems to be considerable variation in the size of the eggs in various specimens and in the eggs of various gonophores of the same specimen. On this point it had occurred to me that perhaps the number developing in a given gonophore might naturally have some influence, but after comparing a considerable number, whether growing singly or in clusters I have not been able to convince myself that such is the case. I am rather inclined to believe that more depends upon the start a given egg may get in growth, and perhaps the state of nutrition in which the given specimen may be at the time, than any other factors.

As a rule eggs which grow singly at the distal end of a gonophore are more nearly spherical, and in consequence I find the cleavage of such eggs much more regular and symmetrical than in cases where two or more are found in the same gonophore and approach maturity at about the same time. This point may be considered in more detail in a later connection under the subject of cleavage.

Growth takes place, as in most hydroids, quite rapidly. With the origin of the gonophores the eggs appear in considerable numbers in the entoderm of the peduncle, as previously stated, and with the later budding of the gonophore bodies either migrate into them, or as seems to be the case in many instances, originate directly from the entoderm of the spadix or lateral walls of the gonophore. At first they have the characteristic aspects of ordinary ovocytes, namely, a very large germinal vesicle, with characteristic chromatin network, a comparatively small proportion of cytoplasm, which is more or less homogeneous in texture, and staining quite uniformly with any of the ordinary plasma stains. Evidences of growth are first indicated by the rapid increase in the mass of the cytoplasm, while that of the nucleus for a time remains apparently unchanged, though later also increasing in mass likewise, though to a much less degree. Nutrition of the ova is at first, indeed throughout so far as I can determine, by direct absorption from the entoderm of the spadix, or to a less degree also from the entoderm of the lateral walls of the gonophore. I find no evidence of the absorption of supernumerary ovocytes involved in the matter of nutrition, and in this respect *C. leptostyla* appears to differ somewhat from *C. squamata*. According to Harm there would seem to be involved both these processes. He says that by the direct assimilation of yolk-like granules from the entoderm cells of the hydranth, and by osmosis from the walls of the gonophore the egg is nourished, and that furthermore, the youngish egg-cells are also nourished by the absorption of ovocytes (*op. cit.*, pp. 11, 12).

The presence of the yolk-like particles to which he refers I have also recognized in the entoderm of the hydranth body at this period. They resemble in all essentials the pigmented yolk granules later found in the fully grown egg, but I have found no evidence that they are ever directly absorbed by the young egg. On the other hand there is ample evidence to the effect that they are gradually broken down and probably liquefied, in which condition they may be easily transferred to the gonophores and absorbed by the young eggs. In an earlier paper ('04) I have directed attention to similar phenomena in the growth of the eggs of Pachycordyle, and it undoubtedly occurs in many others.

HERMAPHRODITISM.

An interesting feature in the reproduction of this hydroid is the fact that occasionally individuals, and perhaps colonies, are found in which gonophores contain both eggs and spermatozoa. Figs. 1, 2; Fig. 5, Pl. IX., show various phases of this somewhat anomalous condition. As will be observed, the elements are in various stages of development, some of the eggs well along toward full growth, and spermatozoa likewise well advanced. In most cases the condition shown at Fig. 5, Pl. IX., was the prevalent one, namely, where one half of the gonophore bore sperms and the other half an egg. In a few cases, however, a well developed egg was found on each side nearly or quite surrounded by sperm-cells.

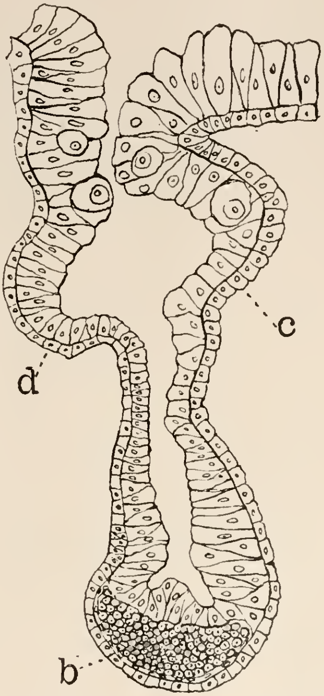


FIG. 1. Longitudinal section through young gonophore, showing at *b* the development of spermary, at *c* and *d* mixed gonads.

Of course, hermaphroditism in itself is nothing strange among animals, whether high or low. Even among hydroids it is quite familiar in the common *Hydra*, though here it is not common to find both organs in active function at the same time on any given individual. I have also found a similar condition in *Hydractinia*, and Bunting ('94), has likewise figured a single case though without giving any details concerning it. In the whole of his extended researches on the "Origin of Sex Cells in Hydromedusæ" Weismann makes no mention, so far as I have observed, concerning hermaphroditism. It would seem somewhat remarkable that he should not have observed some indications of such a condition if it were at all common. Indeed, though having found repeated cases of it in *Clava*, I am disposed to consider it as a rather rare phenomenon in this group.

One other feature in connection with the subject must be noted, namely, that among the several cases, the ova were found in every case in distinctly male gonophores. Among hundreds of female gonophores examined there was not the slightest evidence of male elements among them. Figs. 1 and 2 show sections through two hermaphroditic gonophores. At *b* Fig. 1 is developing a typical male gonad, while at *c* and *d* are shown what are evidently destined to be mixed gonads. Similar conditions are also shown in Fig. 2, which is a camera sketch of a section across the entire hydranth and gonophores.

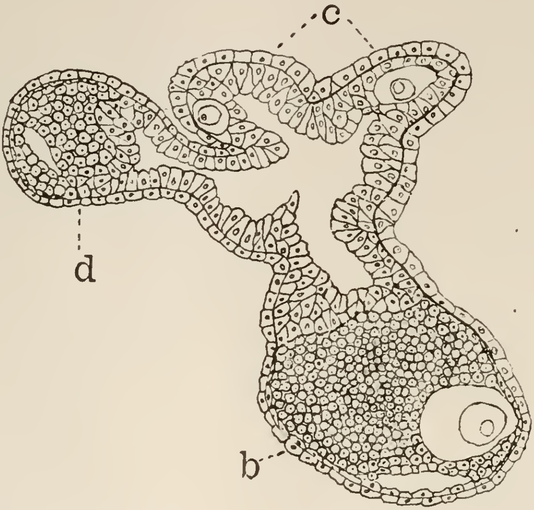


FIG. 2. Cross-section of hydranth showing development of gonophores. *b*, hermaphrodite gonophore ; *c*, early stages of same ; *d*, spermary.

It has long been known that among actinians a form of hermaphroditism, involving successive sexual rhythms, known as protandry or protogyny, occurs. Duerdon ('04), in his studies of West Indian corals has found warrant for believing that protogyny is the predominant condition, since "spermaries have never been found alone, but always associated with large numbers of ova ; on the other hand, many polyps have been found with ova alone, often few in number, as if sexual maturity were but beginning." At the same time he quotes from Mr. Stanley Gardiner, who having studied a large number of developmental stages in *Flabel-*

lum rubrum, has been able to show that in this form spermaries arise first on the mesenteries and that ova appear later, when the production of sperm acini ceases. "The ova grow enormously with the final result that the mass becomes entirely female, consisting usually of two or three large ova, flattened on their sides against one another and occupying the whole area of the former testes."

Hermaphroditism is also known among Scyphomedusæ, Wright having described in some detail the chief features in the case of *Chrysaora hyoscella*. He says, "Large individuals are hermaphroditic, but smaller ones are found which are unisexual, the male or female element being suppressed. Small Chrysaoras (about four inches in diameter), have no ovarian bands in their pouches, which only contain masses of the grape-like bodies (testes), and tentacles before mentioned."

This would seem to imply that here again we have protandrous hermaphroditism, the spermaries developing first, and later the ovaries. Incidentally, it may be observed in passing, that this author in his account of the development of the eggs of this medusa was much impressed with the absence of any germinal vesicle. "The ova of *Chrysaora hyoscella* do not present, at any stage, a trace of the germinal vesicle, — objects which are so readily detected in the ova of other polypoid Zoophytes."

Haeckel ('79), who has also studied the development of the medusa, was able to confirm Wright's account as to hermaphroditism. "In Uebereinstimmung mit letzteren habe ich gefunden, dass junge Chrysaoren rein männlich vorkommen, solche mittleren Alters meisters Hermaphroditen sind, und endlich ganz alte Thiere meisters nur weiblich sind, oft noch mit Ueberresten männlicher Organe."

So far as I have been able to discern in the case of *Clava*, there is no evidence to indicate the operation of either of these oscillating phases of sexualism. I have specimens taken at all times of the breeding season and have found no tendency toward the one or the other. I am rather disposed to regard it as an expression of a mutative impulse, in response to which in other forms, such as those already cited, these interesting features became established.

Organization of the Egg.

It has long been known that simple though the egg may be it must, nevertheless, be regarded as potentially highly complex. Concerning the early views of His, Whitman, Flemming and other earlier investigators, no attempt will be made here to give special citations. The later experiments of Roux, on the development of the frog's egg, supplemented by similar experiments by Driesch '95, served to emphasize still further the general view here stated.

The still later investigations of Wilson ('03, '04), and Conklin ('05^1, '05^2, '05^3), have given a new impulse to researches along this line, and have clarified and measurably harmonized the conflicting views of earlier observers.

So far as I am aware, no one has shown any evidence of anything of a similar character in the organization of the eggs of cœlenterates. In commenting on the account given by the present writer of the cleavage of the egg of *Pennaria* Conklin ('05^3), has suggested the probability that predetermining factors must be present in some form. "Even in such eggs as that of *Pennaria* it is certain there must be determining factors somewhere, if not in the cytoplasm then in the nucleus, which determines that the egg shall develop into a pennaria rather than into some other animal; it is further evident that these determining factors must be present in the cytoplasm at a relatively early stage, if not in the very beginning of development" (p. 215).

Hence in my studies on hydroid eggs rather particular attention was given to this point with the hope that some evidence might be found for or against the views in question. In my work on *Pennaria* ('04^3), while no direct evidence of cytoplasmic differentiation was found in the living egg, there was found in some cases after fixation by certain reagents, more particularly the picro-sulfuric solutions, what seemed to be a stratified, or concentric arrangement of the cytoplasm. At first I was inclined to regard this as probably significant of such locally differentiated matter as might go to form the ectoderm. But since this condition was not found to be constant it was regarded as probably an artifact, due to the action of the reagent. A recent examination of my earlier preparations has not suggested any change of this opinion. Indeed, as pointed out before, the extremely erratic and anomalous behavior of these eggs during cleavage would seem to render extremely difficult, if not impossible, any predetermining factors in either cytoplasm or nucleus, whose influence could be maintained during the varying and indeterminate process of development. While we may readily admit that, as Conklin has suggested, there must be factors present which determine that the egg shall develop into a *Pennaria*, still this does not compel the conclusion that therefore they must be definitely localized. It is simply a matter of heredity ; and if it be true as generally contended that this is a problem of chromosomes ; and if, as I have shown in the case of both *Pennaria* and *Eudendrium*, the chromatin may be more or less dispersed throughout the entire cytoplasm during maturation and early cleavage, then definite localization in one or the other is not involved in Conklin's sense of the term. However, it must be regarded as a question of fact, and so far as evidence exists in the present case it would seem to be opposed to the theory of localization.

During June and July of the past summer I carefully studied the living eggs of *Clava*, and with this point still clearly before me. As pointed out in an earlier section, the eggs originate in the entoderm of the gonophore, and grow by direct nutrition derived from the cells of that tissue. A study of the eggs in various stages of growth revealed the appearance at a certain stage of development of a delicate, bluish pigment, which gradually accumulated in amount as the eggs approached maturity. This was carefully observed in the living specimens and has since been studied in sections after a variety of fixations. At first the pigment makes its appearance in the immediate region of the nucleus, about the time that body takes its place at the outer periphery of the egg. This is shown in Fig. 1, Plate I. From the nuclear region the pigment extends as a crescentic disc outward, forming later a peripheral zone which finally extends over the entire egg, though this rarely occurs until cleavage has made some progress.

An interesting fact observed in this connection was that the amount, or at any rate the color-intensity, of the pigment differed considerably in different specimens. This was particularly the case in colonies which had been kept in the aquarium for a few days. In these the pigmentation was appreciably less intense than in specimens freshly collected. There were, however, notable exceptions in this respect among various specimens under natural conditions. The same has been observed in *Pennaria*, and is, indeed, a fact more or less well known in many animals. Conklin ('05[1], p. 13) has cited similar cases among ascidians observed by VanBeneden and Julin ; in some species two very differently colored eggs being produced, one yellow, the other gray. Both are said to develop normally and in the same manner, giving rise to larvæ whose entoderm cells are of the same respective colors.

In the case of *Clava* there is no such distinction as this, though the presence of more or less pigment has apparently no effect upon the normal development of the embryo. In another point there is also some measure of similarity, namely, in that as development proceeds, the pigment which was at first distinctively peripheral in position, seems later to become transferred to the entoderm of the larva. This, however, as will be seen later, is due not to any shifting of this matter from one region to another, but simply its resorption by the more rapidly developing ectoderm, while as yet the entoderm is only partially differentiated.

Whether this pigmentary zone formed a definite germinal area, and its gradual development was an expression of the differentiation of this area, or whether it might not be simply the results of cytoplasmic metabolism associated with the formation of yolk substance, or whether it might not, perhaps, be in some way associated with the phenomena of maturation, seemed for a time somewhat uncertain. A study of the matter more critically in its cytological aspects soon sufficed to discredit the last alternative, namely, that it was in any way associated with maturation. Furthermore, the appearance of the pigment was too early in the history of the egg to involve the operation of any maturation phenomena.

Again, several series of facts conspired to discredit the probability of the first alternative, namely, that it was in any sense a differentiation of germinal substance. Of these the following may be particularly mentioned : (1) The entire absence of any correlation between the pigmentary zone and the course of cleavage ; (2) the continuous development of pigment, even after the completion of cleavage, and after the ectoderm has been clearly established ; (3) the staining reactions of the granules after hardening is comparable, point by point, with that of yolk granules ; (4) finally, the granules are gradually reduced and resorbed with the growth of the embryo. It is in this way that their disappearance from the ectoderm above referred to is to be explained.

I think that we may therefore conclude that, at least so far as the problem is concerned with *Clava*, its solution is unquestionably in the negative. If further warrant be needed for this view I believe it will be found in the later history of these pigmentary granules as given in the following account.

Origin and Growth of Pigmentary Granules.

As already noted the origin of these pigmentary granules is in the immediate region of the nucleus, and about the time this body reaches the outer periphery of the egg. At first they are of extremely small size, about 0.5 micra in diameter, later growing and reaching in some cases a diameter of 3 to 3.5 micra. For some time they were entirely overlooked in prepared material, owing to improper fixation. Only after fixation with picro-acetic (or to less degree with Petrunkevitch) solutions and staining with iron-hæmatoxylin on the slide were they adequately differentiated so as to be readily studied. Wilson gives a similar account of this technic in reference to the eggs of *Dentalium* (cf. *Jr. Exp. Zool.*, Vol. I., p. 9, Figs. 10–13, explanations).

As the granules continue to grow larger there may be distinguished within their substance what appear to be vacuoles, usually a single one within each granule, occupying an eccentric position. In some respects they exhibit nucleolar-like features, especially in their staining reactions and in their vacuolation. Montgomery ('98) has made similar observations on nemerteans and believes they indicate some sort of genetic relationships between nucleoli, yolk balls and granules. My own observations have not seemed to confirm this last point, though in the case of *Hydra* there are not lacking evidences which I think would give strong confirmation of Montgomery's views. This point may have further consideration in connection with observations upon the history of the nucleolus.

Concerning the real nature of these granules there arises the query, are they katabolic products, associated with some vital wastes incident to the cytoplasmic activities of growth, or are they not rather anabolic in character, highly nutritive proteid bodies, analogous to yolk matter and of similar import? The latter is by far the more probable view, though there are points of difference as compared with the usual formation of such nutritive matters. For example, in most hydroid eggs which have come under my observation the development of yolk granules has no appreciable relations to nuclear influence, and seems to be for the most part developed and deposited chiefly at the vegetal pole of the egg; while in the present case, as has been shown, they seem to arise and develop chiefly in the nuclear area and only at a late period are found at the vegetal pole. However, I am inclined to believe that this is not a serious difficulty. It will be observed that the vegetal pole lies in immediate contact with the spadix of the gonophore, and that the reception of nutritive matter by the growing egg is from this source. Of course, this nutritive matter is in the form of liquid, and so long as the egg is continuously receiving it in this way there is no occasion for further metabolism into the more solid reserve of yolk. The remoter animal pole where such reserve would first be needed begins the anabolic process first, and with the gradual suppression of the nutritive activities of the spadix the process of proteid anabolism would extend into that area.

According to Harm (*op. cit.*, p. 19), the development of this yolk matter in *C. squamata* presents some rather sharp contrasts as compared with the above. For example, instead of developing more or less gradually, and spreading from the nuclear region over the surface of the egg, he finds it arising somewhat suddenly and equally throughout the entire egg. "Bald nachdem die Eizellen Glockenkern erreicht haben, beginnt in ihnen die Dotterbildung, die an allen Stellen zu gleicher Zeit einsetz." He also finds that before the formation of yolk granules the eggs are reddish in color. "Während die lebende Eizelle vor der Dotterbildung röthlich erscheint, zeigt nacht derselben eine blaugraue Fährbung." This point is confirmed in the case of *C. leptostyla*, in which essentially the same process takes place.

Perhaps a few words may be added as to the significance of the development of pigment in connection with these yolk granules. I have in recent papers ('04[1], '04[2]), submitted certain views as to this subject and it may suffice in general to refer to those discussions. In the former it was said that pigments in organisms might appear under three aspects: (1) Those directly serviceable to the organism, as in chlorophyll, hæmoglobin, etc.; (2) as waste products, which embrace probably the more numerous of organic pigments, such as guanin, melanin, etc.; (3) as reserve products, of which the lipochromes are typical. In all probability the various pigmentary matters found in eggs belong to the third of these classes. And here undoubtedly should be classed the pigmentary granules of *Clava*, and other similar pigments of hydroid ova. In the second paper attention was directed to a special case, that of *Pachycordyle*, already referred to, in which one may trace the various stages in the growth of the egg and the formation of the pigmentary bodies. Here as in *Clava* there can hardly be reasonable doubt that the process is a gradual and progressive anabolism, so far as the granules themselves are concerned, but it must still be a somewhat open question as to the exact relation of pigmentation thereto. May it not be probable that here, as in many of the more active phases of metabolism in which pigments are more or less evident expressions of excretory, or waste products, the pigment itself, though associated with anabolic activities, is an expression of the correlative process of catabolism? In other words, that even in those constructive processes involved in the storage of reserve matters, whether as proteids, fats, or whatever they be, there is involved the inseparable process of energy bestowed, and that as one of the signs of such energy its imprint is left in these pigmentary elements? Such I am inclined to believe is what actually happens. And as the nature of these processes differ more or less in various organisms so the pigmentary signs of waste will likewise differ. Hence the purple pigment of the eggs of *Clava*, the pinkish of *Pennaria*, the reddish of *Eudendrium*, etc.

MATURATION.

Concerning this phase of development there is comparatively little to be said in the case of *Clava*. I have studied as critically as the nature of the egg would allow the behavior involved in maturation both in living and preserved material, and in a very large number of preparations, but with almost wholly negative results. That is, I have found the phenomena to be so obscured by the opacity of the cytoplasm, or by the pigment matter in the yolk, or as seems to me a still further probability, namely, the extremely fugitive character of the phenomena, as to render them indistinguishable. I have had occasion to emphasize this matter in several earlier papers dealing with the subject. The observations made upon the eggs of *Pennaria* have been duplicated, almost point by point in the present case. Of course, in the egg of *Clava* there is the added difficulty that all the phenomena occur within the closed gonophore. In sections, however, this fact ought to offer no serious obstructions to their detection, yet the results, as in the former, are quite as uncertain and in most cases absolutely lacking.

In the case of *Clava squamata*, Harm (*op. cit.*, p. 23) describes the phenomena in some detail, and gives almost diagrammatic drawings of the several stages. However, as will be further shown in connection with the cleavage phases, there are so many points of difference between these species that the ova may perhaps belong to very different classes so far as their character and texture are concerned. From the fact that in several particulars I have been able to confirm the observations of Harm it does not seem probable that they are so greatly different as might be implied.

I shall briefly describe the principal features which it has been possible to certainly determine, leaving others open to further inquiry or study.

As pointed out in an earlier section, about the time the ova approach full size the nuclei are to be found close to the outer periphery as will be seen in several of the accompanying photographs. It will also be observed that the eggs occupy closely the entire space of the gonophore, and that, therefore, the nuclei in coming in contact with the gonophore wall become more or less flattened. It is at this stage that the first indications of maturation becomes apparent, namely, the shrinking and gradual disappearance of the nuclear membrane. This is particularly well shown in Fig. 2, Pl. IX. About the same time, or in some cases slightly before, there is also an evident dissolution of the chromatin network, unaccompanied by any indications of chromosomes. In this respect these eggs closely resemble those of *Eudendrium* and *Pennaria*. The most painstaking attempts to differentiate these bodies by staining operations have as frequently failed. It seems to me we are forced to the conclusion that the appearance of dissolution is indeed a fact, and that at this stage there is a general dissipation of chromatin, and perhaps other nuclear matter, into the cytoplasm.

Behavior of the Nucleolus. — Usually at about this stage marked changes take place in the nucleolus. In several instances it has been found to migrate bodily from the germinal vesicle into the cytoplasm where it is gradually dissipated and probably assimilated. At this point I find my observations closely in accord with those of Harm (*op. cit.*, p. 24). "Wahrend also hier der Nucleolus in toto vor der Polkorperchenbildung aus dem Keimblaschen heraustritt, um vom Eidotter aufgenommen und resorbirt zu werden, verbleibt er in anderen Fallen in demselben und zerfallt dort in mehrere Kugelchen."

Just prior to this migration of the nucleolus into the cytoplasm it was found to show varying degrees of vacuolation, in advanced stages of which it was often seen to partially shrink and collapse, as if there had been a loss of nucleolar substance. Something of this may be observed in several of the photographs already referred to.

Conditions very similar to these I have elsewhere described in connection with other hydroid eggs (*op. cit.*, 04[1], p. 562). Montgomery has cited many similar features which had come under his own observations, as well as observations of a like character made by many others. Those who are particularly concerned will find his discussion exceedingly interesting and suggestive, as well as including a valuable summary of evidence bearing on this problem. I may say, however, in passing that I have failed to find any indications in the present case of the metamorphosis of the vacuolated portions of the nucleolus into yolk granules, such as Montgomery has described. On the other hand the whole of the nucleolar substance seems to be directly dissipated throughout the cytoplasm and indistinguishably assimilated by it.

Polar Bodies.—With the phenomena already described there have been found in a few cases what seemed to be polar bodies. But it was impossible to distinguish the presence of any mitotic mechanism. In every case the nuclear matter was devoid of any trace of chromatin, or, as just suggested, mitotic figures. In a few cases the formation of these bodies was observed in living eggs, and these showed essentially the same features. The nuclear matter being in close contact with the gonophore wall, small rounded portions seemed separated from the larger part, and could be distinguished close under these retaining membranes for only a short time, when they gradually disappeared, apparently resorbed, as I have suggested in former studies upon *Eudendrium* and *Pennaria.* In this respect also my observations correspond with those of Harm, though I have in no case been able to confirm his account of the phenomena of mitosis. "Die Resorption der Polkörperchen durch die eizelle sehr schnell vor sich gehen, da ich dieselben zur Zeit, wenn der weibliche Pronucleus besteht, nicht mehr habe nachweisen können."

The results in the present case, as in those of *Eudendrium* and *Pennaria*, already referred to, as well as in certain others, serve to suggest the query whether, indeed, there may not be great variation as to phenomena of maturation, even perhaps to the extent of the suppression of the more conspicuous aspects associated with it in higher forms. It may be regarded as the wildest biological heresy to even remotely suggest that in some eggs these physical phenomena might be entirely absent, but such an impression has grown upon the present writer for some time and increases with each further case, such as that under consideration. If we may have normal nuclear division without mitosis in the early embryonic history, and this I believe to be fairly beyond doubt; and if we may have differentiation without cleavage, now likewise admittedly true; and if, furthermore, we may have prior to either of these phenomena in development the dissipation of both chromatin and other nuclear matter throughout the cytoplasm, another fact of which I can no longer doubt, then it does not seem a far call to the assumption that the reduction phenomena of maturation may well be accomplished without any of the complex and spectacular processes of mitosis. Why should the egg go through with the physical process of extruding the polar globules if they are to be immediately resorbed by the cytoplasm ?

I do not overlook the circumstance that the opacity, and in the present instance pigmentation, of the egg may establish an obstacle so formidable as to render accurate observation extremely difficult, as I have previously admitted, still the fact remains that carefully preserved and stained sections of hundreds of eggs have failed to afford convincing evidence of the presence of these bodies in distinguishable form, except in doubtful cases already referred to. It would seem somewhat remarkable that there should be all the differential results of staining upon other cell features while it should be uniformly lacking in these, usually among the most readily demonstrated.

Yet another feature bearing on this point will be found in the details of cleavage, during which there is every appearance of spontaneous nuclear reorganization throughout large portions of the egg at almost the same time, the last detail of which may be followed. The facts will be described in a later section, though it seems well to call attention to their significance in relation to the matter under consideration.

Cleavage.

In a general way cleavage in these eggs corresponds with that of other hydroids having similar gonophores, such as *Tubularia*, *Hydractinia*, etc. The earlier work of Ciamician ('79), and Brauer ('91), on *Tubularia mesembryanthemum*, and the later work of Allen ('00), on *T. crocea*, afford good examples of the type of cleavage here referred to. In a recent paper ('04^1), the present writer has briefly reviewed the results of Ciamician and Brauer, and it is unnecessary to discuss here in any detail these features. Suffice it to say that Brauer found what he regarded as two rather distinct types of cleavage. The first more or less regular and equal ; the second irregular and indefinite, involving for a time an internal nuclear proliferation, followed later by the spontaneous division of the cytoplasm into a corresponding number of blastomeres. While my own observations (*op. cit.*) did not fully confirm those of Brauer they abundantly proved the general proposition that cleavage is not uniform, either in mode or progress. The work of Allen (*op. cit.*) showed even more conclusively the variation of this feature in *T. crocea.*

The same thing is true in the eggs of *C. leptostyla.* While there is here much more uniformity than in either of the former cases the range of variation is still very considerable, as a glance at the various figures will abundantly show.

FIGS. 3, 4, 5. Camera sketches of phases of cleavage, from life.

The account of Harm (*op. cit.*, p. 28) concerning cleavage in *C. squamata* is in very marked contrast in its general aspects. Both in his descriptions and figures there is a most striking regularity and definiteness in the cleavage. He very briefly refers to a condition which he considers exceptional and abnormal, but which unless I greatly mistake must be much more common than he is disposed to think. It is quite similar to a condition referred to by Brauer, and regarded by him as likewise exceptional, but which I have showed to be more or less common.

Again, Harm describes in some detail what he regards as a more or less definite rotation of the blastomeres during earlier cleavage, in some measure comparable with phenomena familiar in the eggs of annelids and molluscs. When attention is directed to the fact that in these hydroids the eggs are usually quite closely confined within the closed walls of the gonophore, and that, whether there be two or even more in a single gonad, they are always more or less flattened against each other or against the spadix, and that the entire course of development takes place within these walls it is difficult to see how any considerable movement of the blastomeres upon each other can be possible. Or, furthermore, how it is possible to have anything like the regulation type of cleavage characteristic of the forms just referred to, unless, perchance, the gonophores of *C. squamata* differ very greatly from those of *C. leptostyla*, which hardly seems probable. At any rate, I have not found it possible to trace any close correspondence to such features in our species, as will be seen from the following account.

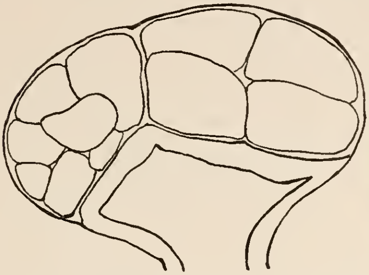


FIG. 6. Camera sketch of gonophore containing two eggs at slightly different stages.

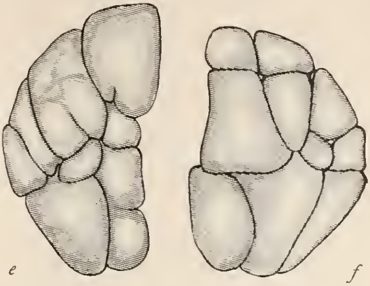


FIG. 7. Camera sketches of two eggs, *e* and *f*, as seen from side.

As stated in an earlier connection, the gonophores of *C. leptostyla* may contain only a single egg or as many as four, though the usual number is two or three. In many cases where but two are found they will be upon opposite sides of the spadix, but frequently also supported side by side at the terminal portion. In either case the eggs are flattened very much on one or more surfaces, as shown in several of the figures (Fig. 7, *e* and *f*). In some cases they may even come to have a biscuit shape, or may be crescentic disks, as also shown in Figs. 3–5. In many cases as the eggs approach full growth there is a tendency to become more or less spherical, especially when occupying singly the terminal portion of the gonophore. Now, I have found that these various conditions have a more or less marked effect upon the mode of cleavage. Where the egg is spherical, for instance, cleavage is usually more or less symmetrical, as shown in Fig. 8. On the other hand, where the conditions maintain a continued pressure the cleavage is very irregular and unequal, as shown in Figs. 6 and 7, drawn from life with the aid of the camera. In Figs. 10 to 18 are shown conditions found in sections of an egg, which are almost exactly equivalent to similar sections obtained in the study of *T. mesembryanthemum* ('04[4]).

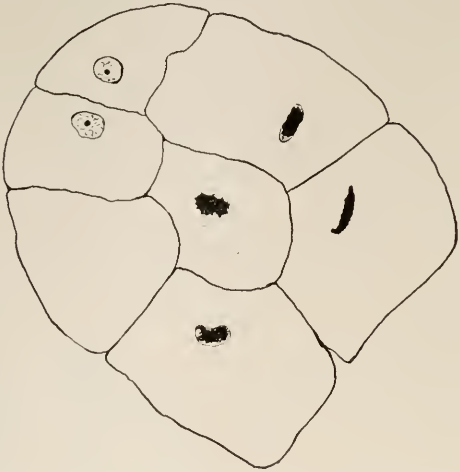
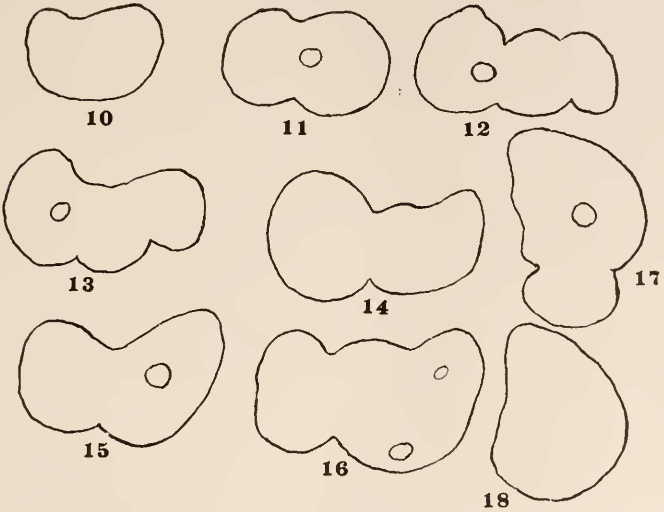


FIG. 8. Sketch of section of egg, showing nearly regular cleavage. In two cells are resting nuclei, and in others various phases of nuclear reorganization.

I think it will be evident, from even a cursory study of the several figures and photographs, that the cleavage in this egg is, like that of *Pennaria*, more or less erratic and indeterminate, and conforms to none of the regular types.

FIG. 9. Sketch of two cells of egg section, showing in one a dumb-bell shaped nucleus in amitotic division.

Another feature of cleavage remains to be considered, namely, one which involves chiefly, perhaps in some cases wholly, the nuclei. In *Eudendrium* and in the species of *Tubularia* referred to above, it has been shown that in a considerable number of eggs there was formed by nuclear proliferation an evident syncytium, and that from this there was later a differentiation of the embryonic tissues without the process of ordinary cleavage. I have found something of the same kind in the case of *Clava*. Occasionally an egg was found among serial sections which showed no evidence of cytoplasmic cleavage, but where internal nuclear proliferation was clearly evident, and the specimens were numerous enough to enable one to definitely determine the phases of tissue differentiation and the formation of the embryo. In these cases were found the same evidences of the origin and organization of nuclei *de novo* which were found in *Eudendrium* and *Pennaria*. It was possible to trace almost every phase of this nuclear organization, from the appearance of the smallest particles of chromatin and their segregation into larger masses to the fully formed resting nucleus with its typical elements in normal relations and proportions.



FIGS. 10–18. Outline sketches of sections of single egg, showing the various distribution of nuclei, shape of egg, etc.

Amitosis and Nuclear Organization. — During the early cleavage, even up to the sixteen-cell stage, no evidence of mitosis has been found. I have already directed attention to its entire absence during maturation also. Now it is interesting to find abundant mitoses during the later cleavage, and during the growth of the embryo. Is it not somewhat remarkable that among the eggs of a given gonophore cluster in various stages of cleavage and later embryonic development there should be found in certain cases abundant mitoses while in others their entire absence? This cannot be attributed, under such circumstances, to differences of fixation or other details of technique, for this has been identical throughout. To what, then, may it be attributed, or how explained? As already suggested in connection with the subject of maturation, it seems to me we are forced to assume the operation of some extremely obscure bio-chemical changes which neutralize alike both acid and basic stains, or that certain phases of the mitotic mechanism may be disguised or actually lacking.

In the light of cumulative evidences along this line I think one may safely assume that the second of these alternatives is the more probable, and the facts here submitted strongly support this conclusion.

During the earlier aspects of development the nuclear phenomena associated with these eggs are extremely indefinite and obscure. During maturation there seems to be an actual fragmentation and dissipation of the nuclear matter throughout the cytoplasm. The first signs of its reorganization appear in the segregation of chromatin-like masses during the early phases of cleavage, about which the cytoplasm becomes organized into cell-like masses, as suggested in an earlier connection. At this time these chromatin masses are extremely irregular in form and size, stain very densely, and appear as indefinite, flocculent patches occupying the center of cytoplasmic aggregates, or cells. They usually assume an elongate, or often dumb-bell shaped form and seem to divide amitotically, followed by the division of the cell. In Fig. 8 is shown a camera drawing of a section of an egg, in which these features are evident. These are very common features during this period of cleavage, and must, it seems to me, be regarded as more or less typical. In two of the cells of Fig. 8 will be observed typical resting nuclei. Whether this is preliminary to the beginning of the later aspects of mitosis I am unable to say, though it seems altogether probable. At any rate, the facts seem clearly to justify the general conclusion that for a time in the early history of the development of this egg nuclear activity differs very greatly from the ordinary forms of mitosis, and appears to involve direct, or amitotic division. During later cleavage abundant mitosis clearly indicates the prevalence of this form of cell division, if, indeed, it may not wholly supersede the other, though its appearance during regenerative activities shows the possibility of its recurrence under various conditions.

I have called attention to similar nuclear phenomena in the development of *Eudendrium* and *Pennaria*, and in the earlier paper ('04^2, p. 267), cited observations of similar sort from several sources. More recently still other cases have come to light, and it seems altogether probable that as facts multiply and attention is focused upon the phenomena cytologists will be forced to take cognizance of this form of cytogeny and give to it something more than a merely incidental place in cellular activities, and assign to it something more than senescent significance.

Among the more recent data bearing upon this point may be cited, first, the observations of Osborn ('04), in connection with the development of *Fasciolaria ;* and second, similar observations by Glaser ('05), on the same organism, which go to substantially confirm the facts noted by Osborn, though with somewhat different phases of interpretation. Still a third series of facts are brought to light by Child ('04) in a paper on "Amitosis in Moniezia," in which he clearly shows the prevalence of this form of cell division in the growth of the reproductive organs and the development of the sexual cells, and expresses the belief that "future investigation will probably show that amitosis is at least as important in the life of the cell as mitosis."

As I have elsewhere pointed out, it is well known that cell division in Protozoa exhibits very different cytological features than do cleavage cells in early ontogeny.

In many, mitosis seems to be entirely lacking, while in most its features are difficult to correlate with the more typical features in metazoa. Just why we should insist upon finding among a class like cœlenterates all the details of cytogenic mechanics more or less familiar in vertebrates or other higher groups of metazoa does not appear to be quite obvious. Moreover, why mitosis should have come to be regarded as absolutely cardinal in biologic faith is likewise uncertain. At any rate, the repeated revisions of creed as to centrosome, chromosomes, prelocalization, etc., should suggest a spirit of tolerance toward facts, whatever their significance.

SYRACUSE UNIVERSITY,
THE ZOÖLOGICAL LABORATORY,
March 1, 1906.

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Explanation of Plate IX.

Fig. 1. Photograph of section of egg under one-twelfth oil immersion, showing the crescent of pigment granules extending peripherally from the nuclear region. This area is very imperfectly shown as compared with the actual condition as seen under the microscope.

Fig. 2. Photograph of section of an egg about the period of beginning maturation. The flattened nucleus at the outer margin is evident, as is also similar change in the nucleolus. There may also be observed the general dissolution of the nucleus. Magnification as in Fig. 1.

Fig. 3. Photograph of section through two eggs in a single gonophore, showing the flattening of the eggs along the line of contact. The cleavage masses may be observed as about the same stages in each egg. While this is often the case, there are exceptions, as shown in some of the text figures.

Fig. 4. Section of egg photographed under one-twelfth oil immersion, showing the more or less syncytial character of the egg at this stage of development.

Fig. 5. Section through a male gonophore, showing on one side the egg, and on the other the mass of spermatozoa, with the body of the spadix occupying the median region of the section.

Fig. 6. Photograph of portion of an embryo about ready to be liberated, showing the entoderm and ectoderm well differentiated, and with cell-like masses of pigmented yolk spheres in the enteron. It may also be observed that the ectoderm is practically free of pigment matter, as pointed out in the text.

I am under obligation to my colleague, Dr. Rogers, for the photo-micrographs illustrating the above features. All were made under the one-twelfth oil immersion lens, with arc light illumination.

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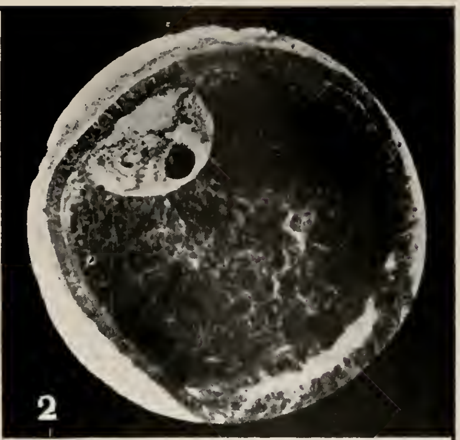

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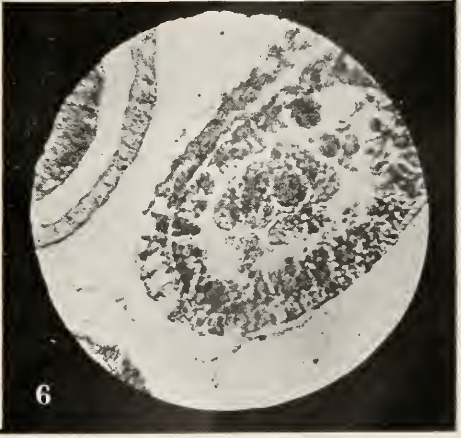

6

REGARDING THE RATE OF GROWTH OF THE AMERICAN LOBSTER.[1]

PHILIP B. HADLEY.

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At the present time when artificial propagation is bidding fair to at least partially check the ever-increasing depletion of many forms of marine animals whose economic value has long sustained a many-sided fishing industry, any facts which may bear directly or indirectly upon the life, habits or development of such forms might seem to be of value. This is especially true of the American lobster (*Homarus americanus*), a knowledge of whose development must influence not only methods of artificial propagation, which is in these days becoming more common, but also state legislation in determining the size and season at which the taking of lobsters shall be allowable.

The Frequency of Molting and the Percentage of Increase.

The rate of growth of a lobster depends primarily upon two factors, the frequency of the molting periods and the amount of increase in length at each molt. To date, the most complete and only satisfactory account of the development of *Homarus* is presented by Herrick[2] who made many observations at Woods Hole on all of the earlier and many of the later stages. Herrick found that young lobsters (stages 2 to 10) in confinement gain from 11 to 15.84 per cent. at each molt, the average in 66 individuals being 13.5 per cent. He assumes that 15.3 per cent. is the average rate of increase both for the young lobsters which grow up in natural environments, and for adults under normal con-

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[1] This paper is presented with the purpose of giving in brief the main facts of a more detailed report on this subject to the Rhode Island Fish Commission. The full account will appear in *The Thirty-Sixth Annual Report of the Rhode Island Commission of Inland Fisheries, 1906.* Reprinted as *Special Paper No. 23, 1906.*

[2] Herrick, *U. S. F. C. Bulletin*, vol. 15, 1895.

ditions. The difference in the above percentages he attributes to the unfavorable conditions of aquarium life. Taking this fact as a basis, and assuming the average length of the first stage lobster to be 7.84 mm., Herrick then constructs the following scheme to show the probable relation between the stage and the size of lobsters from the time of hatching through the thirtieth molt:

Stage.	Length.	Stage.	Length.	Stage.	Length.
1	7.84	11	32.55	21	135.17
2	9.04	12	37.54	22	155.86
3	10.42	13	43.28	23	179.70
4	12.02	14	49.90	24	207.20
5	13.86	15	57.53	25	238.90[1]
6	15.98	16	66.34	26	275.45[2]
7	18.42	17	76.49	27	317.59
8	21.24	18	88.19	28	366.16
9	24.49	19	101.68	29	422.21
10	28.23	20	117.24	30	486.81[3]

[1] 9.5 inches. [2] inches. [3] 19.1 inches.

Regarding the probable *frequency* of molts, Herrick assumes that a lobster molts fourteen to seventeen times during its first year of life, and that in this time it attains a length of two to three inches. From this and other detailed considerations, Herrick finally concludes that a lobster ten and one-half inches long is between four and one-half and five years old, the higher degree of probability being in favor of the lower estimate.

The observations made by the writer and others at the experiment station of the Rhode Island Fish Commission at Wickford, R. I., though differing to some extent from the results obtained by Herrick at Woods Hole, may serve to throw further light on the rate of growth of lobsters in their natural environment, and give some hint as to the conditions which modify it.

The record of the rate of growth of the early stages (one to ten) include observations upon several hundred young lobsters whose definite stage and approximate age was, for the most part, known. Individual records were started immediately after the molt from the third to the fourth stage and were carried on as long as either the weather conditions or the term of life of the young lobsters permitted. In most cases the young individuals were confined in separate compartment cars which furnished a very natural environment, and rendered it easy to make observations at any time. The facts concerning the development of the early stages may be more tangible when presented in the following table :

Wickford Lobsters.

Stage.	Length.	Stage-period.	Per cent. Increase.
1	8.2 mm.	2 days	
2	9.6	4 days	17.0
3	11.4	5 days	19.2
4	13.5	12[1] days	18.6
5	16.0	11[1] days	15.0
6	18.8	12.5 days	19.9
7	22.5	14 days	21.0
8	26.5	15.5 days	17.0
9	32.0	21 days	21.0
10	37.9	25 days	17.0
			Total average 18.3

Woods Hole Lobsters.

Stage.	Length.	Stage-period.	Per cent. Increase.
1	7.84 mm.	1– 5 days	
2	9.20	2– 5 days	17.3
3	11.10	2– 8 days	20.6
4	12.60	10–19 days	13.3
5	14.20	11–18 days	12.7
6	16.10	14 days	13.3
7	18.60		15.5
8	21.03		12.5
9	24.50		16.5
10	28.03		14.0
			Total average 15.3[2]

The foregoing demonstrates the fact that the average stage-period of the Wickford lobsters is less than the stage-period of the Woods Hole lobsters. Further that, while the percentage of increase at each molt for the Woods Hole lobsters (kept in aquaria) was only 13.5 or 15.3, the amount of increase for the Wickford lobsters was over 18 per cent. in the individuals recorded above, while in the case of a group of individuals which had been especially selected (*i. e.*, the smaller and weaker specimens were

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[1] The fifth stage-period is often shorter than the fourth because the water at Wickford is usually the warmest during the fifth stage-period.

[2] This percentage is greater than in the case of another group of 66 lobsters in which Herrick obtained a result of 13.67 per cent.

thrown out) the average amount of increase for the first ten stages was 20.9 per cent. In this last instance the lobsters in the respective stages were consequently much larger than those in the group of Wickford lobsters tabulated above. Their average measurements, however, are presented in the following table:

Stage.	4	5	6	7	8	9	10
Length (mm.).	14.4	17.0	20.5	24.6	31.3	37.0	45.0
Stg.-per. (days).	11.7	11.2	12.2	13.5	15.1	21.0	25.1
Per cent. increase.		18.0	20.6	20.0	27.2	18.5	21.6

These and other observations would demonstrate that there are great variations in the rate of development of lobsters, not only in different localities, and under different conditions of environment, but also in the same locality and under identical conditions. Furthermore, that there is a tendency manifested in those individuals which are slightly above the normal in size and strength, to increase the advantage which they have already gained. This advantage, lodged in the fourth stage lobster, may be no more than a millimeter, but this slight gain compounded through numerous successive stages gives, even the tenth and eleventh stage, a decided lead which is never again lost and which may be observed in the last mentioned group.

Continued observations upon the later stages (from the tenth on) prove that not twelve to seventeen stages, as calculated by Herrick, but an average of twelve stages are passed during the first year of the lobster's existence. We may trace the future development of the young lobster through the later successive stages as follows: September finds the average individual, hatched the previous June, in the ninth stage and with an average length of 32 mm. He passes into the tenth stage in the latter part of September, with a corresponding length of 37.9 mm. In the latter part of October or the first of November he enters the eleventh stage with an increase to 45 mm. Through the months of November, December, January, February and March he lies dormant, passing into the twelfth stage some time in April or the first part of May. Thus it appears that a lobster one year old is in the twelfth stage and has an average length of 53 mm. There are always exceptions to this rule, — instances where an individual may occasionally pass into the twelfth stage before the winter months. Such specimens sometimes manifest an increase of 28 per cent. in passing a single stage. These lobsters are, however, usually among those which were hatched early in the season, and are not very common.

From observations upon the yearling lobsters it becomes apparent that the young creature molts on the average of four times during its second year of life. The thirteenth stage is entered some time in July or August, with a corresponding length of 62 mm. In the latter part of August he molts for the thirteenth time and now covers 73 mm. The entrance to the fifteenth stage occurs in October of the second year. No further change takes place until the following April; that is to say that the average lobster passes its second winter in the fifteenth stage, length 86 mm. (3⅜ inches). By the middle of June we find the young lobster, now approximately two years old, in the sixteenth stage, and with a length of 102 mm. ($4\frac{1}{12}$ inches).

Observations on the molting periods of lobsters over two years old make it apparent that the entrance to the seventeenth stage takes place some time in the late summer of the third year. The lobster generally molts again before the winter months of the same year into the eighteenth stage with a length of 141 mm. (5⅝ inches). No further change is experienced until the following April.

After the seventeenth or eighteenth stage the percentage of increase at each successive molt undergoes a gradual diminution as the molting periods become less frequent. The amount of increase for lobsters about 6 inches in length appears to be in the neighborhood of 15 per cent. Thus continuing, we find that the young lobster passes its third winter in the eighteenth stage, molts again in the spring (usually in April) and by June, when approximately three years old, has a length of 162 mm. (6½ inches).

In lobsters of 7 inches and over we find a still smaller percentage of increase at each molt; 11 or 12 per cent. represents with a fair degree of accuracy the average percentage of increase in length for lobsters between 7 and 10 inches.

Further observation reveals the fact that lobsters over 6 inches in length do not molt oftener than twice in a year; once in the spring or early summer and once in the autumn. Thus the average lobster enters the twentieth stage some time in the autumn of his fourth year and at this molt increases from 162 mm. to 181 mm. (7 1/4 inches). In the late spring or early summer of the following year the lobster, now approximately four years old, enters the twenty-first stage with a corresponding length of 200 mm. (8 inches).

If the case is not one of a young female bearing external eggs (very rare in lobsters of this length), we may expect another molt the following autumn and consequently find the lobster in the twenty-second stage now with a length of 222 mm. (8 7/8 inches). In all probability the molting periods of the male and female remain the same until past the nine-inch length. Therefore, the entrance to the twenty-third stage probably takes place just before, or at any rate soon after, the lobster becomes five years old. The corresponding length is 247 mm. (9 7/8 inches).

By the time this length is reached many of the female lobsters are sexually mature and are bearing external eggs. Owing to this fact, from this time on the rate of growth of the females must be much diminished. This is due no doubt to the checking of the growing process, a phenomenon which very naturally precedes the spawning period; also to the length of time (ten to eleven months) the eggs are carried. The male lobsters, on the other hand, maintain their former rate of development so that by the twenty-fourth stage the average male lobster has a length of 275 mm. (11 inches) and cannot be much less than six years old. In the case of the females, however, which have borne eggs since the nine-inch stage, the eleven-inch limit cannot be attained in a shorter period than eight years.

This discrepancy in the rate of growth of the male and female lobsters from this time on, is undoubtedly the explanation of the fact that, in nearly all individuals in which the sex has been observed, the "giant" lobsters have been of the male variety. There are few data on the rate of growth of large lobsters but it is probable that after the ten-inch size has been attained, the lobster does not molt oftener than once in a year; and after the fifteen-inch stage not oftener than once in two years. Regarding

Stage No.	Sex.	Age.	Length, mm.
1	Male and		8.2
2	Female.	3 days.[1]	9.6
3	“	7 days.	11.4
4	“	12 days.	13.5
5	“	24 days.	16.0
6	“	36 days.	18.8
7	“	7 weeks.	22.5
8	“	9 weeks.	26.5
9	“	3 months.	32.0
10	“	5 months.	37.9
11	“	9 months.	45.0
12	“	1 year.	53.0
13	“	1 yr. 1 mo.	62.0
14	“	1 yr. 3 mo.	73.0
15	“	1 yr. 6 mo.	86.0
16	“	2 yrs.	102.0
17	“	2 yrs. 3 mo.	121.0
18	“	2 yrs. 6 mo.	141.0
19	“	3 yrs.	162.0
20	“	3 yrs. 6 mo.	180.0
21	“	4 yrs.	200.0
22	Male.	4 yrs. 6 mo.	222.0
“	Female.	4 yrs. 6 mo.	“
23	Male.	5 yrs.	247.0
“	Female.	6 yrs. 5 mo.[2]	“
24	Male.	6 yrs.	275.0[3]
“	Female.	8 yrs. 4 mo.	“
25	Male.	7 yrs.	300.0
“	Female.	10 yrs. 4 mo.	“
26	Male.	8 yrs.	327.0
“	Female.	12 yrs. 4 mo.	“
27	Male.	9 yrs.	356.0
“	Female.	14 yrs. 4 mo.	“
28	Male.	10 yrs.	380.0
“	Female.	16 yrs. 4 mo.	“
29	Male.	12 yrs.	406.0
“	Female.	18 yrs. 4 mo.	“
30	Male.	14 yrs.	431.0
“	Female.	20 yrs. 4 mo.	“
31	Male.	17 yrs.	457.0
32	“	20 yrs.	480.0
33	“	23 yrs.	505.0
34	“	26 yrs.	525.0
35	“	29 yrs.	546.0
36	“	33 yrs.	568.0[4]

[1] Age at entrance to the stage.

[2] Assumes that the lobster spawns for the first time in the summer of its sixth year, and that the eggs hatch the following summer.

[3] 11 inches.

[4] 22¾ inches.

the growth of "giant" lobsters, it appears reasonable to believe that the molting process does not occur oftener than once in three years; and this is a small estimate. The amount of increase in these specimens at a single molt cannot be over four or five per cent. and is often inappreciable. The shells of these huge lobsters present every appearance of great age and give testimony to a life of inactivity. Using as a basis the observations which led to the foregoing conclusions, the writer has compiled a table showing the estimated rate of development of lobsters from the time of hatching to the attainment of the greatest known size. While the data on the first twenty stages have their ground in actual observation, the records of the later stages have been deduced from less positive evidence, and are, to a great extent, speculative. The great variation in the size of lobsters, even of the same age and stage, render it well-nigh impossible to tell off-hand the age of any adult lobster. On the other hand, the size of large numbers of individual lobsters of a certain age must remain not far from a general average, on a basis of which, the approximate age of large numbers of individuals can be determined with a fair degree of certainty. It is this average, together with the correlated age, that the writer has attempted to formulate in the preceding table:

Influences on the Rate of Growth.

Among the influences which modify the rate of growth of young lobsters under natural or artificial conditions, are to be mentioned especially the following: temperature, food supply, light, parasites, injuries and individual physiological peculiarities. It is probable that the water temperature and physiological condition are the most influential for young lobsters in the ocean. The others enter largely into consideration in the problems of artificial propagation. The frequency of the molting periods and, secondarily, the amount of increase in length at each molt, is directly dependent upon and determined by the prevailing temperature of the water; a difference of twelve degrees may cause the period of growth to the fourth stage to be over twice as long as normally. Thus we find great variations in the rates of growth of lobsters at different points on the Atlantic coast. For this reason it is very probable that the lobsters in the warm waters of Narrangansett Bay may attain marketable size (10½ inches in Massachusetts) much sooner than do the Massachusetts or Maine lobsters.

It is apparent through other observations that the effects of strong lights and, as shown by Emmel,[1] the mutilation of appendages, exert an influence detrimental to the development of the lobster in the early stages. Excessive sunlight in cases where the lobsters were exposed superficially in the water, appears to cause not only a marked increase in the duration of the stage-periods, but also a diminution in the percentage of increase in length at molts ; and furthermore, a generally less healthy condition in the lobsters themselves. This may be brought about either directly, by inhibiting the body processes and general metabolism, or indirectly, by favoring the excessive growth of diatoms, algæ and protozoa which, under certain conditions, may accumulate on the body and appendages, to such an extent as to prevent nearly all activity. It is also observable in this connection, that food supply may play an important rôle in determining the size of the young lobster.

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[1] V. E. EMMEL ; "The Regeneration of Lost Parts in the Lobster," *The Thirty-Fifth Annual Report of the Rhode Island Commission of Inland Fisheries, 1905.*

NOTE ON THE INFLUENCE OF TEMPERATURE UPON THE RATE OF THE HEART-BEAT IN A CRUSTACEAN (CERIODAPHNIA).[1]

T. BRAILSFORD ROBERTSON.

1. Arrhenius has shown[2] that the velocity of a chemical reaction increases very much more rapidly with increasing temperature than any known physical phenomenon. The velocity of a chemical reaction increases about 10 per cent. per degree centigrade rise in temperature while molecular velocity, the electric conductivity of a wire, the elasticity of a solid, the viscosity of a fluid, surface-tension, etc., are much less affected by the same rise of temperature. The viscosity of a fluid, for example, only diminishes about 2 per cent. per degree centigrade rise in temperature. The viscosity of a gas, according to Maxwell's equation for air[3] should increase about 6.7 per cent. per degree centigrade rise in temperature at 15° C.

2. The fact of the extreme variability of chemical reactions with temperature has been applied to ascertain whether given biological phenomena involve chemical reactions or not. Arrhenius[4] and van t'Hoff[5] have shown that the quotient

$$\frac{\text{velocity of reaction at } T_{n+10}}{\text{velocity of reaction at } T_n}$$

is equal to about 2, T_n being the absolute temperature. That is to say, the velocity of a chemical reaction increases about 100 per cent. per ten degrees. Hertwig[6] has found that the rate of development of frog's eggs increases very rapidly with rise of temperature. At Dr. Loeb's suggestion C. D. Snyder[7] investi-

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[1] From the Rudolph Spreckels Physiological Laboratory of the University of California.

[2] *Zeitschrift f. Physik-Chemie*, 1899, Bd. 4, s. 226.

[3] "Theory of Heat," 1872, p. 279.

[4] *Loc. cit.*

[5] "Études de dynamique chimique," p. 112, etc.

[6] "Die Zelle und die Gewebe," Bd. II., S. 119.

[7] University of California Publications, Physiology, Vol. 2, p. 125.

gated the influence of temperature upon the rate of the heart-beat and found that the rate of the beat in the heart of the terrapin (*Clemmys marmorata*) is almost exactly doubled by ten degrees rise in temperature between the temperatures 10° C. and 32.5° C. — at lower temperatures the rate is more than doubled by a rise of 10° while at higher temperatures the rate is somewhat less than doubled by the same rise in temperature. Loeb[8] has found that the velocity of artificial maturation in the eggs of Lottia is more than doubled by raising the temperature from 8° C. to 18° C. Other investigations on the influence of temperature upon biological phenomena are being carried out in this laboratory. These observations do not prove that the above-mentioned biological phenomena are entirely chemical in character, but they afford indication that chemical reactions are involved although not to the exclusion of possible concurrent physical changes.

3. It appeared to me of interest, in connection with experiments on the influence of electrolytes on the rate of the heart-beat, of which an account will appear at an early date, to ascertain the influence of temperature upon the rate of the heart-beat in the transparent fresh-water crustacean Ceriodaphnia (?). The organism, after washing in tap-water, was laid in a drop of tap-water in the depression on the glass top of an Englemann gas-chamber, the temperature being regulated by running warm or cold water through the chamber. A thermometer was fitted into the chamber so that the bulb lay directly under the depression in which the organism was placed. A few minutes was always allowed to elapse before the rate of the beat was registered in order to allow the organism to attain the same temperature as the bulb of the thermometer. The beats at room-temperature and at higher temperatures are so rapid that they cannot be counted but have to be recorded by tapping a key which completes a circuit including a signal-magnet, which thus registers a mark upon a revolving drum for every beat, the time being taken with a stop-watch.

4. The following are the experimental results.

[8] University of California Publications, Physiology, Vol. 3, p. 1.

EXPERIMENT 1.

Temperature.	Beats per Second.
24.75°	6.53
20.75°	4.43
15.75°	2.47

EXPERIMENT 2.

Temperature.	Beats per Second
15°	2.53
19°	3.46
23°	4.39
27°	6.11

EXPERIMENT 3.

Temperature.	Beats per Second
15°	2.63
17°	2.95
19°	3.67
21°	4.87
23°	5.05
25°	6.59
27°	6.10
29°	7.375

$$\frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 2.51; \quad \frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 2.07; \quad \frac{\text{Rate at } 29^\circ}{\text{Rate at } 19_\circ} = 2.01.$$

EXPERIMENT 4.

Temperature.	Beats per Second.
13°	2.22
15°	2.88
17°	3.46
19°	2.95
21°	3.91
23°	5
25°	5.71
27°	6.23
29°	6.15

$$\frac{\text{Rate at } 23^\circ}{\text{Rate at } 13^\circ} = 2.25; \quad \frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 1.98;$$

$$\frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 1.80; \quad \frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.08.$$

EXPERIMENT 5.

Temperature	Beats per Second.	Remarks
11°	2.31	Irregular pauses in diastole accompanied by very slight quick beats.
13°	——	Still irregular.
15°	4.12	Regular.
19°	4.79	"
21°	6.15	"
25°	6.22	"
29°	6.17	"
31°	7.01	Gills stopped

At 33° the whole animal went into convulsive tremors, and at 35° the heart stopped permanently.

$$\frac{\text{Rate at } 21^\circ}{\text{Rate at } 11^\circ} = 2.66\,; \quad \frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 1.51\,;$$

$$\frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 1.29\,; \quad \frac{\text{Rate at } 31^\circ}{\text{Rate at } 21^\circ} = 1.14.$$

EXPERIMENT 6.

Temperature.	Beats per Second.	Remarks.
11°	2.11	Beat irregular
13°	2.53	" "
15°	2.53	" regular
19°	2.67	" "
21°	4.05	" "
23°	4.80	" "
25°	5.56	" "
29°	6.30	" "
29°	5.33	After a fall and subsequent rise in temperature.

$$\frac{\text{Rate at } 21^\circ}{\text{Rate at } 11^\circ} = 1.92\,; \quad \frac{\text{Rate at } 23^\circ}{\text{Rate at } 13^\circ} = 1.90\,; \quad \frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 2.20\,;$$

$$\frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.36\,; \quad \frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} \text{(second observation)} = 2.00.$$

EXPERIMENT 7.

Temperature.	Beats per Second.
11°	1.64
15°	2.38
17°	2.63
19°	2.98
*21°	4.53
21°	4.55
21°	4.48
25°	5.33
27°	5.4
29°	5.15

$$\frac{\text{Average rate at } 21^\circ}{\text{Rate at } 11^\circ} = 2.76\,; \quad \frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 2.24\,;$$

$$\frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 2.05\,; \quad \frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.06.$$

The beat was regular throughout the observations in this experiment. The observations were made in the following order, 21°, 21°, 29°, 27°, 25°, *21°, 19°, 17°, 15°, 11° — hence the rate of beat at 21° was counted three times, twice at the beginning of the experiment and once after taking the animal through a series of temperature-changes none of which were extreme — it will be observed that the three observations at 21° are in very good agreement.

Experiment 8.

Temperature.	Beats per Second.	
11°	1.82	
15°	2.95	
17°	3.78	
19°	4.09	
*21°	4.375	
21°	4.15	Average = 4.40
21°	4.65	
25°	4.67	
27°	6.67	

$$\frac{\text{Average rate at } 21^\circ}{\text{Rate at } 11^\circ} = 2.41\,; \quad \frac{\text{Rate at } 25^\circ}{\text{Rate at } 15^\circ} = 1.58\,;$$

$$\frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 1.76.$$

The beat was regular throughout the experiment. The organism was taken through the following temperatures in the following order: 21°, 21°, 29° (beats not counted), 27°, 25°, *21°, 19°, 17°, 15°, 11°—hence the rate of beat at 21° was counted three times, twice at the beginning of the experiment and once after taking the animal through a cycle of temperature-changes none of which were extreme—the three determinations of the rate at 21° are in good agreement with each other and with those in experiment 7.

Experiment 9.

Temperature.	Beats per Second.
19°	3.27
21°	5.12
29°	6.25

The organism was taken through the following temperatures in the following order, 31° (not counted), 29°, 21° and 19°.

$$\frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 1.91.$$

EXPERIMENT 10.

Temperature.	Beats per Second.
29°	6.15
21°	3.06
21°	3.23
19°	2.86

$$\frac{\text{Rate at } 29^\circ}{\text{Rate at } 19^\circ} = 2.15.$$

The beat was regular throughout, but the temperature had been raised above 29° before the observations at 21° were taken.

EXPERIMENT 11.

Temperature.	Beats per Second.
17°	2.38
21°	4.04
27°	5

$$\frac{\text{Rate at } 27^\circ}{\text{Rate at } 17^\circ} = 2.10.$$

The observations were taken in the following order, 21°, 17°, 27°.

EXPERIMENT 12.

Temperature.	Beats per Second.
13°	2.28
21°	4.57
23°	5

$$\frac{\text{Rate at } 23^\circ}{\text{Rate at } 13^\circ} = 2.19.$$

5. The average value of the coefficient $\frac{\text{Rate at } T_{n+10}}{\text{Rate at } T_n}$ for all the observations made was 2.03. The results are therefore such as to confirm Snyder's observations, referred to above, in so far as they apply in general to rhythmically contracting tissues and to lead us to conclude that a chemical reaction is involved — as I have assumed in previous papers.[1]

6. The rate of the beat in different individuals at 21° is remarkably constant, provided they have been treated in the same manner and have not been subjected to any extreme temperatures — this is particularly well shown in experiments 7 and

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[1] *Transactions of the Royal Society of South Australia*, Vol. XXIX., p. 47; *Plüger's Arch. f. d. ges. Physiologie*, Bd. 110, s. 610.

8. I have repeatedly confirmed the fact that the rate of the beat at 21° is about 4.5, with a possible variation of about 5 per cent. The organisms were all taken from the same vessel, in which they were kept; but they differed widely in size and development, some containing eggs and others containing well-developed embryos. Ceriodaphnia therefore affords homogeneous material for experiments on the heart-beat.

TWO DISTOMES.

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1. Pneumonœces complexus, N. S., a New Frog Distome.

This description is based on the examination of several preserved specimens which were received from North Carolina marked "From the mouth of *Rana pipiens*."

P. complexus is a rather elongated worm, between 5 mm. and 8 mm. in length, and in the widest portion, about 1.7 mm. wide. The thickness is about .71 mm., a cross-section being elliptical in outline. The widest region is just forward of the middle, and from here it tapers very slightly toward the hinder end, which is rounded and blunt. Toward the forward end it tapers more rapidly, sometimes giving the worm almost the appearance of having a neck. The oral sucker is subterminal and .4 mm. in diameter. The acetabulum is 2.4 mm. from the anterior end of the animal and very slightly smaller than the oral sucker, being .38 mm. in diameter. Both suckers are sessile. All the specimens examined were smooth, *i. e.*, without spines. This may have been due, however, to maceration. Of the five species of this genus described by Stafford (1902), all are covered with spines except one, *P. longiplexus*, which is smooth. The determination of this point with respect to *P. complexus* will depend finally upon the examination of a living or freshly-killed specimen. In the specimens examined, however, there were patches where the cuticle seemed to be intact. It was very thick and there was no trace of spines. Thus the evidence seems almost conclusive that the worm is a smooth one.

The mouth is easily discernible in the center of the oral sucker, and leads to the muscular, bulb-like pharynx. The œsophagus is about .5 mm. in length. It then branches, forming the two intestinal cæca, which extend to within .4 mm. of the posterior end of the body. The cæca are rounded tubes about .15 mm. in diameter, and are without branches.

The excretory vesicle, which is tubular and in cross-section about the size of one of the intestinal cæca, is extremely long. The median portion extends from the excretory pore, which is terminally located at the posterior extremity, to a position just posterior to the acetabulum. Here it branches, forming the two crura, which extend outward and forward to about the plane of the posterior end of the œsophagus. The excretory vesicle is extremely thin-walled, and almost impossible to be seen except in sections. It is dorsal in position throughout its entire extent.

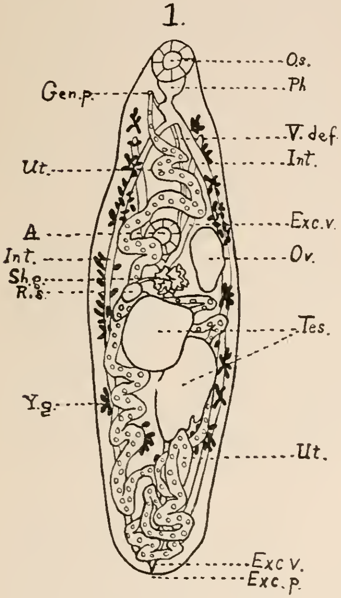
The testes are two large, very prominent organs just posterior to the middle of the animal. They are irregularly ovate in outline and are somewhat lobed. They do not both lie in the same plane, the left one usually being posterior to, but overlapping, the right. The posterior one is usually somewhat longer than the anterior, being 1.4 mm. long, while the anterior one is 1.1 mm. long. Each is about .92 mm. wide and about .78 mm. thick. The vas deferens from the left testis runs forward to the left of the right testis, lying between it and ovary. Just anterior to the shell gland it is joined by its fellow from the right testis, and from here a slightly convoluted tube extends to the genital pore. There is no cirrus or cirrus sac. The genital pore is a minute opening on the ventral side, in the same transverse plane as the pharynx and to the right of the median plane.

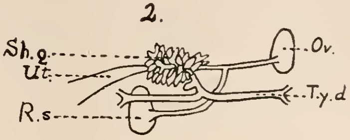
The ovary is a somewhat elongated organ considerably smaller than either testis. It is usually on the left side and slightly posterior to the acetabulum. Occasionally it is on the right side, and when it is so placed the testis on that side is posterior to the other. It is about .7 mm. in length, .32 mm. in width, and .32 mm. thick (dorso-ventrally). The oviduct runs from the ovary into the shell-gland where there is an ootype. The shell-gland lies in the middle of the body just posterior to the acetabulum.

The receptaculum seminis is a sac about one-half as large as the ovary. It lies just forward of and lateral to the right testis. Its duct runs forward and to the left, meeting the oviduct at its entrance into shell gland. There is no Laurer's canal.

The yolk glands are numerous, being placed in clusters along both sides of the body from the position of the pharynx to the posterior extremity of the hinder testis. There are five or six distinct groups or clusters on each side, each group being composed of 6 to 20 glandular bodies. They are ventrally placed and follow the general course of the intestine.

The tube from the forward clusters on each side meets that from the rear clusters just inside the intestinal cæcum and in a transverse plane just posterior to the ootype. The transverse tubes meet near the median plane, well toward the dorsal body-wall, and from here a short tube leads to the yolk reservoir.





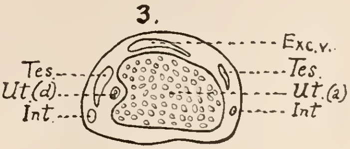


FIG. 1. *Pneumonœces complexus*, ventral aspect. *O. s.*, oral sucker; *A.*, acetabulum; *Ph.*, pharynx; *Int.*, intestinal cæcum; *Exc. v.*, excretory vesicle; *Ut.*, uterus; *Tes.*, testes; *R. s.*, receptaculum seminis; *Gen. p.*, genital pore; *Ov.*, ovary; *Sh. g.*, shell gland; *V. def.*, vas deferens; *Y. g.*, yolk gland.

FIG. 2. Diagram of female genital organs in *P. complexus*. *Ov.*, ovary; *Sh. g.*, shell-gland; *Ut.*, uterus; *R. s.*, receptaculum seminis; *T. y. d.*, tranverse yolk duct.

FIG. 3. Cross-section of *Renifer elongatus* in region of testes. *Ut.* (*d*), uterus, descending; *Ut.* (*a*), uterus, ascending; *Tes.*, testis; *Int.*, intestinal cæcum; *Exc. v.*, excretory vesicle.

From the yolk reservoir a tube extends diagonally forward and towards the ventral body wall joining the oviduct after its entrance into the shell gland. The yolk reservoir is a pear-shaped sac about .12 mm. in length.

The uterus is a continuation of the oviduct, running to the posterior end of the animal, then forward to the genital pore. It winds about, filling the spaces between the other organs, and in the case of adult worms often nearly covers them. It is circular in cross-section, and about .15 mm. in diameter. In the adult animal this tube is filled with eggs which as they ripen take on a dark brown color, and give the worm a peculiar mottled appearance. There are no lateral longitudinal folds as in the case of most of the other species of this genus.

P. complexus is closely related to *P. similiplexus*, described by Stafford (1902) under the name of *Hæmatolœchus similiplexus.* The principal differences between the two species are that in *P. complexus* the testes are very much larger than in *P. similiplexus ;* there are no lateral longitudinal folds of the uterus ; and the eggs are considerably smaller, being .029 mm. by .014 mm. as compared with .039 mm. by .019 mm. in *P. similiplexus.* In outer characters, — size, shape, suckers, etc., — there is a close similarity between the two species. *P. similiplexus* is covered with spines, while *P. complexus* is probably smooth. In this respect *P. complexus* resembles *P. longiplexus.* In respect to the folds of the uterus it most closely resembles *P. medioplexus.* The ascending and descending coils seem however to have less regularity that in that species.

The other species of this genus are universally reported as being found in the lungs of frogs, while, as has already been noted, these specimens were labelled as having been found in the mouth of the leopard frog. Upon inquiry I found that these distomes are rarely or never found in the mouth of a living or freshly-killed animal. If, however, the frogs are killed by chloroform, and especially if they are left for some time in the killing jar, the worms, still alive, may frequently be found in the mouths of the dead animals. They may be thrown from the lungs by the struggles of the dying animal, or they may find their way to the mouth in their efforts to escape from a dead host. At any rate the above facts explain their being found in the mouth of the frog, and leave every probability that *P. complexus* is a lung parasite.

II. RENIFER ELONGATUS PRATT.

This worm was first described by Pratt (1903) from a single specimen, mature but not yet fully grown. The object here is simply to supplement that description, by a comparison with a full-grown specimen. The worm from which the following measurements were taken was selected as the largest of about 40 or 50 specimens at hand. This worm is 5.5 mm. in length, and 1.7 mm. wide at the widest part, while that described by Pratt was 3 mm. in length, and .68 mm. in width. The thickness of the two worms is .93 mm. and .4 mm., respectively.

The oral sucker is round and about .35 mm. in diameter; the acetabulum is decidedly elliptical in outline, extending .82 mm. transversely and .6 mm. longitudinally. The corresponding measurements given by Pratt are the following: oral sucker; length, .32 mm., width, .33 mm.; acetabulum; length, .4 mm., width, .36 mm. The relative positions of the suckers on the animal are of course the same. The relative sizes of the various parts of the digestive tracts of the two worms correspond to relative sizes of the worms themselves.

The most marked difference between the two worms are the greatly increased size of the ascending limb of the uterus, and the consequent displacement of the other organs in the larger worm. In the region just back of the testes, the uterus occupies nearly two thirds of the space within the body walls, and the excretory vesicle is pressed flat against the dorsal wall, extending from one lateral margin to the other. In the region of the testes there is but little diminution in the size of the uterus, and the testes are pressed outward toward the lateral body walls, they themselves being apparently somewhat flattened (see Fig. 3). Forward of the acetabulum, the uterus rapidly decreases in size as it approaches the genital pore.

The eggs were approximately the same size as those in the smaller worm. The measurements given by Pratt are .035 mm. by .02 mm. The measurements in the larger worm were .034 mm. by .021 mm.

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BIOLOGICAL BULLETIN

THE YPSILOID APPARATUS OF URODELES.

INEZ L. WHIPPLE.

Introduction.

Articulated to the anterior margin of the pubis of many salamanders, there is a cartilage which, from its peculiar Y shape, is known as the *Cartilago ypsiloides.* This cartilage has in general been assumed, by those who have described and figured it, to be identical with the anterior paired or unpaired cartilaginous process, the *Processus epipubicus* of the *Proteidæ* and *Derotremata.* Thus Wiedersheim in his *Grundriss* (1893, p. 165), says, after speaking of the paired nature of the anlage of the pelvic girdle, "Dies hervorzuheben ist namentlich auch wichtig im Hinblick auf die Morphologische Bedeutung jenes Abschnittes, den ich als *Processus epipubicus* bezeichnet habe. Die ursprünglich paarige Natur desselben tritt bei dem Becken von *Proteus* und *Amphiuma* zeitlebens deutlich hervor.

"Auch bei den *Derotremen* und *Salamandrinen* findet sich am vorderen Beckenrand in der Medianlinie ein Knorpelfortsatz, der als *Epipubis* zu betrachten ist, und in manchen Fällen lässt sich dessen directer Zusammenhang mit der eigentlichen Beckenplatte noch deutlich nachweisen. Insofern aber liegt bereits ein Fall von sogenannter abgekurzter Entwicklung vor, als der *Processus epipubicus* hier nicht mehr paarig, sondern als ein unpaarer Auswuchs sich an seinem Vorderende erst secundär gabelig theilt."

Gegenbaur, also, in his "Vergleichende Anatomie" (1898, Vol. I., p. 550), after describing the pelvic girdle of *Necturus* with its long anterier process says that "Derselbe Teil bei Salamandrinen als medianer terminal gegabelter Fortsatz erscheint, das soganannte *Epipubis.* Aus der Vergleichung dieser beiden Zustände geht hervor, dass das *Epipubis* bereits in der Platte des Pubis besteht und nicht als besonderer Fortsatz auftritt. Seine Entstehung geht sonach aus einer bilateralen Reduction eines Teiles der ventral Beckenplatte hervor."

Hoffmann (Bronn's "Their-reich," Bd. 6, II., Amphibien, 1873–8, p. 77), says in his description of the pelvic girdle of *Urodeles*, "Der vordere Rand des Schamsitzbeins verlängert sich nach vorn in eine mediane Spitze. Bei *Proteus* und *Menobranchus* ist diese Spitze mit der ventralen Platte continuirlich verbunden, wahrend sie bei den anderen Urodelen durch Syndesmose damit innig zusammenhängt. Dieser ventrale Fortsatz, welcher bei *Proteus*, *Menobranchus*, und *Amphiuma* nur sehr kurz ist, verlängert sich bei den anderen Urodelen in die ventrale Muskelmasse und spaltet sich vorn gabelförmig in zwei divergirende Schenkel (*Cartilago ypsiloides*). Bei *Cryptobranchus japonicus*, wo dieser ventrale Fortsatz der knorpeligen *Epiphyse* der Schambeinplatte aufsitzt, ist desser rechter Schenkel in drei Sipfen [*sic*] gespalten, welche jedoch am linken Schenkel fehlen."

In all of the above quoted authorities it is implied or assumed that the epipubic cartilage (*Cartilago ypsiloides*) occurs universally in *Urodeles*. Wiedersheim ('75), however, in his work on *Salamandrina perspicillata* and *Geotriton fuscus* (p. 142), under the heading *Cartilago ypsiloides* expresses himself as greatly surprised at finding no trace of the cartilage in *Geotriton*, adding, "Wo also die Erklarung zu suchen ist, ist mir dunkel gebleiben, doch wäre vielleicht von der Untersuchung der Larven, welche mir im Augenblick nicht bei der Hand waren, noch etwas zu erwarten"; and recently Miss Emerson in her work on the anatomy of *Typhlomolge rathbuni* (1905) mentions the absence of the *Cartilago ypsiloides* in that species.

The following tabulated results of my own dissection of the pelvic region of salamanders[1] shows, moreover, that the *Cartilago ypsiloides* is far from being universal in occurrence:

Cartilago ypsiloides, present.	*Cartilago ypsiloides*, absent.
Triton alpestris,	*Desmognathus fusca*,
Triton helveticus,	*Batracoseps attenuatus*,
Diemyctylus viridescens,	*Spelerpes ruber*,

[1] The term salamander is used here as synomymous with the suborder Salamandrida, comprising all of the Urodeles except the Derotremata and Perennibranchiata.

Cartilago ypsiloides, present.	*Cartilago ypsiloides*, absent.
Amblystoma opacum,	*Spelerpes bilineatus*,
Amblystoma punctatum,	*Spelerpes guttolineatus*,
Amblystoma jeffersonianum,	*Spelerpes porphyriticus*,
Amblystoma talpoideum,	*Spelerpes longecaudus*,
Salamandra maculosa,	*Manculus quadridigitatus*,
Salamandrina perspicillata.	*Plethodon cinereus*,
	Plethodon erythronotus,
	Autodax lugubris.

In those forms in which the cartilage is present it exhibits the typical Y-shaped character, and is movably articulated to the anterior edge of the pubis. In the species given in the right-hand column there is no trace of the cartilage whatsoever. These results show that the presence of a *Cartilago ypsiloides* is closely correlated with the presence of lungs, although there seem to be two exceptions, viz., *Amblystoma opacum* and *Salamandrina perspicillata.* The first of these, however, rests upon an error, since the *Amblystoma opacum*, like the others of its genus, possesses large and well developed lungs, although through the authority of Lönnberg ('96) this species has for several years been placed among lungless forms. It is impossible to say through what appearances Lönnburg was deceived, but the presence of well developed lungs has been repeatedly demonstrated by me through dissection and physiological experiment. The most plausible hypothesis is that he was mistaken in his species, an error extremely likely to occur in the study of this group.

Marked differences in the activities of lungless and lunged salamanders when in the water, a subject which will receive full discussion later, further corroborate the view that the function of the ypsiloid cartilage is correlated with that of the lungs. In fact, it was this difference in activity which first called my attention to the difference in anatomy and led me to make an extensive study of the ventral pelvic region of *Urodeles.*

PART I. ANATOMY OF THE YPSILOID REGION IN URODELES.

A. *Description of Adult Forms.*

1. *Diemyctylus viridescens.*—In *Diemyctylus*, as in all *Urodeles*, the muscular abdominal walls are differentiated into the four typical layers, viz., *Musculus obliquus externus*, *M. obliquus internus*, *M. rectus abdominis* and *M. transversalis*. The external oblique is strongly developed, showing the typical outer and inner laminæ. The outer has lost its metamerism except in its origin; its fibers, which form a strong, continuous, muscular sheet, are mainly inserted into a wide, ventral aponeurosis, and the posterior bundles into ilium and pubis. The rectus abdominis consists of a narrow band of muscle, lying on either side of and contiguous to the linea alba and covered ventrally by the aponeurosis of the obliquus externus. It is very primitive in character, consisting of a series of myotomes, separated by well formed myocommata. The obliquus internus is almost vestigial and in some individuals I was unable to find it. Its very thin layers of fibers arise mainly by digitations from myocommata beneath the deeper layer of the external oblique, although the more posterior bundles have their origin in the anterior edge of ilium and pubis. The fibers of this muscle extend very obliquely, anteriorly and ventrally and are inserted into myocommata. At the edge of the rectus abdominis muscle the fibers of the internal oblique seem in some cases to become continuous with those of the rectus, in other cases to pass dorsal to the rectus, which, however, they only slighly overlap.

The transversalis, even more than the obliquus externus, consists of a continuous muscular sheet, its metamerism being evident only in its origin. It is inserted ventrally into an aponeurosis which is somewhat narrower than that of the obliquus externus and which lies on the inner (*i. e.*, dorsal) surface of the rectus abdominis. This aponeurosis narrows abruptly in the two metameres anterior to the pubis, and ends at the pubic symphysis in a point.

In the two myotomes immediately anterior to the pelvic girdle there is considerable muscular differentiation, which may be most easily shown by a series of dissections. Fig. 1 shows a ventral view of the posterior abdominal region, with the skin and external oblique muscle removed from the left side. In the mid line, extending anteriorly from the pubic symphysis through the width of a single myotome is the stem portion of the *Cartilago ypsiloides* (*y*), the two lateral arms of which are hidden by the rectus abdominis. The first myotome of the rectus abdominis, counting anteriorly from the pelvic girdle, is differentiated superficially into two portions, the more ventral and lateral of which (*ra*) extends outward with its fibers converging to their insertion into a process at the outer edge of the pubis, which I shall call the *lateral process* of the pubis. This portion is well differentiated into a distinct muscle, and in its insertion is closely associated with the posterior portion of the external oblique. The second portion (*rb*) is partially covered by the first, and its fibers slant medially to their insertion into the ventral surface of the stem of the ypsiloid cartilage and the anterior margin of the pubis. When the more ventral of these two muscles is removed, it is found that the medial portion of the deeper muscle takes its origin from the lateral arm of the ypsiloid cartilage, which extends obliquely nearly across the second myotome. In Fig. 2 both of these muscles have been removed, as well as that portion of the second myotome which lies over but unattached to the *Cartilago ypsiloides*. On the right side in the figure may be seen two muscles connected with the cartilage. One of these, which may be designated the *M. ypsiloideus posterior* (*yp*), is a strongly developed muscle which arises from the anterior margin

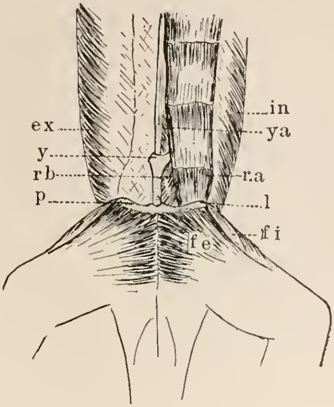

Fig. 1. Ventral view of *Diemyctylus viridescens*, posterior abdominal region, × 3. The external oblique muscle has been removed from the left side. Abbreviations: *ex*, M. obliquus externus; *fe*, M. pubo-ischio-femoralis externus; *fi*, M. pubo-ischio-femoralis internus; *in*, M. obliquus internus; *l*, lateral process of pubis; *p*, pubis; *ra*, and *rb*, differentiations of the first myotome of the rectus abdominis; *y*, ypsiloid cartilage; *ya*, M. ypsiloideus anterior.

of the pubis, its thickest portion having its origin in the lateral process. The fibers of this muscle diverge slightly, and are inserted into the edge of the lateral arm, and the inner (*i. e.*, dorsal) surface of the stem of the *Cartilago ypsiloides.* Lying, as it does, dorsal to the main mass of the rectus abdominis, this muscle is almost continuous at its lateral boundary with the obliquus internus. It is the muscle which has been often called the pyramidalis. Thus Hoffmann described it as a part of the general urodele musculature and homologized it with the pyramidalis of human anatomy.

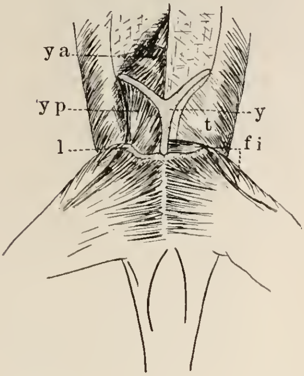

FIG. 2. Ventral view of *Diemyctylus viridescens*, posterior abdominal region, × 3. All of the abdominal muscles have been removed except the tranversalis muscles and the ypsiloid muscles of the right side. Abbreviations not previously explained: *t*, M. transversalis abdominis; *yp*, M. ypsiloideus posterior.

The other ypsiloid muscle, *M. ypsiloideus anterior* (*ya*), arises from the medial portion of the second myocomma, and from the linea alba of the first metamere, and its fibers extend obliquely outward and posteriorly to their insertion into the anterior edge of the lateral arm of the cartilage.

The transversalis muscle shows a wide range of individual variation in its relation to the ypsiloid cartilage. In some of the specimens dissected none of its fibers were inserted into the cartilage. The posterior narrowing of the aponeurosis of the transversalis in this region, however, causes the edges of the aponeurosis to lie closely parallel with the outer edges of the ypsiloid cartilage, and in the majority of the specimens dissected some of the fibers of this muscle had become inserted into the cartilage, the ends of the lateral arms being the region where this insertion most frequently occurs. The extent of the insertion of this ypsiloid portion of the transversalis varies, however, from one which involves the outer portion of the arm only, to one which extends along nearly the entire length of the cartilage, both arm and stem (Fig. 3, *tb*). Almost invariably a few fibers of the muscle immediately anterior to the cartilage extend farther medially than those of the rest of the muscle and may even be inserted into the second myocomma in association with those of the *M. ypsiloideus anterior* (Fig. 3, *ta*).

It should perhaps be emphasized that the entire musculature of the ypsiloid cartilage, while in all cases strongly developed, shows a considerable amount of individual variation, as if the apparatus were one of relatively recent origin and still in the experimental stage of physiological adaptation. The most definite of the muscles involved is the *M. ypsiloideus posterior*.

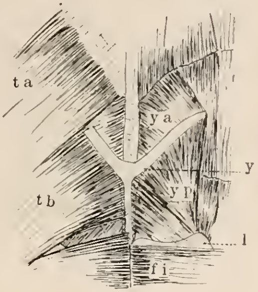

FIG. 3. Dorsal view (*i.e.* from within body cavity showing the muscles attached to the ypsiloid cartilage of *Diemyctylus viridescens*, ×5. On the right side the anterior part of the M. pubo-ischio-femoralis internus has been cut away. Abbreviations not previously explained: *ta*, the portion of the transversalis inserted into the second myocomma; *tb*, the portion of the transversalis which is inserted into the ypsiloid cartilage.

In addition to the above described musculature of the *Cartilago ypsiloides*, the posterior portion of the stem of the cartilage forms the origin of the anterior portion of the *M. pubo-ischio-femoralis internus*. This muscle takes its origin mainly from the mid ventral line of the pubo-ischium, and that portion which arises from the ypsiloid cartilage extends between the posterior ypsiloid muscle and the transversalis (Figs. 1, 2, and 3, *fi*).

2. *Triton helveticus*, *T. alpestris*, *Salamandra maculosa*, *Amblystoma opacum*, *A. punctatum*.—Although representing different subfamilies, these forms so closely resemble *Diemyctylus* in the anatomy of the ypsiloid region that they may be grouped together in this comparison. The *Tritons* most closely resemble *Diemyctylus*, the correspondence part for part being almost exact. *Salamandra* and the *Amblystomas* have a relatively less strongly developed external oblique, while the rectus abdominis is broader and more powerful and the internal oblique more strongly developed. The differentiations in connection with the ypsiloid cartilage (Fig. 4), are, however, practically the same as in *Diemyctylus*. The *M. ypsiloideus anterior* presents less deviation from the longitudinal course of the rectus abdominis, almost all

of its fibers proceeding from the myocomma instead of the linea alba. The transversalis shows about the same range of variation that was described in *Diemyctylus ;* the edges of its aponeurosis coincide more exactly with those of the cartilage and in one very muscular specimen of *Amblystoma punctatum*, this muscle was strongly inserted into the cartilage along the whole length of its arm. In the same individual the cartilage was very large, as its arm crossed the second myotome, and the lateral portion of the *M. ypsiloideus anterior* rose from the third myocomma thus giving this muscle an origin from two myocommata.

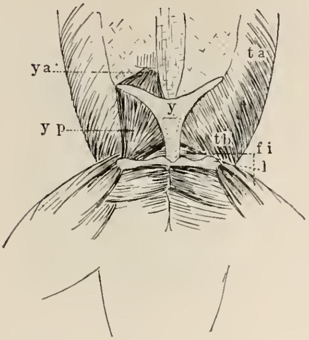

FIG. 4. Ventral view showing the ypsiloid apparatus of *Amblystoma punctatum*, × 2. Dissection and abbreviations as in Fig. 2.

In all of these genera as in *Diemyctylus* the *M. pubo-ischio-femoralis internus* takes its origin partly from the posterior end of the ypsiloid cartilage.

3. *Salamandrina perspicillata.* — This species, which, so far as is known, is the only lungless member of the subfamily *Mecodonta*, is sufficiently different in its abdominal musculature from the lunged forms already described, to require a separate discussion. The outer, deeper portion of the rectus abdominis (the rectus profundis of Maurer's nomenclature), is highly specialized into a retractor of the tongue (*M. pubo-hyoideus*). It lies in a sheath, in which it moves freely, since although it possesses myocommata corresponding to those of the remaining portion of the rectus abdominis, these are wholly disconnected from those of the main mass of somatic muscles and thus do not correspond with the latter during all phases of muscular contraction. Aside from this highly specialized region, the abdominal muscles of *Salamandrina* are poorly developed. The external oblique muscle seems reduced almost to a mere sheet of connective tissue, and is evidently functional more as a support for the abdominal wall than for any muscular activity. The internal oblique I have been unable to demonstrate. The rectus abdomianis is thin and its two halves are separated by a very wide line

alba. It does not show in the myotome anterior to the pubis the superficial differentiation noted in the forms already described. Neither is any of this superficial portion attached to the ypsiloid cartilage.

The musculature of the *Cartilago ypsiloides* is otherwise quite similar to that of *Diemyctylus*. The anterior ypsiloid muscle, is, owing to the great width of the linea alba, inserted into the distal portion only of the arms. The relation of the *M. pubo-ischio-femoralis internus* to the ypsiloid cartilage is the same as that already described in the preceding cases.

4. *Desmognathus brimleyorum*, *D. fusca*, *Spelerpes ruber*, *S. bilineatus*, *Plethodon erythronotus*, *P. cinereus*.— In none of these forms is there a *Cartilago ypsiloides*. The musculature of the posterior abdominal region gives, however, decided evidence of the former existence of an ypsiloid apparatus.

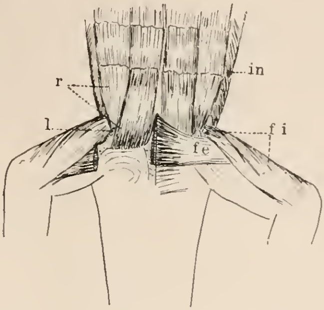

FIG. 5. Ventral view of the posterior abdominal region of *Desmognathus brimleyorum*, × 2. The M. obliquus externus has been removed from both sides and the M. pubo-ischio-femoralis externus from the right side.

The abdominal wall shows a more primitive musculature than *Diemyctylus*, the metamerism being much more strongly marked. The rectus abdominis is broad and powerful. In the myotome (Fig. 5, *r*) immediately anterior to the pubis there is the same superficial differentiation that *Diemyctylus* and other lunged forms exhibit. There is one difference, however, which is due to the fact that the anterior border of the pelvic girdle is deeply incurved on either side of the ventral midline so that the border presents a conspicuous median and two lateral processes (Fig. 6, *l* and *m*). As a result of this the medial portion of the rectus abdominis extends much farther posteriorly than the lateral portion, since it is inserted into the ventral surface of the girdle somewhat posterior to this incurved edge.

In all of the lungless forms dissected the deeper portion of the first myotome of the rectus abdominis shows a well defined though not at all strongly developed portion (Fig. 6, *ypv*) which arises from the lateral process of the pubis. From this origin the fibers diverge to their insertion mainly into the linea alba, although in most cases a few are inserted into the first myocomma. When we consider the *Cartilago ypsiloides* of the lunged forms, with its stem in the linea alba and its arms diverging at the first myocomma, it seems very probable that this muscle in the lungless forms is a vestigial *M. ypsiloideus posterior* which persists even after the disappearance of the cartilage to which it was originally attached. In some specimens a few fibers of this muscle were found to extend to the second myocomma and in one specimen of *Desmognathus brimleyorum* a separate little muscle (Fig. 6, *yav*) consisting of a few fibers only was found in the region of the second myotome arising in the linea alba and converging obliquely outward and posteriorly. These variable evidences of differentiation from the second myotome suggest, of course, the probable vestiges of the *M. ypsiloideus anterior.*

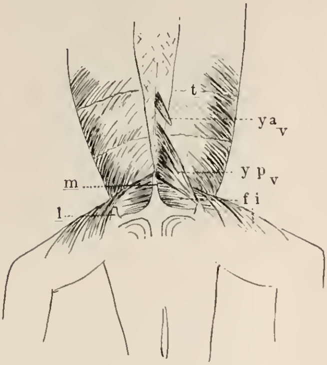

Fig. 6. Ventral view of the posterior abdominal region of *Desmognathus brimleyorum*, × 2. All of the abdominal muscles have been removed except the transversalis muscles and the vestigial ypsiloid muscles of the left side. Abbreviations not previously explained: *m*, median process of pubis; ya_v, vestige of anterior ypsiloid muscle; yp_v, vestige of posterior ypsiloid muscle.

Still further evidence of the former existence of an ypsiloid cartilage in lungless forms is furnished by the fact that in its absence the anterior portion of the *M. pubo-ischio-femoralis internus* originates from the linea alba in the exact region corresponding to the origin from the posterior part of the stem of the ypsiloid cartilage in lunged forms.

5. *Cryptobranchus allegheniensis.* — In *Cryptobranchus* the ypsiloid cartilage is very well developed and its articulation with the pubis displays marked mobility. It differs somewhat in form from that of the salamanders, in that its lateral arms are very much longer and rapidly broaden toward the outer ends so that they are spatulate in shape (Fig. 7). The cartilage begins to

fork slightly anterior to the first myocomma and the lateral arms extend obliquely across the third myotome.

The musculature of the cartilage is interesting. Superficially, the first myotome of the rectus abdominis is imperfectly differentiated into two portions, and the medial fibers not only of the first but of the second myotome are inserted into the stem of the cartilage, the more anterior fibers of the second monotome, however, being inserted into the ventral surface of the cartilage at the very base of the arms.

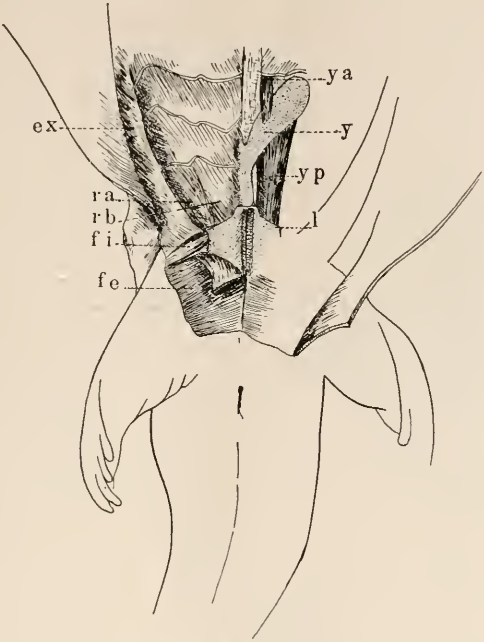

FIG. 7. Dissection of *Cryptobranchus alleghenicnsis* showing the ypsiloid cartilage and the muscles associated with it, × 2½. The external oblique and rectus abdominis muscles have been removed from the left side. Abbreviations as in previous figures.

The deeper layers show a strongly developed *M. ypsiloideus posterior*, differing from that of the forms already described in that its insertion into the *Cartilago ypsiloides* extends only about two thirds the length of the arm, the thin, expanded, outer third of the arm being free from all muscular attachment. The most medial fibers of the third myotome extend as a small bundle from the third myocomma to an insertion into the middle of the ventral surface of the lateral arm of the cartilage. This differentiation from the third myotome is the only representative of a *M. ypsiloides anterior*, and except for its insertion it is not at all distinct from the adjoining fibers of the myotome.

None of the fibers of the transversalis are inserted into the ypsiloid cartilage. The edges of its aponeurosis in this region, however, are parallel with the cartilage and separated from it by a space about equal to the width of the cartilage itself. Moreover, the stem and proximal third of the arms of the cartilage are firmly bound to the aponeurosis; the portion of the cartilage involved in this attachment is much thicker than the free, distal, expanded region of the cartilage which lies in the third myotome in a sort of sheath between the aponeurosis of the transversalis and the deeper layers of the rectus abdominis and has neither muscular nor aponeurotic attachments.

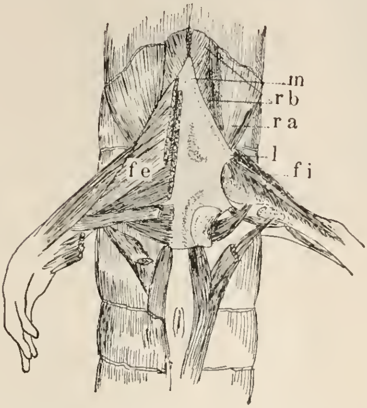

FIG. 8. Dissection of *Necturus maculatus* showing a ventral view of the pelvic region, with the *M. pubo-ischio-femoralis externus* of the left side removed, × 1. Abbreviations as in previous figures.

[From an unpublished drawing by H. H. Wilder.]

The origin of the anterior portion of the *M. pubo-ischio-femoralis internus* does not involve the ypsiloid cartilage, although it extends to the extreme anterior margin of the pubo-ischium.

It should be mentioned in connection with this description of *Cryptobranchus allegheniensis* that Hyrtl ('65) has described the ypsiloid cartilage of *Cryptobranchus japonicus* as lacking bilateral symmetry in that the right lateral arm is subdivided into three branches while the left is simple. This description was, I suppose, based upon a single individual and the condition was quite probably an abnormal one.

6. *Necturus maculatus.* — In *Necturus* there is no vestige whatever of a *Cartilago ypsiloides.* The pubo-ischium is produced anteriorly into a long median point (the epipubic process of previous writers), the sides of which slant gradually posteriolaterally to the outer angle of the pelvis where a lateral process is slightly developed (Fig. 8). The first myotome of the rectus abdominis muscle is correspondingly narrow at the ventral midline, and much wider toward its lateral boundary. Its fibers are inserted along the entire anterior margin of the pubo-ischium and show a differentiation into a medial (*rb*) and a lateral portion (*ra*), the latter inserted into the lateral portion of the pubis. In some specimens this lateral portion is more distinct than in others, and occasionally assumes the character of a semi-independent muscle, as in *Diemyctylus.* There is no indication whatever of a muscle dorsal to the main mass of the rectus abdominis and inserted into the linea alba, to suggest a vestigial ypsiloid musculature. The *M. pubo-ischio-femoralis internus* moreover, has its origin wholly from the pubo-ischium instead of arising in part from the linea alba as it does in the case of the lungless salamanders. There is therefore absolutely no indication of the previous existence of a *Cartilago ypsiloides* in this species.

7. *Amphiuma means.* —*Amphiuma means* shows a similar failure of all trace of an ypsiloid apparatus. The pubo-ischium lacks a mid-ventral symphysis in this form and there is only a slight anterior prolongation of the cartilage on either side of the mid-ventral line where the two halves of the girdle are in contact. The muscular differentiation in this region is, however, practically similar to that already described for *Necturus.*

8. *Siren lacertina.* — This species not only shows no trace of a pelvic girdle and appendages, but, as might be expected, there is also no muscular differentiation to indicate the former presence of an ypsiloid apparatus.

B. *Development of the Ypsiloid Apparatus.*

My material for the study of the development of the ypsiloid apparatus was somewhat limited. It consisted of (1) larvæ of *Amblystoma opacum* — various stages from 37 to 50 mm. in length ; (2) a series of horizontal sections of the larvæ of *Triton* *alpestris*, length from tip of snout to cloaca, 13 mm.; (3) small specimens of *Diemyctylus viridescens* in the terrestrial stage, 32–68 mm. in length; (4) larvæ of *Spelerpes ruber*, and *Spelerpes bilineatus;* (5) larvæ of *Desmognathus fusca* from 17.5–25 mm. in length.

The methods employed in the study of these larval forms were (1) dissection of the larger ones, prolonged staining in methylene blue being used in some cases to bring out the cartilage; (2) staining in borax carmine and clearing *in toto* the ventral wall of the posterior part of the body cavity including the pubo-ischium and proximal portion of the femur; (3) horizontal serial sections of the ventral body wall; and (4) transverse serial sections of the posterior part of the body. In the case of each of the younger stages all three of the latter methods were used.

In 37 and 42 mm. long *Amblystoma opacum* larvæ there is no trace of the ypsiloid cartilage. The two halves of the pubo-ischium are quite separate. The muscular abdominal walls show the two primitive laminæ (obliquus externus profundus and obliquus internus) with the rectus abdominis as a ventral continuation of both. In the larger specimens the obliquus externus superficialis and the transversalis appear as secondary developments in the form of very thin laminæ. There is a noticeable difference in size of fiber between the medial well differentiated portion of the rectus abdominis and the more lateral region which grades imperceptibly into the deep external oblique on the outside and the internal oblique within. The latter region of the rectus abdominis (*i. e.*, the rectus abdominis profundus), like the two primitive laminæ with which it is continuous, consists of large fibers two or three times the diameter of those constituting the medial portion of the rectus. The latter are thus easily recognized both in sections and in the *in toto* preparations.

All of these early stages show that in the somite immediately anterior to the pubo-ischium, the inner portion of the rectus abdominis is differentiated into a muscle which deviates sharply from the general longitudinal course of the rectus abdominis. Its fibers, which are small like the rest of the medial portion of the rectus abdominis, arise in the lateral processes and along the anterior edge of the pubis and extend obliquely medially to be inserted partly into the linea alba of the first somite. From the point where the first myocomma joins the linea alba, the insertion of the muscle follows an outwardly curving line which ends about half way across the second myotome, and thus with the insertion of the corresponding muscle of the opposite side maps out the exact location of the future *Cartilago ypsiloides*. This muscle is evidently the *M. ypsiloideus posterior* and is at this stage the only definite indication of an ypsiloid apparatus.

In *Amblystoma opacum* larvæ of 50 mm. I find that the ypsiloid cartilage has appeared. It possesses practically the adult form and relationship, but is very thin, especially toward the ends of the lateral arms. The stem of the cartilage is quite separate from the pubo-ischium which at this stage still consists of two wholly separate lateral halves. I was unable to obtain larvæ of *Amblystoma opacum* between 42 and 50 mm. in length and can therefore make no statement concerning the earliest appearance of the ypsiloid cartilage. Its entire absence in the 42 mm. stage, however, considered in connection with its complete formation in the 50 mm. stage in which the two halves of the girdle are still separate, points conclusively to the origin of the ypsiloid cartilage independently of the pelvic girdle as a chondrification of the linea alba of the first somite and of the deeper portion of the first myocomma. The possibility of such chondrification of regions of muscular attachments upon which a special strain is brought is well established, and the lack of correspondence in this case between the transverse direction of the myocomma and the curved form of the arms of the cartilage may be looked upon as expressing a resultant of forces, since the arms of the cartilage tend somewhat to follow the direction of the edge of the aponeurosis of the transversalis, the posterior portion of which, we have already seen, is usually eventually inserted into it.

The horizontal series of sections of the *Triton alpestris* larva show a well developed ypsiloid cartilage. This is wholly separate from the pubo-ischium though, as in *Amblystoma*, articulated with it. The two halves of the pubo-ischium are at this stage still quite separate.

All of the specimens of terrestrial *Diemyctylus* which I dissected show the ypsiloid cartilage well developed and with practically the same muscular attachments as in the adult.

From my necessarily limited study of larval forms, it appears, therefore, that (1) ypsiloid cartilage is of later origin than the differentiation of the muscles associated with it, and (2) that it arises as an unpaired structure in association with these muscles and at a time when the girdle itself still exhibits its paired nature. *It cannot, therefore, be interpreted as having arisen from the epipubic process, but must be regarded as an independent chondrification in association with differentiations of the innermost portion of the rectus abdominis muscle in the two somites immediately anterior to the pelvic girdle.*

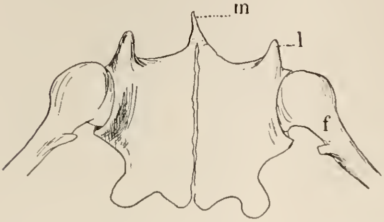

Fig. 9. Ventral view of the puboischium of *Desmognathus fusca* larva (21 mm.) obtained by maceration, × 25. Drawn with camera. Abbreviations: *f*, femur; *l*, lateral process; *m*, median process, at this stage the only median part.

Larvæ of both *Spelerpes* and *Desmognathus* show, as do those of lunged forms, an early differentiation of the *M. ypsiloideus posterior.* As in the adult lungless forms the fibers of this muscle insert, in the absence of the ypsiloid cartilage, into the linea alba of the first somite.

I had thought it possible that these larval stages might even show a vestige of the ypsiloid cartilage itself, and there is, in fact, some indication that such may be the case though I have as yet been unable to obtain the stage necessary to absolutely prove it. The union of the two halves of the pubis begins at the extreme anterior end, thus forming the future median anterior process of the pubis. This union (Fig. 9) appears to occur, not as a direct fusion of the two halves by the process of chondrification of the connective tissue between them, but rather by a fusion of each half with a median unpaired anlage, which lies in the linea alba anterior to the girdle. Thus in transverse section this median portion shows no trace whatever of a paired nature, a fact which is especially significant when the condition is compared with that of *Necturus* larvæ in which the median anterior process of the pubis (*Processus epipubicus*) shows a paired nature even to its extreme end. However, I have not succeeded in obtaining either a *Spelerpes* or a *Desmognathus* of a stage just previous to the formation of this connection between the two halves of the pubis, and I am not sure, therefore, that the median unpaired portion ever exists as a separate cartilage arising like the ypsiloid cartilage anterior to the pelvic girdle and independent of it. If it has such a separate origin it is undoubtedly a vestige of the stem of the ypsiloid cartilage.

C. *The Homology of the Ypsiloid Apparatus.*

There are two diametrically opposed views as to the homology of the ypsiloid cartilage. One of these is that indicated by the quotations given earlier in this paper, viz., that the *Cartilago ypsiloides* is the homologue of the median anterior process of the pubis (*Processus epipubicus*) such as is found either single or paired in certain of the *Perennibranches* and *Derotremes*. Moreover, this homology is extended to include the similarly situated process in various *Selachians*, *Ganoids* and *Dipnoans*. This opinion as to the homology of the ypsiloid cartilage has been held very strongly by C. K. Hoffmann ('73–'78) and R. Wiedersheim ('92) and corroborated by certain observations of H. Riese ('91). Aside from general similarity of location of the ypsiloid cartilage and the *Processus epipubicus* the homology is apparently based upon a continuity of the cartilage tissue of the pubis with that of the stem of the ypsiloid cartilage. This condition is, as has been shown, not the usual one in the adult, although Riese ('91) found it to exist in *Tylototriton verrucosus*, and Wiedersheim has noted in the case of old individuals of other species a condition which he designates as a secondary fusion. Wiedersheim says, however, regarding the adult condition, that "Man bei histologischer Untersuchung in Allgemeinen viel häufiger auf verbindende Knorpelbrücken zwischen der Hauptmasse des Beckens und dem Epipubis stösst, als man dies nach der einfachen Präparation mit Messe und Pincette erwarten sollte."

In *Triton alpestris* larvæ Wiedersheim found, as I have done, that the ypsiloid cartilage arises by an independent anlage, but in larvæ of *Triton helveticus* and in a 26 mm. Axolotl he found the cartilage element continuous, although he adds: "Diese

Verbindungszone bestand im vorliegenden Fall nur ventralwärts und werde weiter dorsalwärts d. h. gegen das Cavum pelvis zu durch zellreiches Bindgewebe ersetzt." He finds, moreover, that "Um diese Zeit stellt das Epipubis eine auf dem Vorderand der Beckensymphyse aufsitzende spitzhöckerige, durchaus unpaare Vorwölbung dar, welche nur langsam zapfenartig nach vorn auswächst, und sich erst verhältnismässig spät in die schon erwähnten zwei Äste gabelt." The movable articulation of the *Cartilago ypsiloides* is then, according to Wiedersheim's interpretation, a secondary condition.

That there is, on the other hand, an apparent inconsistency between this idea of the homology of the *Cartilago ypsiloides* and the well-established fact of the paired nature of the anlage of the pelvic girdle, Wiedersheim at least tacitly admits when he says: "Die Verwischung des ursprünglichen Verhaltens prägt sich nämlich bei Salamandrinen in dreifacher Weise aus, erstens darin, dass hier von einer paarigen Anlage des Epipubis ontogenetisch nichts mehr nachweisbar ist, zweitens, dass zwischen diesem und dem übrigen Becken häufig eine Kontinuitätstrennung besteht, und drittens endlich, dass das kopwärts schauende Ende des Epipubis eine secundäre Formänderung, eine Gabelung, erfahren hat."

A further, and, in my opinion, insurmountable objection to this homology lies in Wiedersheim's own statement that the ypsiloid cartilage is of later origin ontogenetically than the girdle and makes its appearance as late as at the time when the midventral symphysis of the halves of the pubo-ischium is taking place.

The second view as to the homology of the *Cartilago ypsiloides* is that held by Bunge ('80) and Baur ('91) that the structure is developed wholly independently of the pelvic girdle to which it becomes secondarily articulated. With regard to this homology Bunge says:

"Es (the epipubis) ist eben eine Gebilde sekundärer Art, das ausschliesslich den Amphibien zukommt, wie ja Ähnliches auch bei anderen Wirbelthieren beobachtet werden kann, z. B. das Hypoischium der Saurier . . . Der Ansicht Wiedersheim's dass der Epipubis als ein erst sekundär von der knorpeligen *Pars* *pubica*, resp. deren Verlängerung zur Symphysenbildung abgegliedertes Gebilde sein kann, da dasselbe sich als einheitlicher Knorpel vor dem proximalen Ende der Symphyse anlegt, gleichfalls nicht zugestimmt werden."

Baur, basing his reason upon the fact of the completely paired origin of the true epigastroid (*i. e.*, epipubic) process, as shown by *Necturus*, for example, draws the following very definite conclusion with regard to this unpaired, more anterior structure, the *Cartilago ypsiloides* :

"I believe the ypsiloid cartilages are of secondary origin, developing independently from the gastroid (*i. e.*, pubic) cartilage. The long epigastroid of the *Chelyiidæ* is homologue to the short epigastroid in *Testudinidæ ;* homologue to the anterior portion of the gastroid cartilage in *Necturus ;* homologue to that portion of the gastroid in salamanders and *Dactyletra* to which the ypsiloid cartilages are connected. I consider these cartilages as a later acquisition and they may develop in any group, *Batrachia*, *Pterosauria*, *Monotremata*, *Marsupulia*." So far, even, as similarity in location between the *Cartilago ypsiloides* and the epipubic process of the lower Urodeles is concerned, I have been unable to find any ground for the homology. In none of the Urodeles in which the *Cartilago ypsiloides* is lacking have I found the epipubic process crossing even a single myotome of the rectus abdominis. In *Necturus*, the form in which the epipubic process is most conspicuously developed, there is merely a corresponding narrowing of the posterior myotomes of the rectus abdominis, particularly the first one (Fig. 8).

Whether the ypsiloid cartilage is ever in any case continuous with the pubis or not, it is very evidently a separate structure, an independent chondrification of the linea alba of much later origin than the pelvic girdle to which it sooner or later becomes articulated. Thus considered, the ypsiloid cartilage presents no obstacle to the idea of the paired nature of the anlage of the pelvic girdle. Its existence is moreover explained quite in accordance with the principle which accounts for the origin of similar structures (*e. g.*, the sternebræ of *Necturus*) in those connective tissue regions where especially strong origin or insertion of the muscle fibers is necessary. I have not had the opportunity to study either the anatomy or the habits of *Dactyletra* in which an apparently similar cartilage to the *Cartilago ypsiloides* is formed. and can therefore express no opinion as to this homology.

That the *Cartilago ypsiloides* of salamanders is homologous with the marsupial bones of *Monotremata* and *Marsupulia* is a view which has been considered so completely established that Dugés ('55) named this cartilage in salamanders the "marsupial cartilage." Huxley also accepted this idea of its homology and it is one of the principal points made by Wiedersheim ('92) in his Phylogenie der Beutelknochen, in which, of course, this interpretation is quite consistent with his idea that both the ypsiloid cartilage and the marsupial bones are differentiations of the epipubic process. Moreover, the acceptance of this homology is indicated in the various names which have been given to the *M. ypsiloideus posterior*, such as pyramidalis (Hoffmann). Leaving out of account the question as to whether the ypsiloid cartilage and marsupial bones are of similar origin so far as the pelvic girdle is concerned, the supposed homology between the two is disproved by their relations to the rectus abdominis muscle. The ypsiloid cartilage lies dorsal to the main mass of this muscle; the marsupial bones are, of course, ventral to it. A comparison of musculature, therefore, shows the lack of homology of the ypsiloid apparatus with the marsupial. The musculature of the ypsiloid cartilage is derived from the deeper layers of the rectus abdominis and from the transversalis; from the very poistion of the marsupial bones, on the other hand, it is evident that the musculature of this apparatus is derived from the superficial abdominal muscles.

The pyramidalis, which has been homologized with the *M. ypsiloideus posterior* is, for example, the most superficial portion of the rectus abdominis. Further, it is on the wrong side of the marsupial bone to make the homology a consistent one throughout, since, if the marsupial bones correspond to the lateral arms of the *Cartilago ypsiloides*, a muscle to be the homologue of the *M. ypsiloideus posterior* must extend from the outer edge of the marsupial bone to the pelvis, not as does the pyramidalis, from its medial side to the linea alba or sternum. The pyramidalis and the posterior ypsiloid muscle are then homologous only in the very general sense that both are differentiations from the rectus abdominis; they are differentiated from different layers and in connection with structures which are not themselves homologous. They are, in other words, independent differentiations occurring in widely separated forms and in response to absolutely different physiological needs.

Part II. The Function of the Ypsiloid Apparatus.

A. *Respiratory Habits of Lunged Salamanders.*

Beyond the statement of the very evident fact that the ypsiloid cartilage furnishes the attachment for certain of the abdominal muscles (Wiedersheim, '75), I have been unable to find, in the literature upon the subject, any explanation of its function. There is, however, as has already been said, so apparent a correlation in the *Salamandrida* between the presence of the apparatus and that of the lungs, that the explanation of its function will involve, first of all, a discussion of the respiratory habits of lunged salamanders.

The more obvious respiratory movements of lunged salamanders when breathing air have been very clearly described by Bruner ('96). In brief, two forms of aerial respiration occur, one merely a bucco-pharyngeal, the other a pulmonary respiration. Both of these may be readily observed in the case of any lunged salamander. The first takes place almost constantly and with great rapidity. It begins with an enlargement of the bucco-pharyngeal cavity by lowering the hyobranchial apparatus; this results in air being drawn in through the nares. Following this inhalation is an exhalation in which the floor of the mouth rises again. These movements follow each other so quickly that the visible effect is a rapid fluctuation of the throat. The mouth remains tightly closed during the entire process, and the respiratory currents make use of the nasal passages alone.

At frequent, though irregular intervals, during bucco-pharyngeal respiration, acts of pulmonary respiration occur. These are easily distinguished externally from the bucco-pharyngeal form by the fact that the depression of the floor of the mouth is a prolonged and exaggerated one, during the latter part of which a contraction of the *M. constrictor naris* occurs. According to

Bruner, the effect of this contraction is to completely close the external naris. My own observations of *Diemyctylus* and the *Amblystomas* made by the aid of a lens do not, however, corroborate this statement, since I have frequently seen the external nares fail to close completely during pulmonary respiration, although there is always an almost complete closure.

As a result of the prolonged depression of the floor of the mouth air is first drawn in through the open nares, as in bucco-pharyngeal respiration. This part of the process is known as aspiration. During the latter part of the act of depression, however, when the external nares are closed, air is drawn from the lungs into the mouth through the opened glottis and the air in the mouth thus becomes a mixture of pure and impure air. This part of the process is termed expiration. When the floor of the mouth rises again some of this mixed air is forced into the lungs, the external nares being still closed. This constitutes the process of inspiration. Finally the external nares are opened again, and the fluctuating movements of bucco-pharyngeal respiration are resumed.

In addition to these two methods of aerial respiration the lunged salamanders which have come under my observation possess, when in the water, an *aquatic* bucco-pharyngeal respiration.

The Gages ('86*b*, '91) have reported such an aquatic respiration for *Diemyctylus* as well as for some of the lower *Urodeles*. Their statements are, however, indefinite as to the exact method by which the water is alternately taken into and expelled from the mouth. O. P. Hay ('89) seems to have made more exact observations upon *Amblystomas*, of which he says that " streams of water are drawn in through the nostrils and this water is then expelled at intervals by the mouth." This is precisely the method of bucco-pharyngeal respiration of water which I have many times verified with a lens by the aid of solid particles (carmine or sediment) suspended in the water in which specimens of *Diemyctylus* and *Amblystoma* were submerged. The muscular act seems to be exactly the same as in the bucco-pharyngeal aerial respiration, but owing to the heavier fluid the act is a much slower one, though varying in depth and rapidity with the activity of the specimen, as the accompanying tabulation of observations shows. Moreover, the expulsion of water takes place through the slightly opened mouth as well as through the nares. *Amblystoma opacum*, which is said to be the most terrestrial of all the *Amblystomas*, showed the least readiness to adopt this aquatic mode of respiration, the pharyngeal movements being very feeble as if they occurred in response to an almost forgotten instinct. They are probably not of sufficient respiratory value to support life, since these specimens die in a short time if compelled to remain in the water.

RECORD OF OBSERVATIONS OF AQUATIC BUCCO-PHARYNGEAL RESPIRATION IN DIEMYCTYLUS VIRIDESCENS.

(In each experiment a different individual was used.)

No. of Experiment.	Average No. of Respiratory Acts per Minute.	Largest No. for One Minute.	Smallest No. for One Minute.	Duration of Experiment in Minutes.	Nature of Respiratory Acts.	Activity of Specimen During Experiment.
No. 1.	21.4	23	19	10	Shallow.	Slightly active.
No. 2.	15.14	20	11	7	Deep.	Inactive.
No. 3.	10.04	13	9	5	[Not recorded.]	Slightly active.
No. 4.	8	18	3	10		

Detailed Record for Each Minute of Experiment No. 4.

	4				Very shallow.	Inactive.
	9				One very deep.	Walking slowly.
	3				Very deep.	Inactive.
	12				Shallow.	Active.
	4				Deep.	Inactive.
	9				Shallow.	Inactive, after a period of activity.
	18				Shallow.	Active.
	6				One very deep, ending in a prolonged gape.	Inactive.
	3				Very deep.	Inactive.
	12				Shallow.	Slightly active, following a period of great activity.

RECORD OF OBSERVATIONS OF AËRIAL BUCCO-PHARYNGEAL AND PULMONARY RESPIRATION IN DIEMYCTYLUS VIRIDESCENS.

No. of Experiment.	Average No. of Bucco-phar. Resp. per Min.	Largest No. for One Minute.	Smallest No. for One Minute.	Average No. of Pulmonary Resp. per Min.	Largest No. for One Min.	Smallest No. for One Minute.	Duration of Observations in Minutes
No. 1.	187.7	213	145	1.1	4	0	10
No 2.	125.75	146	104	[Not recorded.]			4

The method of change of respiratory habit necessitated by the transition from one median to the other is interesting. When a *Diemyctylus* which has been breathing air is submerged in water, bucco-pharyngeal respiration of water begins almost at once. *Amblystomas*, being less thoroughly aquatic, postpone this change of habit for a longer or shorter time. The nares in this case are at first tightly closed and if the animal is kept submerged for only a few minutes it may not establish aquatic respiration at all. When a specimen which has fully established the aquatic habit of respiration is taken from the water there is evinced more or less mechanical difficulty in reëstablishing the aërial habit. This difficulty arises from the fact that the nasal passages are filled with water which must be removed before rapid, unimpeded respiration of air can occur. The efforts to do this involve forced and greatly exaggerated depressions of the floor of the mouth, a device which may prove efficacious in two ways, first, by drawing the water from the nasal passages into the mouth, and second, by drawing from the lungs a supply of air which can be used to force the water out of the nasal passages through the external nares. The transition from aquatic to aërial respiration may thus involve much effort and a considerable loss of time. I have observed specimens of *Diemyctylus* to consume ten minutes or more before perfectly normal aërial respiration was established. *Amblystomas* make the transition more quickly.

In connection with this mechanical difficulty of rapid transition from aquatic to aërial respiration, some lunged salamanders, notably *Diemyctylus*, have acquired for use when in the water a modification of the ordinary method of pulmonary respiration. Frequently air must be taken into the lungs during the brief period when by a rapid swimming to the surface, sufficient momentum has been acquired to force the head for an instant out of the water. It is evident that under these conditions the nostrils are utterly useless as air passages, as they are filled with water. Moreover, even if they were empty of water, or could be emptied in so brief a time, the ordinary method of drawing in air through such narrow passages is far too slow to be made use of here. The method employed is, therefore, a quick, gulping motion by means of which the water in the mouth is replaced by air. This is immediately followed, as the head again returns into the water, by a forcible swallowing motion as a result of which the air is forced from the mouth partly into the lungs and partly out through the nostrils. Of those species the habits of which I have studied, *Diemyctylus viridescens* accomplishes with the greatest ease the act of taking air into the lungs in this way, an observation quite in harmony with the fact that *Diemyctylus* has the reputation of being the most thoroughly aquatic of our American salamanders.

The Gages ('86*a*, *c*) have shown that, in general, an animal having a mixed aquatic and aërial respiration depends mainly upon the latter for its supply of oxygen. I am not convinced, however, that this is of necessity true in the case of lunged salamanders living under aquatic conditions. *Diemyctylus* and the *Amblystomas*, it is true, not only swim frequently to the surface and take in air, but, if it is possible, will partly crawl out of the water and for a shorter or longer time each day will breathe air normally. However, to test the absolute physiological necessity for aerial respiration, I experimented as follows: Several specimens of *Diemyctylus* were enclosed in small wire cages which were immersed to a depth of about 15 inches in a small tank of running water. To prevent the collection of bubbles of air upon the inside of the wire, the cages were frequently shaken to remove the bubbles while they were still too small to be used in breathing and thus vitiate the experiment. To ensure this frequent agitation during the night when personal attention to the matter was inconvenient, I used the simple device of placing a large and lively specimen of *Necturus* in the tank with the cages. The *Necturus*, being nocturnal in its activities, accomplished quite as efficiently the duty of keeping the cages free from air as was done during the day time by my own exertions. The precaution was taken, moreover, to make it impossible for any activity of the *Necturus* to lift the cages out of the water.

For periods varying from seven to ten days specimens of *Diemyctylus* were thus kept completely submerged and they remained in an active condition and apparently suffered no inconvenience as a result of the experiment. The capillaries of the skin, however, as observed by means of a lens, were much more distended with blood than those of a specimen which had meanwhile lived a free aquatic life with access to the air. Apparently the skin, which, being supplied with capillaries may be looked upon as an accessory respiratory apparatus, had proved itself, in the emergency, equal to the extra demand made upon it.

An interesting effect of the disuse of lungs in this experiment showed itself in the great difficulty with which specimens, thus confined to the water, reëstablished the habit of filling the lungs with air when they were released from their imprisonment. Ordinarily, when a *Diemyctylus* swims to the surface and takes in air by the gulping process already described, there is an abundant visible proof of the fact that air has entered the lungs in the increase in girth of the body and especially in the immediate increase in buoyancy to such an extent that the specimen which before had been able to sustain itself in the water only by active swimming, suddenly becomes lighter than water and passively floats. When, however, specimens which had for several days been prevented from using their lungs were once more set free in the water and swam to the surface, although great gulps of air were taken, there was not the usual subsequent increase in buoyancy and the air escaped immediately in large quantities from both nostrils as the head sank again below the surface of the water. The effort to fill the lungs was repeated many times interspersed with intervals of rest lasting 15 or 20 minutes, so that several hours elapsed before any effect seemed to be produced upon the disused lungs. One could from the fruitless efforts of the animals imagine the lungs in a collapsed condition, the inner surfaces in contact with each other, and therefore resisting the entrance of air; and such, indeed, was found to be the case in other specimens which had been similarly confined under water and then killed without having had access to the air.

B. *The Hydrostatic Habits of Lunged Salamanders.*

Having discussed the various methods of respiration of lunged salamanders we are now prepared to describe those particular habits which involve, as will be shown, the use of the ypsiloid apparatus. Since my more extended observations have been made upon *Diemyctylus viridescens*, this species is the one which will be referred to almost exclusively in the following discussion.

As has been said, the adult *Diemyctylus* is the most aquatic of our American salamanders. To anyone who has observed, even casually, the activities of this little animal in the water, its absolute ease under aquatic conditions must have been evident. If the specimens are in a deep aquarium this physical ease is very readily observed. Occasionally they may be seen at the bottom where they walk about or take rapid little swims to higher levels from which, as soon as the swimming motions cease, they passively sink to the bottom again. More often they may be found at the very surface of the water where they float with great ease or rest upon the aquatic plants, sometimes supporting themselves upon these by the fore limbs and lifting the entire head above the water. Frequently, moreover, they will be seen suspended in the water at a greater or less depth, where they have the power to swim lazily to and fro with hardly a perceptible muscular action, to paddle about, using all four feet as propellers, or to dart swiftly through the water by means of a rapid lashing with body and tail, the legs meanwhile being closely pressed to the sides of the body. For many minutes they will sometimes remain absolutely motionless in the water, the body kept in place by the mere contact of a foot, or even of a single toe, with some plant or other stationary object.

These facts indicate that while the specific gravity of *Diemyctylus* is never far from one, it varies slightly, as shown by the passive sinking, suspension or floating of the body at different times. What are, then, the mechanical means by which these changes in buoyancy are accomplished?

If our observations begin with a *Diemyctylus* at the bottom of the aquarium, with a specific gravity greater than one, it will be found that sooner or later the animal will swim rapidly to the surface, and, by the modified process of pulmonary respiration already described, will take into the lung sufficient air to cause the body to float (Fig. 10, *b*), the minute portion of the back which appears above the surface bearing witness to the fact that the specific gravity has now become slightly less than one. The animal may remain in this condition for a longer or shorter time. If he swims to a lower level, the moment his motions cease his body rises slowly to the surface again. Usually, however, the increase of buoyancy is soon followed (within a minute or two) by the emission from the lungs through the mouth of one tiny bubble of air after another, seldom more than two or three in all, until his buoyancy is so perfectly adjusted that his specific gravity is exactly one. In this condition he can go about at ease, or remain motionless at any depth, and it is apparently only when he desires to sink to the bottom and remain there with some stability that by the emission of still more air the specific gravity is made sufficiently great to serve the purpose. There always occurs, however, a gradual loss of buoyancy even when there is no further emission of air, a loss which I have never observed to be made good until the animal again swims to the surface and takes in more air.[1]

Diemyctylus shows a still further delicacy of adjustment to its aquatic environment, since, under any condition, whether floating, suspended in the water or resting on the bottom, there is the power to change, without the slightest swimming motion, the direction of the long axis of the body. This adjustment may be best observed when the animal is suspended motionless in the water; since then all other factors which produce change of position are eliminated. The usual position of *Diemyctylus* when thus suspended is one in which the anterior end of the body slants slightly downward (Fig. 10, *b*). From this position the whole body, without the slightest bending, may swing through a vertical angle of perhaps 30° until the head is directed upward instead of downward. This change of direction is accompanied by a striking change in the shape of the animal. When the poise is such that the head slants downward, there is a pronounced bulging of the lateral and ventral walls of the posterior third of the body cavity, particularly noticeable in the angle between the ilium and the vertebral column, as though some mechanism within were exerting an outward pressure. As the

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[1] This phenomenon of loss of buoyancy without the emission of air is worthy of careful investigation. It is probably not attributable to a mere compression of air (as in the case of the air-bladder of the fish), since there is no subsequent increase of buoyancy without taking in more air. It must be due to an actual loss of gas from the lungs, probably owing to the excess of the volume of oxygen used over that of CO_2 and other gases given back to the lungs.

body swings upward, however, there occurs a marked constriction of the posterior part of the abdominal cavity, often so pronounced that the ventral wall (that is, the ypsiloid region) is drawn sharply upward (Fig. 10, *a*). In this condition the ventral contour of the body exhibits an angle between this posterior and the more anterior region. These changes of shape may best be seen in a specimen which has not been fed for several days, since they are partially masked by the presence of masses of food in the digestive tract.

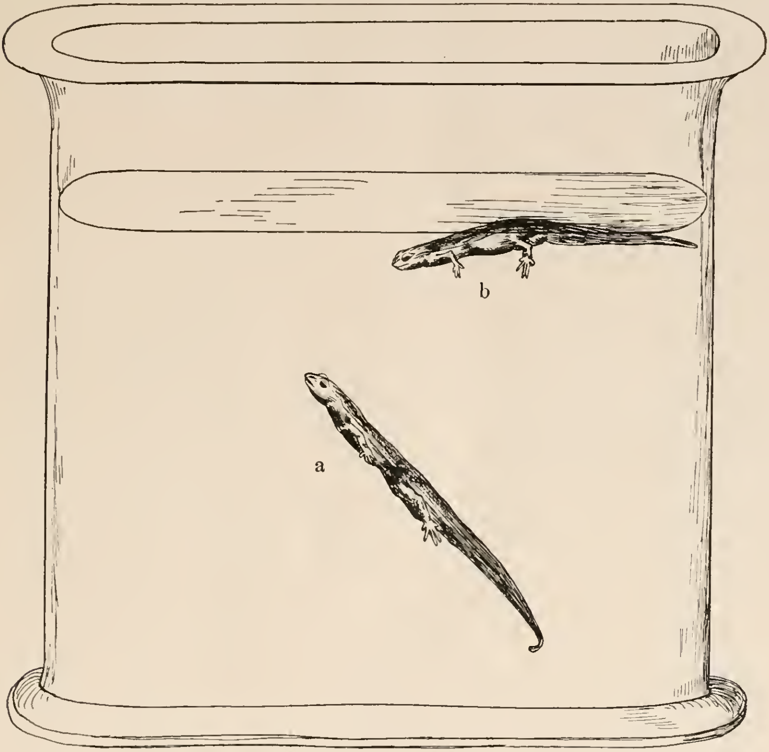

FIG. 10. *Diemyctylus viridescens;* (*a*) showing the body directed upward in swimming as a result of the compression of the posterior portion of the body cavity through the action of the ypsiloid apparatus; (*b*) showing a characteristic floating position with the posterior portion of the body cavity expanded and the anterior end of the body depressed.

They occur, moreover, not only during this inactive change in the direction of the long axis of the body, but changes of direction during active swimming involve a constant exhibition of corresponding changes of contour ; the constriction of the posterior abdominal region occurs when the swimming motion is upward (Fig. 10, *a*), the prominent bulging when the motion is downward. Further, a sudden change from a downward to an upward direction is *preceded* by an exaggerated constriction of the posterior part of the cavity, an act which conspicuously involves the sudden vigorous inpulling of the ypsiloid region. Evidently *there is in operation some mechanism for controlling the direction of the body, whether at rest or in motion, through the control of the relative buoyancy of anterior and posterior ends.*

C. *The Hydrostatic Mechanism of Lunged Salamanders.*

Turning now to the anatomy of the ypsiloid region, the explanation of this hydrostatic mechanism becomes very simple. The contraction of the *M. ypsiloideus posterior* exerts a strong pull upon the whole ypsiloid cartilage. The origin and insertion of this muscle are, however, so nearly in the same plane as the fulcrum (the articulation of the ypsiloid cartilage with the pubis) that it seems at first a question as to whether the cartilage would be bent upward or downward (*i. e.*, dorsally or ventrally) by the contraction of this muscle alone. It must be remembered, however, that the muscle is inserted into the upper (dorsal) surface of the stem of the cartilage and also that the more strongly developed portion of the muscle has its origin in the lateral portion of the pubis, a region which owing to the convexity of the body is slightly higher (more dorsal) than the insertion of the muscle and the articulation of the cartilage. Moreover, outside of the whole apparatus there are muscular walls (external oblique and rectus abdominis) which would resist any tendency to bend the cartilage downward, and with origin and insertion on so nearly the same plane as the fulcrum it requires only a slight resistance of this sort to turn the scale. Other muscles, moreover, are attached to the ypsiloid cartilage and coöperate with the ypsiloideus posterior to determine the direction of motion. The contraction of the ypsiloid portion of the transversalis exerts a decided upward (dorsalward) pull upon the ypsiloid cartilage while the anterior ypsiloid muscle, pulling upon the arms of the cartilage from the linea alba, at least lends a certain steadiness to the apparatus while at the same time it coöperates with the ypsiloideus posterior and the ypsiloid portion of the transversalis to pull the arms strongly inward.

Corroboration of the above explanation is furnished by those occasional specimens which happen to have been preserved with the ypsiloid muscles contracted. These cases show that the effect of the concerted contraction of the muscles associated with the ypsiloid cartilage is not only to bend the stem of the cartilage upward (dorsally) at its articulation with the pelvic girdle but to curve the flexible arms upward and inward (medially). Evidently the result of the contraction of the three pairs of muscles connected with the ypsiloid cartilage is a decided constriction of the posterior region of the abdomen and a consequent compression of the organs contained within it.

To understand in what way this action of the ypsiloid apparatus controls the relative buoyancy of the anterior and posterior regions of the body, the shape and position of the lungs must be considered. The lungs of *Diemyctylus* are exceedingly simple structures, mere sacs with no trace of the usual amphibian condition in which the cavity is subdivided by partial partitions. It seems impossible, in fact, that such very simple structures with so small a supply of blood can justify their existence merely as respiratory organs. In shape, also, the lungs of *Diemyctylus* are peculiar. Narrow anteriorly, they widen gradually and round off quite abruptly at the posterior end. The whole form is most adequately described, perhaps, as club-shaped. The statement often given as to the size of the lungs (viz., one third to one half of the length of the body cavity) I find quite incorrect when the observations are made upon freshly killed specimens. If the lungs of such a specimen be inflated through the glottis not even sufficiently to float the body in water (and therefore not unduly), subsequent dissection shows that the lungs extend the entire length of the body cavity so that their rounded, bulging, free ends lie on either side in the angle between the ilium and the vertebral column.

It is thus easy to see the cause of the bulging of the lateral and ventral walls of the posterior part of the body cavity.

Moreover, it is also evident that with the lungs inflated and with no muscular constriction of this posterior region this portion of the body will possess greater relative buoyancy than the anterior portion, or in other words the long axis of the body will assume its ordinary position with the anterior end slanting downward. As soon, however, as the ypsiloid apparatus is brought into action, the resulting pressure upon the posterior abdominal organs becomes exerted upon the clavate ends of the lungs thus forcing the air in them forward. The effect is to immediately increase the buoyancy of the anterior region of the body and diminish that of the posterior region.

On the other hand, when the muscles relax, the pressure of the air in the lungs, as well as the elasticity of all the parts concerned, causes the return of the air to the posterior region again and the bulging of the body wall in this region occurs as before. It seems probable that the superficial portion of the rectus abdominis which is attached to the ventral side of the ypsiloid cartilage (its fibers extending from the lateral arms to the stem and to the anterior margin of the pubis), may assist in straightening the curved ypsiloid cartilage, since when the cartilage is in the bent condition these fibers lie upon its convex (ventral) side.

Thus the ypsiloid cartilage and the muscles connected with it constitute, together with the lungs, the mechanism by means of which the relative buoyancy of anterior and posterior ends of the body may be controlled. One needs only to witness the constant use of this hydrostatic apparatus by *Diemyctylus* to understand how completely the absolute ease of the animal under aquatic conditions is due to its power to control the direction of its body by means of the rapid adjustment of the relative buoyancy of anterior and posterior ends. The ypsiloid apparatus is thus of vital importance in the free-swimming aquatic life of a species which, like *Diemyctylus*, depends for its food supply upon its ease of movement in water at any depth.

Opportunity has not been afforded me to study extensively the aquatic activities of lunged forms other than *Diemyctylus*. Several specimens of *Amblystoma punctatum* and *Amblystoma opacum*[1] have, however, been observed with regard to this point. Both

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[1] See p. 257 for statement with regard to the lungs of *Amblystoma opacum*.

of these species, although capable of much less perfect adjustment to aquatic life, resemble *Diemyctylus* in the fact that the lungs are used as hydrostatic organs. Almost the first act of an *Amblystoma* when it is placed in deep water is to swim to the top and take in the air sufficient to float the body. I have not observed an *Amblystoma opacum* to become sufficiently at home in the water to do more than to remain floating at the surface. *Amblystoma punctatum* will, however, after a little while, appear quite at ease, crawling about the bottom, floating at the surface, or swimming around with much freedom.

Although the greater thickness and breadth of the rectus abdominis of *Amblystomas* prevent the visible exhibition of the action of the ypsiloid region during aquatic life, changes of shape of the posterior lateral walls of the body are often observable. Upon one occasion a specimen of *A. punctatum* was observed floating in a horizontal position at the surface of the water. Suddenly there was a violent contraction of the posterior abdominal walls particularly noticeable in the lateral region, and immediately the position of the body became so nearly vertical that the head was sufficiently protruded from the water to make aerial respiration through the nostrils possible. This observation not only proved that the ypsiloid apparatus is functional in the control of the hydrostatics of *Amblystoma punctatum*, but it suggests the application of its action as a means for bringing the floating body into such a position that the respiration of air may occur. In the case of imperfectly aquatic forms this use of the mechanism might at times be extremely important. For example, *Amblystoma opacum* will frequently, if compelled to remain in the water, take this same almost vertical position at the surface with the nostrils out of the water and is thus able to breathe air.

That the lungs of *Amblystomas* are of greater importance as respiratory organs than are those of *Diemyctylus* is evidenced by the fact that they are more complicated in structure and therefore present a much larger respiratory surface. They are, however, like the lungs of *Diemyctylus*, of sufficient length when moderately inflated to extend the entire length of the body cavity and would therefore lend themselves readily to the hydrostatic function in connection with the ypsiloid apparatus.

With regard to the relative importance of the lungs of salamanders as respiratory and as hydrostatic organs, it is a significant fact that in no case have I found that a *Diemyctylus* or an *Amblystoma* which was out of the water and using its lungs normally in air-breathing, had sufficient air in the lungs to float the body when it was dropped into water. Almost the first act under these circumstances is to swim to the top and take in a quantity of air sufficient to float the body. This indicates plainly the secondary adaptation of the lungs as organs of buoyancy and it is easy to see how in the case of a species like *Diemyctylus* which has become thoroughly aquatic, the hydrostatic function might become of so much greater importance than the respiratory as to account for the apparent degeneration of the lungs as respiratory organs which is indicated by their simplicity of structure. Moreover, it is easy to understand how a mechanism such as the ypsiloid apparatus for controlling relative buoyancy of the anterior and posterior ends of the body, while useful to any lunged form for the longer or shorter periods during which it normally stays in the water, would become especially perfected in its action in the case of a thoroughly aquatic species.

D. *Negative Evidence Furnished by Lungless Salamanders.*

In corroboration of the above conclusions as to the function of the ypsiloid apparatus of lunged salamanders, we have the negative evidence furnished by the habits of lungless forms in which, with the single exception of *Salamandrina perspicillata*, the *Cartilago ypsiloides* is apparently lacking.

These forms have, of course, no hydrostatic powers. They are thus, unlike the lunged salamanders, incapable of a comfortable, free-swimming existence at any depth, but owing to lack of hydrostatic organs they must remain for the larger part of the time at the bottom. As Camerano ('94, '96) has pointed out, although certain lungless species may be more or less aquatic, their activities, even when in the water, are terrestrial. Various species of *Spelerpes*, *Plethedon* and *Desmognathus*, for example, will at first, when placed in an aquarium, swim to the surface, then around and around the edge of the aquarium, as if seeking a means of escape, but the instant that active swimming ceases, the body sinks clumsily and heavily to the bottom where they remain until disturbed, or until another effort is made to escape.

Consistently with the lack of hydrostatic apparatus, lungless forms show on the whole, little power to adapt themselves to aquatic life. Most of them are terrestrial in habit, some of them as, for example, *Plethodon cinereus* and *P. glutinosus*, being found far from any water supply, while the arboreal *Autodax* furnishes an extreme illustration of total abandonment of aquatic life. Those species, which, like *Desmognathus*, live along the banks of small streams, apparently never seek deep water, nor do they remain long submerged in shallow water, but often are found lying with the body in the water and the head (or at least the nostrils) out.[1]

Lungless forms, moreover, exhibit less adaptation to aquatic life in their respiratory powers, since unlike the lunged forms there is practically no aquatic bucco-pharyngeal respiration. When the animal is submerged, the nostrils, which have been widely open during aërial bucco-pharyngeal respiration, close at once and, so far as I have been able to carry my observations, the nares remain closed as long as the animal is in the water. In a few cases I have observed occasional feeble movements of the floor of the mouth, which were undoubtedly attempts at bucco-pharyngeal respiration, but even then the external nares were closed and the water was both drawn in and expelled through the slightly opened mouth.

Spelerpes ruber proved to be the most aquatic of all the lungless forms with which I experimented. One specimen lived for weeks at the bottom of the aquarium and was never observed to attempt to come to the surface except when disturbed. On the other hand, specimens of *Desmognathus fusca* invariably escape from the water when not caged, while *Plethodon glutinosus*, *Spelerpes guttolineatus* and *Spelerpes bilineatus* make frantic attempts to do so, but since they do not possess the power to crawl up the surface of the dry glass as *Desmognathus* does, their efforts are unsuccessful. This aversion to aquatic life, is, however, apparently not due to an actual physiological need, for specimens of *Desmognathus fusca*, *Plethodon glutinosus* and *Spelerpes*

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[1] See my article now in press on the "Naso-labial Groove of Lungless Salamanders."

guttolineatus suffer no physiological inconvenience when compelled to remain under water as in the experiment described above in which they were confined in wire cages immersed in running water for a week or more without access to air. They invariably, however, as soon as released, swam to the surface of the water and tried to escape, thus showing a strong instinct to seek terrestrial conditions even though their physiological needs were satisfied. It cannot, therefore, be argued that the aversion to aquatic life is due to lack of lungs and correlated ypsiloid apparatus but rather that the long continued terrestrial habit has resulted in the loss of these structures. An aquatic lungless form like *Spelerpes ruber* must then be regarded as having secondarily reacquired its aquatic habits.

Salamandrina, a lungless form which possesses an ypsiloid apparatus, is an interesting exception but by no means an embarrassing one, since it belongs to a wholly different group of salamanders and thus represents a case of analogical resemblance. It might be expected *a priori* to show less divergence in structure from the lunged salamanders than do the members of the families *Plethodontidæ* and *Desmognathidæ*, since its departure from the habits of the rest of its own family, the *Pleurodelidæ*, is presumably comparatively recent. Thus we find that it still possesses arytenoid cartilages and rudiments of lungs. Similarly the ypsiloid apparatus persists, though Wiedersheim ('75) called attention to the fact that the cartilage is less strongly developed than in the case of *Triton* in which it usually undergoes more or less calcification. There are additional evidences in the condition of the muscles of the region (already described) that slight degeneration of the apparatus has taken place.

Of course a secondary adaptation to some other function might tend to preserve the apparatus, but as I have not yet had the opportunity to observe the living *Salamandrina* I can make no statement as to the probabilities of such secondary adaptation.

E. *The Hydrostatic Functions of Perennibranches and Derotremes.*

The following table expresses briefly the conditions of lower *Urodeles* with reference to the possession of lungs and ypsiloid apparatus, together with a general description of body form and habits :[1]

Name.	Lungs.	Ypsiloid Apparatus.	Form of Body.	Habits.
Necturus.	Present.	Wholly lacking.	Short and broad with compressed tail.	Lives largely on bottom. Floats only under abnormal conditions, *i. e.*, when water becomes foul.
Proteus.	Present.	Cartilage lacking; facts concerning musculature unknown.	Slender with compressed tail.	Facts unknown.
Siren.	Present.	Wholly lacking.	Slender and eel-like.	Burrows and swims.
Typhlomolge.[2]	Lacking.	Cartilage lacking; facts concerning musculature unknown.	Slender.	Crawls about at bottom.
Axolotl.	Present.	Present.	Short and broad; tail compressed.	Swims.
Cryptobranchus.	Present.	Present.	Short and broad; tail compressed.	Swims; is said to come frequently to the surface for air.
Amphiuma.	Present.	Wholly lacking.	Slender and eel-like.	Burrows, swims with great ease and comes frequently to the surface for air.

A comparison of the facts given in this tabulated form shows that the following classifications may be made :

1. Forms with lungs and ypsiloid apparatus — *Axolotl, Cryptobranchus.*

2. Forms with lungs but without an ypsiloid apparatus — *Siren, Necturus, Proteus, Amphiuma.*

3. Forms with neither lungs nor ypsiloid apparatus — *Typhlomolge.* Of the first group it may be said that since *Axolotl* is a larval form of a salamander which has lungs and an ypsiloid apparatus, its condition is exactly what one would expect to find

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[1] The statement of facts relating to the habits of these forms is the result of my own observation only in the case of *Necturus* and *Amphiuma*. In other cases the information has been derived from various scientific works.

[2] Miss Emerson (1905) has shown valid reasons for regarding *Typhlomolge* the permanent larval form of one of the *Plethodontidæ*.

from the facts already given as to the development of the ypsiloid apparatus in other *Amblystoma* larvæ. Moreover, the lungs are doubtless functional as hydrostatic organs and the ypsiloid apparatus probably serves its usual purpose in controlling this function.

I have not had the opportunity to observe the habits of *Cryptobranchus*. From the descriptions which have been given of its habits, however, one can readily believe that its lungs and ypsiloid apparatus are important, functionally, as hydrostatic organs. The large size of the ypsiloid cartilage and the well developed state of its muscles is, in itself, an indication of its functional value. Moreover, the body is relatively short and the three posterior somites of the trunk, that is, the region which would be constricted by the action of the ypsiloid apparatus, form a sufficiently large proportion of the entire length of the body to render such constriction effective.

With regard to the second group two general types of body form may be observed — the short, stout, heavy body of *Necturus*, and the eel-like form such as *Amphiuma* and *Siren*. Camerano ('96), has expressed his belief that in all these forms the lungs have an important hydrostatic function. My own observations of the living animals have been confined to *Necturus* and *Amphiuma*. The former I have never observed to float except upon one or two occasions when the water has become very foul. Under ordinary conditions the *Necturus* in captivity stays at the bottom of the aquarium, often hiding in crevices between rocks. Occasionally, especially if much disturbed, it will swim to the surface and take in air through the mouth by a gulping motion. This is usually followed by an immediate escape of air through mouth and gill-slits as the animal sinks slowly to the bottom. Undoubtedly the natural habitat of *Necturus* is at the bottom; it has, therefore, no use for an apparatus controlling the hydrostatic function of the lungs. Moreover the total lack of all traces of an ypsiloid apparatus indicates at once that the species has not descended from one with such an apparatus, since the muscular vestiges in wholly lungless forms show how very slowly the degeneration of such an apparatus occurs. Neither can we believe that the *Necturus* is a permanent larval form of a lunged salamander, since such a larval form would certainly show traces of an ypsiloid apparatus. In this connection it may be noted that H. H. Wilder in a footnote to Miss Emerson's recent work on *Typhlomoge* (1905) stated that Kingsbury's (1905) suggestion that *Necturus* may be a permanent larva of one of the *Plethodontidæ* is untenable, since all of the *Plethodontidæ* are lungless. It now seems that in view of its lack of ypsiloid apparatus, *Necturus* is ruled out of all possible claim as a salamander larva.

My observations of *Amphiuma* give evidence of greater hydrostatic powers than in the case of *Necturus*. While this animal, like *Necturus*, spends its time largely at the bottom of the aquarium, burrowing if the mud is sufficiently soft, it occasionally comes to the surface of the water to breath air. To accomplish this, the tip of the snout is thrust out of the water, the body being sustained at the surface by its own active serpentine movements. Air is taken into the lungs by the process of pulmonary respiration already described for lunged salamanders. As the air enters, the buoyancy of the body increases perceptibly, often until the body actually floats. I have seldom, however, observed a specimen to retain this buoyant condition for more than a few minutes. It will swim down, allowing bubbles of air to escape as it goes, until it rests with its usual stability upon the bottom. I have sometimes observed, however, during these few minutes, marked constrictions of anterior or posterior body regions with corresponding changes of buoyancy of these regions, such changes apparently aiding somewhat in directing the eel-like motion of the animal. From my own somewhat limited observations I should conclude, however, that the lungs of the *Amphiuma* subserve mainly the respiratory function although there is a possibility of use for hydrostatic purposes. In any case it is very evident that an ypsiloid apparatus affecting as it does, only two or three body somites, would be practically useless as an accessory hydrostatic apparatus in the case of an animal with a long eel-like body comprising a very large number of somites like that of *Amphiuma*. This fact would in itself account for the lack of such an apparatus in these slender, eel-like forms, *Amphiuma* and *Siren*. The lack of all vestige of both ypsiloid cartilage and muscles does not however as in the case of *Necturus* preclude the possibility of descent by degeneration from some higher lunged form, since the entire pelvic region shows numerous signs of degeneracy.

With regard to *Typhlomolge*, Miss Emerson (1905) has already shown conclusively the probability that this form is a permanent larva of a lungless salamander. Unfortunately I have not at hand the means for ascertaining whether in this form, as in the known *Plethodontidæ*, vestiges of ypsiloid muscles occur, but Miss Emerson mentions the failure of the cartilage as one of the characteristics of *Typhlomolge*. My proof that the use of the ypsiloid cartilage is correlated with the hydrostatic function of the lungs, therefore merely strengthens Miss Emerson's argument that *Typhlomolge* is the larva of a lungless form.

In conclusion, I wish to acknowledge my indebtedness to Dr. Harris H. Wilder for much practical assistance in the preparation of this paper.

SUMMARY.

1. The ypsiloid apparatus is, with the exception of *Cryptobranchus*, confined to the suborder *Salamandrida*. It has arisen in response to the physical need of controlling the direction of the body in water through the adjustment of the relative buoyancy of the anterior and posterior ends. Its function is therefore closely correlated with the hydrostatic function of the lungs.

2. In origin the ypsiloid cartilage is independent of the pelvic girdle. Its stem arises as a chondrification of the linea alba of the somite immediately anterior to the pelvic girdle. The arms are more complex in origin since the process of chondrification involves not only the myocomma anterior to the above named somite but also the outer edge of the aponeurosis of the transversalis muscle.

The *Cartilago ypsiloides* is therefore not homologous either with the *Processus epipubicus* of the lower *Urodeles* or with the marsupial bones of certain mammals.

3. In the *Plethodontidæ* and *Desmognathidæ*, in which the lungs have wholly degenerated, a correspondingly complete degeneration of the ypsiloid cartilage has occurred, although

vestiges of the ypsiloid musculature remain to indicate the former possession of the apparatus.

4. This interpretation of the function of the ypsiloid apparatus throws some light upon the systematic position of certain of the lower *Urodeles*. The more obvious conclusions are :

a. That forms with lungs but without vestiges of an ypsiloid apparatus, and with no evidence of degeneration in the pelvic region (*e. g.*, *Necturus*) are neither degenerate forms, nor permanent larvæ of any of the *Salamandrida*.

b. That the absence of the ypsiloid cartilage considered in connection with the absence of lungs in the case of *Typhlomolge* is in full accord with the conclusion [Emerson, 1905] that *Typhlomolge* is the permanent larva of some lungless salamander.

c. That the presence of a functional ypsiloid apparatus in *Cryptobranchus* indicates that *Cryptobranchus* lies near the line of descent of the *Salamandrida*.

Smith College, Northampton, Mass.
February 1, 1906.

Postscript.

Since the above article was written, a paper on the Anatomy of *Cryptobranchus allegheniensis* by Reese has appeared in the *American Naturalist*, Vol. XL., No. 472. In this article the following statement is made :

"Anteriorly the pubis is prolonged into a long, cartilaginous *epipubis*, which, instead of being forked as in the Japanese salamander and some other Amphibia, is a straight rod, slightly broadened and flattened at its distal end and somewhat enlarged, both laterally and dorso-ventrally at its attached end. The union of the pubis and epipubis is a close one, but allows considerable freedom of motion."

The results of my own dissections (p. 264) are so completely at variance with this description of Reese's that I can but feel that he was mistaken in the form and character of the part in question. I have, however, based my description upon three specimens only, and it is possible that we have here to do with a case of marked individual differences ; but that all of my specimens should have the typical Y-form, while all of Reese's were rod-shaped, does not seem probable. I. L. W.

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THE OSMOTIC RELATIONS BETWEEN FISHES AND THEIR SURROUNDING MEDIUM (PRELIMINARY NOTE).[1]

FRANCIS B. SUMNER.

The effects upon fishes of changes in the density and salinity of the surrounding medium involve numerous problems of great physiological importance. Why is an extreme change of density so fatal in some cases and so harmless in others? And is it the change of *density* which is responsible for the harmful effects after all? May not salt water be *toxic* in a narrower sense to fresh-water fishes and *vice versa?* In any case, what is the immediate cause of death? Are the limiting membranes of a fish permeable to both water and salts, or are they only semi-permeable? Or are they perhaps impermeable to both? And are all of the limiting membranes alike in this regard? Likewise is their condition the same for all species and under all circumstances? These are closely related questions. They have received many and quite contradictory answers. It is hoped that the experiments here discussed have contributed something toward their solution.

The first of these experiments were chiefly concerned in determining whether a given change in water density was harmful to a given species of fish, records being kept of the rate of death. In a second series, weight determinations were made with a view to ascertaining whether such changes in the density of the surrounding medium were accompanied by appreciable osmotic effects upon the fishes. Third, it was sought to discover whether the membranes were permeable to water only or to salts as well. The passage of salts from the fishes into the surrounding water was tested chemically, and likewise the salt content of the tissues of fishes of several species under different conditions was determined. Finally a series of experiments was performed with a view to discovering whether such osmotic changes were confined

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[1] A more complete account of these experiments is in course of publication by the Bureau of Fisheries and will, before long, appear in the Bulletin of that bureau.

to the gills or whether the general integument of the body were likewise concerned.

Altogether the results of about 150 experiments have been taken into consideration in arriving at the conclusions here presented. These experiments were carried on during the summers of 1904 and 1905 at the biological laboratory of the Bureau of Fisheries at Woods Hole, Mass., and during the spring of 1905 at the New York Aquarium. Acknowledgments are due to the officials of the Bureau of Fisheries for facilitating the progress of this work; and to the director of the New York Aquarium, Mr. Chas. H. Townsend, who placed at my disposal a room equipped for research, and provided me with abundant material throughout. My thanks are likewise due to Prof. W. C. Sabine, of the department of physics of Harvard University, for valuable criticism, and to Mr. D. W. Davis, assistant at the Fisheries Laboratory, for help during the earlier portion of the work.

Full details of these experiments, including the methods employed, and the precautions taken, must be deferred to the more extended paper which will soon appear. In the meantime, the principal results may be summarized as follows:

1. *Certain brackish and salt-water fishes were unable to survive even a gradual transfer to pure fresh water, though enduring an abrupt transfer to water of a very low degree of salinity. Thus fresh water, as such, proved fatal to these fishes, the degree of abruptness of the change being of secondary importance.*

These conclusions are drawn from experiments upon the three local species of killifishes (*Fundulus heteroclitus, majalis* and *diaphanus*), together with the allied species, *Cyprinodon variegatus;* likewise the white perch (*Morone americana*), cunner (*Tautogolabrus adspersus*), tautog (*Tautoga onitis*), sculpin (*Myoxocephalus octodecimspinosus*), and winter flounder (*Pseudopleuronectes americanus*). The death of a varying (often a large) proportion of specimens of *F. diaphanus*, when transferred from mildly brackish (sp. gr. 1.002–1.006) to pure fresh water was certainly unexpected, since this species in nature is not confined to brackish waters, but is indigenous to lakes and streams far from the coast. *F. heteroclitus*, likewise, is known to occur at times in fresh water; but the writer has found (*contra* Garrey[1]) that in nearly every

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[1] BIOLOGICAL BULLETIN, Mar., 1905.

experiment the entire lot died throughout a period of from less than a day to several weeks.

In addition to a simple reversal of the normal medium (salt-water fishes in fresh water and *vice versa*) experiments were conducted upon *acclimatization*, which was found to retard, but not to prevent, the fatal effects of fresh water ; likewise with *water of a very low degree of salinity*, which gave some of the most striking results to be recorded ; with *fresh and salt water in alternation*, in which case the fatal effects of the former were diminished or annulled ; and with *distilled water*, which soon proved fatal to *F. heteroclitus* (the only species used). *Surface abrasions* (extensive removal of scales) hastened the death of *F. heteroclitus* in fresh water, but only exceptionally led to the death of fishes returned to full-strength sea water (again, *contra* Garrey)[1], and wrought no harm to fishes placed in very dilute brackish water (three to four per cent. sea water), despite the fact that the latter was without doubt strongly hypotonic to the fish.

2. *Considerable changes of weight were found to result, in many cases, from changes in the salinity (hence the osmotic pressure) of the surrounding medium.*

The weighing operations were conducted with the following species : *Fundulus heteroclitus*, *F. majalis*, *F. diaphanus*, *Myoxocephalus octodecimspinosus*, *Microgadus tomcod*, *Pseudopleuronectes americanus*, *Stenotomus chrysops*, *Ameiurus nebulosus*, *Leuciscus erythrophthalmus*, *Morone americana* and *Oncorhynchus tschawytscha*. During these experiments, the fishes were kept unfed, and it is needless to add that abundant control experiments were performed in order to determine the normal rate of loss through waste. The changes of weight following changes in the density of the surrounding medium were frequently surprisingly great, at times as much as five per cent. or more in a single day. In many cases, moreover, they were not accompanied by any apparent harmful effect upon the fishes. With a very few exceptions, the changes were such as to indicate that they were the result of osmotic action. Thus, as a rule, the fishes *gained* in weight only in solutions known to be decidedly hypotonic to their body fluids, while with few exceptions a significant *decrease* only occurred in

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[1] *Op. cit.*

those cases in which they were transferred from a hypotonic or isotonic medium to one which was strongly hypertonic. Negative results were indeed encountered at times, but very few which could be regarded as contradictory.

Extensive *surface abrasions* did not facilitate the influx or efflux of water. The changes of weight in *dead* fishes were such as to show that factors other than osmosis were concerned. Dead fishes of most of the species used were found to gain in weight in water of any degree of salinity up to the strength of normal sea water.

3. *Considerable changes in the salt* (*chlorine*) *content of the body were likewise found to result, in many cases, from changes in the salinity of the water.*

The problem here involved was attacked from both sides. In the first place, the passage of salts (strictly speaking, of *chlorides*)[1] into fresh water from fishes taken from salt or brackish water was tested chemically. In the second place, the salt content of the tissues of various fishes which had lived in water of various degrees of salinity was likewise determined.[2] It was found that the results from these two methods presented some striking points of agreement, though the latter proved, on the whole, to be much more satisfactory. These changes in the chlorine content of the body were frequently astonishing in their magnitude. *F. heteroclitus* by both methods was found to part with about twenty-five per cent. of its chlorine in the course of a single day. The loss of chlorides from the body was, however, found to occur at a steadily diminishing rate.

The following table indicates the percentages of chlorine found in specimens of *F. diaphanus* from brackish water (their habitat locally) and in those kept for varying periods in fresh and in salt water. The series is certainly suggestive.

Fresh water.........	11 days (8 fishes employed).........	0.085
	3 days (4 fishes employed).........	0.108
	1 day (4 fishes employed).........	0.112

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[1] Mohr's silver nitrate titration method was here employed. The gain or loss in the proportion of chlorine was held to be indicative of the behavior of the various saline ingredients of sea water.

[2] It is needless to state that all fishes used in these two series of tests were previously thoroughly rinsed in fresh water.

Brackish water (sp. gr., 1.002) (5 lots of 4 fishes each), 0.134 [or 0.142][1]

Salt water (1.023) { 5 days (2 fishes employed) 0.143
10 days (3 fishes employed) 0.151

It will be seen that the last of these figures is about 78 per cent. greater than the first! And yet the fishes were all alive and apparently well at the time they were killed for analysis. It will be likewise seen that whichever figure be regarded as the more correct one for the brackish-water fishes, the latter agree much more closely with the salt-water than with the fresh-water individuals (the comparison being of course between the extreme members of the series). It must be added in strict fairness, however, that in two different tests fishes kept for only *one* day in sea-water gave a much higher percentage of chlorine than those kept for five or ten days. For this apparent anomaly I believe that a satisfactory explanation can be given, but this has been deferred to my longer work.

A series of figures somewhat similar to the above was obtained from experiments with the white perch.

Experiments with both *F. heteroclitus* and *F. majalis* agreed in showing a great difference between the effects upon the chlorine content of the body of pure fresh water and water having a certain small percentage of salt. This difference is extremely significant in view of the difference, already mentioned, in their effects upon the life of the fishes. Moreover, it was further found that the average percentage of chlorine contained in the salt-water fishes analyzed was of the same order of magnitude as that of water containing just enough salt to maintain the fishes in health. That such water could not have been even approximately *isotonic* with the body fluids of these fishes seems evident from the cryoscopic determinations of other investigators.

4. *Careful control experiments excluded the possibility that the water or salts entered or passed from the body through the alimentary canal, leaving as the only probable alternative an osmotic exchange through one or more of the external membranes.*

The alimentary canal, and indeed the whole abdominal viscera, together with the washings from the body cavity, were found in

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[1] This second figure is the mean which results when one very questionable determination is included. It is inserted for the sake of strict fairness. The other averages are in each case derived from *all* of the fishes tested.

some cases to yield less chlorine than passed from the body of a living fish in the course of a few hours. Likewise those fishes whose bodies were analyzed gave approximately the same percentages of chlorine whether or not the alimentary canal was included in the analysis. It must be remembered also that the fishes employed in these experiments had, in all cases, been kept unfed for some days previously.

5. *In certain fishes, at least, it was found that the membranes chiefly concerned in such exchanges were those of the gills.*

In the case of certain specimens, salt water was passed through the gills by means of a rubber tube placed in the mouth, the body being bathed with fresh water; while in others the arrangement was reversed, the gills receiving fresh water and the general integument salt. In six experiments with the carp it was found that a considerable loss of weight occurred in all of those cases in which the former conditions obtained, while the weight remained practically stationary in those cases in which the conditions were reversed.

A complete historical review of previous researches in this field of physiology would be beyond the scope of the present paper. The investigations of Fredericq,[1] Bottazzi,[2] Rodier,[3] Garrey[4] and Greene[5] agree in showing that the blood of marine teleosts is far from being isotonic with the surrounding sea-water, but that it has an osmotic pressure which is roughly about one half that of the latter. But it has likewise been shown (Fredericq, Greene, *op. cit.*) that the osmotic pressure of the blood of salt-water teleosts is considerably higher than that of fresh-water ones, though this fact has been almost lost sight of in the zeal to prove that the internal medium is not isotonic with the external, and that its osmotic pressure is *relatively* constant. Indeed it does not seem to have been generally appreciated that there is a certain correlation between the inner and outer fluids, both as regards osmotic pressure and salt content; and certain authors have been free to state that the membranes of teleost fishes form an effective barrier against osmotic changes. Fredericq makes this assertion

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[1] *Archives de Biologie*, 1904.

[2] *Archives italiennes de Biologie*, 1897.

[3] Cited by Fredericq, 1904.

[4] BIOLOGICAL BULLETIN, 1905.

[5] Bulletin U. S. Bureau of Fisheries, 1905.

broadly, while Garrey says of *Fundulus heteroclitus:* "The integument and gills are therefore impermeable." Garrey is cautious enough, however, not to postulate an *absolute* impermeability, either for *Fundulus* or for teleosts in general. Greene, though he finds that the osmotic pressure of the blood of the Pacific salmon undergoes a decrease of about 17½ per cent. when the fish ascends a river to spawn, is nevertheless doubtful whether osmotic exchanges with the surrounding water are responsible for this decrease.

In view of my own experiments, however, we are certainly not justified in concluding from the absence of osmotic equilibrium between the fish and its environment that no osmotic interchanges normally occur. On the contrary, abundant experiments seem to prove that both water and salts may, under certain conditions, be transmitted in either direction without any harm resulting to the fish. These conditions seem impossible to state in advance for a given case. In general we may say that:

1. *Measurable changes in weight result only from considerable changes in the surrounding water, but*—

2. *Not all such changes of density suffice to produce changes of weight, even when the fish is transferred to a medium which is known to be strongly hypertonic or hypotonic to its own body fluids.*

3. *Changes in the salinity of the water may or may not result in changes in the salt content of the body.*

4. *Changes in the bodily salt content may or may not be accompanied by changes in weight.*

5. *Neither the changes in weight nor in salt content are at all proportional to the changes in the density of the external medium.*

It would appear that there is normally a tendency on the part of the fish to resist osmotic changes and to maintain the fluids of the body at a definite degree of concentration. Under various conditions, however, this resistance is overcome and a certain degree of permeability is established. This is generally a differential permeability, resulting in osmosis and consequent changes of weight. In such cases, however, the membranes are not strictly semipermeable, but transmit salts in some measure. Indeed it would seem that at times the permeability is indiscriminate, in which case the salts may diffuse freely, but no changes in weight occur. These various *changes continue until a new level of stability is established, after which the normal resisting power of the fish reasserts itself and no further alteration occurs as long as the medium is constant. Complete osmotic equilibrium between the fish and the water is probably never attained except in waters having roughly a medium degree of salinity. The osmotic pressure of the "internal medium" fluctuates within a much narrower range than that of the "external medium."*

The foregoing conclusions are intended to apply only to normal fishes. It seems certain that the enfeeblement of the fish may result in an increased permeability of the membranes, which, in turn, would doubtless result in a further enfeeblement of the fish. The death of those fishes which cannot withstand transfer to a medium very different from that to which they are accustomed is thus probably in part a cause and in part an effect of these changes. Death is accompanied (perhaps in some cases caused) by a giving way in the power to resist an abnormal degree of osmotic exchange. The body becomes water-soaked (if in fresh water) or dehydrated (if in salt). The difference between the more hardy and the more delicate species in this regard seems to lie partly in the resisting power of the limiting membranes (chiefly those of the gills); partly, also, in internal differences, such as composition of blood, etc., which determine whether or not a given influx or efflux of water or salts shall prove fatal.

The actual cause of death following a change in the salinity of the water seems to differ in different cases. With those fishes which succumb rapidly with but a slight change of weight (*e. g.*, scup), it is unlikely that any appreciable alteration occurs in the tissues at large. Such changes are probably confined to the blood, perhaps, as Bert[1] held, to that in the gill capillaries, in which case death may result from asphyxiation (Bert,[2] Mosso[3]). In those cases, on the contrary, where the fatal effects are not manifested for some days, it seems likely that the manner of death is different. In the case of *F. heteroclitus*, it was found in most instances that the endosmotic flow of water had ceased, and that a secondary *decrease* in weight had ensued, within one

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[1] *Comptes Rendus de l'Académie des Sciences*, 1871, 1883. [2] *Op. cit.*

[3] *Biologisches Centralblatt*, 1890.

or two days after transfer to fresh water. On the other hand, it will be remembered that fishes of this species commonly did not die for a considerable number of days, while many survived for a week and some even for several weeks. Again, it will be recalled that the fatal effects of fresh water upon this and some other species were nullified by the admixture of a very small percentage of salt water. Analyses showed that in this latter case there was little or no decrease in the salt content of the body. A rough approximation was pointed out between the percentage of salts in this faintly saline water and that in the fishes themselves. *All of these facts point to the conclusion that one factor in the death of salt-water fishes in fresh water is the extraction from their tissues of an amount of salts sufficient to reduce the percentage below a certain necessary minimum.*

If the question be asked: Why are not fresh-water fishes thus affected in their own medium? it is replied that their membranes have been adapted to resisting such an extraction of salts. It is perhaps also true that the irreducible minimum of salts in these species is lower than in the case of salt-water ones. In any case, the percentage actually present is, on the average, less (Atwater,[1] Katz,[2] Quinton[3] and several others).

Whether or not salt water ever has a *toxic* effect, in the narrower sense, upon fresh-water fishes cannot be stated definitely. Bert denied that such was the case, but he is not entirely consistent in this position. In view of the fatal effects upon salt-water fishes of some of the individual components of sea salt, when taken separately (Loeb,[4] Siedlecki[5]), it seems quite possible that sea-water may act as a poison to fresh-water organisms, independently of any osmotic effects. Indeed both of the last-named writers have shown that it is the chemical nature of the solutions used rather than their osmotic pressures which determines, in many cases, whether or not they shall prove fatal.

College of the City of New York,
March 27, 1906.

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[1] Report U. S. Com. Fish and Fisheries for 1888 (1891).

[2] *Archiv für die gesammte Physiologie*, 1896.

[3] "L'eau de mer, milieu organique," Paris, 1904.

[4] *American Journal of Physiology*, 1900.

[5] *Comptes Rendus de l'Académie des Sciences*, 1903.