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MORPHOLOGICAL NOTES

FROM THE BIOLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

EDITED BY WILLIAM K. BROOKS, ASSOCIATE PROFESSOR OF MORPHOLOGY.

[The papers which are named below contain abstracts of researches on material which was collected during the seasons of 1888 and 1889 at the Laboratory of the U. S. Fish Commission, and I take this opportunity to thank the Commissioner, Professor Marshall McDonald, for the opportunities for study which he has afforded me and the investigators named:—Brooks, Salpa and Pyrosoma; Watase, Cleavage of the Ovum, Migration of the Retinal Area; Wilson, Development of the Sea Bass; Morgan, Embryology of Pycnogonids; Bigelow, Physiology of Caravella, Sense Organs of Pelagidae; Andrews, Notes on Sipunculus; Herrick, Development of the Lobster.—W. K. BROOKS.]

On the Relationship between Salpa and Pyrosoma.
By W. K. BROOKS.

Part 4 of Volume XXIII of the *Jenaische Zeitschrift* contains a most thorough and exhaustive memoir by Seeliger on the development of Pyrosoma (Zur Entwicklungsgeschichte der Pyrosomen, mit tafel XXX–XXXVII), in which the author confirms, in all essential particulars, the accounts which Huxley and Kowalevsky have given us of the process of budding in Pyrosoma. Seeliger's account is much more minute and detailed than the older papers, and is a most valuable contribution to our knowledge of the subject, and in fact it appears to be so complete as to leave nothing more to be done, but it shows also that the older accounts were perfectly accurate, although they were less exhaustive than Seeliger's researches.

In a paper which I published in 1886, in Volume III of the "Studies from the Biological Laboratory of the Johns Hopkins University," on "The Anatomy and Development of the Salpa Chain," I showed that, after the secondary complications due to crowding and pressure are allowed for, the process of budding in Salpa is strictly comparable in every essential particular with that which had been described in Pyrosoma by Huxley and Kowalevsky; that "the Salpa chain is a single series of animals like the Pyrosoma-stolon: the middle plane of the stolon the same as those of the Salpae; that the right halves of all the bodies arise on the right half of the stolon, and their left halves on its left, and that they are not formed by budding from its walls but by the direct conversion of its tissues and cavities into those of the Salpa, and that the process is directly comparable, in every particular, with the published accounts of what occurs in Pyrosoma." (Page 472.)

My reason for publishing the paper was, as I then stated, the appearance of Seeliger's paper on the budding of Salpa (Die Knospung der Salpen, von Oswald Seeliger, *Jen. Zeitschrift*, XIX, 1885), and I showed that this author, like all the others who had written on the subject, had gone hopelessly astray in the interpretation of his sections, and that his account of the process of budding in Salpa is of no more value than those given by earlier writers.

I am pleased to learn from his new memoir on Pyrosoma that Seeliger now holds the view which I have advocated, as to the essential similarity between the Salpa-stolon and that of Pyrosoma, but I am surprised to find that the only reference to my work on the subject is the statement that I have "emphasized" this resemblance—"Die Umbildung der eingelnen Segmente des Stolo (of Pyrosoma) zu einer volständigen Pyrosoma verläuft,

wie schon Brooks betonen konnte, sehr ähnlich mit den Vorgängen in der Salpen entwicklung." (Page 613.)

It seems to me that this is a very inadequate recognition of the fact that I pointed out the resemblance in detail, in contradiction of Seeliger's own statement of the case, and in opposition to all other published accounts.

During the last year I have been engaged in studying the Salpae which were collected by the U. S. Fish Commission Steamship Albatross, and those collected by Professor Libbey on the U. S. Fish Commission Schooner Grampus, and as these collections furnish the material for the comparative study of the process of budding, I have been able to amplify and complete my work on the subject, and to illustrate it by comparisons between different species. I am now preparing the illustrations for a memoir on the budding of Salpa, which will be ready for publication this fall. Two species, collected by the Grampus, are peculiarly favorable for studying the minute details of the process. One of them, which was brought up on the thermometer tubes of the Grampus, is very similar to, if not identical with S. pinnata of the Mediterranean. It is not a true Salpa, as it differs from the ordinary species of this genus in many structural features. It is especially valuable for the study of the process of budding, as the Salpae gradually increase in size from the base to the tip of the stolon, and it is therefore peculiarly valuable for studying the histology of the process of budding, and for tracing the development of the various organs.

A second species, also collected by the Grampus (S. clotho), is peculiarly favorable for studying the anatomy of the chain, since the secondary complications which are brought about by crowding are more easily intelligible than they are in any other species which I have studied. This is due to the fact that the young Salpae attain to a larger size and to more perfect development before crowding takes place, than they do in other species.

The study of these two species, and of Seeliger's beautiful figures of Pyrosoma, show that the resemblance between Salpa and Pyrosoma is even more perfect and complete than I had supposed, as it extends to all the details of structure.

Karyokinesis and the Cleavage of the Ovum. By S. WATASE.

(This paper contains an abstract of that portion of my researches on the embryology of Cephalopods which relates to the early stages. It is based upon the material which I collected in the summers of 1888 and 1889 at the laboratory of the U. S. Fish Commission at Wood's Holl).

The study of the developing ovum presents two important topics for consideration, viz.: (1) The external phenomenon of cleavage in its relation to the organization of the adult or the larva; (2) The phenomenon of nuclear division in its relation to the more general problem of Karyokinesis in animals and plants. The former is essentially the phenomenon manifested in the cytoplasm, and the latter in the nucleus of the ovum.

Since, however, in a developing ovum, the cleavage furrows may be entirely absent, while no development of the ovum is possible without the division of the nucleus, we may safely assume that the fundamental feature of the egg-cleavage is the division and distribution of the chromatic substances derived from the original segmentation nucleus or from the male and female pronuclei. The system of cleavage furrows, when such exists, indicates in a superficial way the paths along which the progeny of the original segmentation nucleus migrate in different directions through successive stages of their development. This migration of the chromatic substances often takes such a definite direction, and the furrows indicative of this internal change reproduce so accurately the paths along which nuclei are travelling that the delineation of the adult or the larval organization becomes discernible in an extremely early stage of development. In fact, several eggs in which the pro-morphological relations have been accurately studied show the anterior and the posterior, the right and the left, the dorsal and the ventral sides, each giving rise respectively to the corresponding sides of the adult organisms, before they show any sign of segmentation.

Leaving the various theoretical considerations bearing on this interesting point advanced by some naturalists, we may accept at present, as a demonstrated fact, that a certain ovum before it begins to segment at all, shows a symmetry which exactly coincides with that of the adult or of the larva, and the dispersion of the chromatic substances derived from the original segmentation nucleus bears a definite relation to the planes of symmetry predetermined in the ovum. If the symmetry, therefore, of a certain ovum first becomes manifest by the appearance of the cleavage furrows, the latter need not necessarily be taken as the determining cause of the symmetry of the ovum itself. The predetermined symmetry of the ovum in such a case may simply have been made apparent for the first time by the cleavage furrows.

The cleavage furrow bears no constant relation to any particular phase of developing ova in different animals. Thus the first furrow of cleavage coincides with the median axis of the adult in such animals as the frog (*Newport, Pflüger, Roux*), the teleostean (*Agassiz and Whitman*), Clavallina (*E. van Beneden and C. Julin*), and the squid, while in Ascaris (*E. van Beneden, Hallez*) the first furrow of cleavage divides the future ectoderm from the future endoderm. The two-cell stage of the squid, therefore, does not correspond in its nature to the two-cell stage of Ascaris, although both are produced by the first act of cleavage.

If we represent by a and b respectively the two chromatic substances which by a series of changes give rise to the future ectoderm and the endoderm with their derivatives, and by a heavy straight line, the plane of the first cleavage furrow, we may represent the cleavage of an ovum like that of Ascaris by $\left(\frac{a}{b}\right)$; the form like that of the squid may be represented by $\left(\frac{a+b}{2}\Big|\frac{b+a}{2}\right)$. A third form of cleavage is conceivable, which may be illustrated by $\left[\frac{\frac{a+b}{2}}{\frac{a+b}{2}}\right]$. The first form of cleavage corresponds approximately to the dorso-ventral division of the ovum; the second, to the right and the left, and the third to the antero-posterior division of the ovum.

In some cases the separation of the ectoderm from the endoderm is accomplished gradually—that is, by a series of Karyokinetic processes and not by a single stroke at the first. In such cases, and others of similar kinds, the plane of the first act of Karyokinesis bears no definite relation to the organization of the adult or of the larva. The regularity and symmetry observable in the cleavage of a certain ovum may be considered as a final outcome of a long series of variations chiefly in relation to the nature, amount, and distribution of food-yolk, and thus subjected to the influence of selection.

If the homology of the cleavage furrows in different ova has to be established it must be founded on the nature of the Karyokinesis which immediately precedes the formation of each furrow, or on the fate of the resulting cells separated by such furrows, not by the order of their appearance in different ova. Tested by this standard, the first cleavage furrow which divides the blastoderm into right and left halves corresponding to the right and left halves of the adult or the larva, as in the case of the teleost, the frog, the ascidian, and the cephalopod, may be considered homologous throughout, but no homology would exist between the first furrows of cleavage in the squid and in Ascaris. Important as this intimate inquiry on the nature of Karyokinesis appears to be in relation to the problem of the general development of the ovum, our knowledge of the subject is very imperfect, and even such a fundamental point as the mechanism of division, by which the distribution of the chromatic substances is effected, is yet far from being satisfactorily settled. To this latter point of the problem I will devote the following, pending a fuller account in my main paper on the development of Cephalopods, which I hope to publish in the near future.

The Mechanism of Karyokinesis.

Whatever may be the difference of opinion in regard to the minor details of the nuclear phenomena known as Karyokinesis, it seems now to be generally conceded that the ultimate purpose of the process is the division of the total quantity of chromatic substances in the nucleus among the daughter cells. To be more explicit, in the analysis of Karyokinetic figure I believe with Boveri, " daz bei jeder Karyokinese die Verteilung der Hälften eines jeder Kernelements auf die beiden Tochterzellen als der *Zweck*, die ganz achromatische Figur aber als das *Mittel*, als der mechanische Apparat zu betrachten ist, um diesen Zweck zu erreichen." (T. Boveri: *Zellen Studien,* II.) E. van Beneden has shown that what is called the achromatic spindle is not a simple structure but consists really of two cones meeting with their bases at the equator of the original nucleus. As will be shown later, this analysis of a spindle into two independent structures is a great step in the advancement of our knowledge on Karyokinesis, for upon the accurate knowledge of the origin of the cone as well as of the mode of its activity, the complex phenomena of Karyokinesis are alone capable of being reduced into a series of connected events. Van Beneden believes that by the *contraction* of these spindle fibres, whose distal extremities are fastened to the chromatin threads, the equatorial chromatin plate is divided into two daughter plates, and each is carried to its proper destination. Boveri concurs with van Beneden on these points from his own independent researches.

If we look, with van Beneden and Boveri, upon the entire achromatic figure as the mechanism by which the division of the chromatic element of the nucleus is effected, it follows that our task of inquiry into the nature of Karyokinetic phenomena is greatly simplified. The behavior of the chromatic substances then, at least during the process of their actual migrations, may be looked upon as a passive phenomenon brought about by the activity of the achromatic mechanism. Although my study on this head is not so complete as I desire, I publish the following, with a hope that it may serve to confirm the view entertained by van Beneden, Boveri, Strassburger, and Guignard, as to the extra-nuclear origin of the achromatic spindle, and I would like to add a suggestion as to the mode of its activity towards the nucleus and the part it plays in the subsequent phenomenon of nuclear division. In short, the subject of the following notes will be the origin and formation of the achromatic spindle, and its behavior towards the nucleus in the segmenting blastoderm of the squid (*Loligo pealii*). For this purpose I find it convenient to treat the subject in the following order:

1.) Formation of the achromatic spindle.
2.) Its behavior towards the nucleus.
3.) Formation of the equatorial chromatin plate.
4.) Separation of the daughter plates and the formation of the interzonal filaments (Mark).

I will attempt to show that the entire sequence of the Karyokinetic phenomena arranged into four orders as given above is due to the continuation throughout of one and the same activity; in other words, the activity which manifests itself in the formation of the achromatic spindle (1) appears also in the production of all the subsequent phenomena (2), (3) and (4). To begin our description with the cell in which the division of its nucleus is completed; the daughter nucleus has at this stage a complete nuclear membrane and the main threads of chromatin are arranged in such a way as to show a slight convergence towards the nearest pole of the spindle. The outline of each nucleus is bean-shaped, with its concave border turned towards the nearest aster. From the aster, whose centre is filled with an amorphous substance showing in my preparation no definite body, as has been described by van Beneden, Boveri, and Platner in other animal cells,

proceeds in all directions a number of achromatic rays, some of them passing through the substance of the daughter nucleus. Those rays which pass through the nucleus seem to become continuous with the interzonal filaments which lie between the two nuclei. Midway in the interzonal filaments lies the division line, which, becoming deeper and deeper, marks out the morphological boundary between the two daughter cells. The contents of each daughter cell, when the division is completed, consist then of the *nucleus* surrounded by the *cytoplasm*, in which is the *aster*, which closely clings around the spherical body of the nucleus. Soon afterwards the daughter nucleus again divides. This division of the nucleus is preceded by the division of the aster, which lies very close to the outside of the nuclear membrane. The central area of the aster becomes divided into two; each centre having achromatic rays around it, there is formed a little spindle between the centres by the meeting of the two systems of rays. It appears, as the length of the rays continually increases, as if the two asters push one another in opposite directions, and the length of the spindle becomes continuously increased. The increase in the length of the achromatic rays of the asters continues, and with it the mutual displacement of the asters in opposite directions. When the asters become so widely separated that the whole nucleus can lie between them, and when their rays no longer interfere with each other, the asters cease to wander and come to lie on the opposite surfaces of the nucleus. Their effect on the latter is soon seen. The rays of the aster grow towards the nucleus and push the more solid portion of the nuclear substance in front of them, while the more fluid portion accumu-
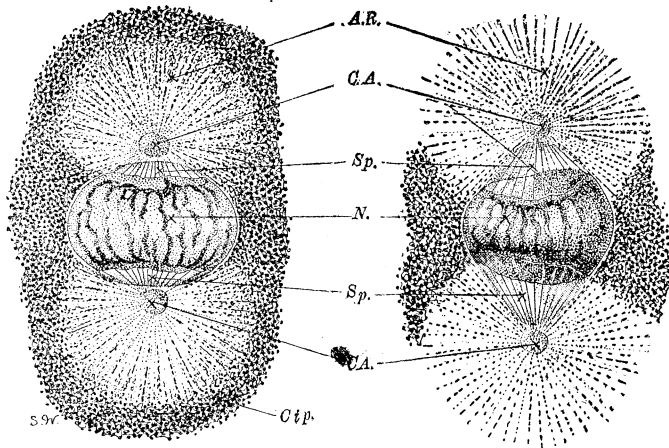
* FIG. 1.   FIG. 2.

lates in the spaces from which the portions of the nucleus have been displaced. (Fig. 1). When viewed from the side, the nucleus appears to be at this stage a biconcave disc, the concavity of the disc being produced by the pressure of the extra-nuclear achromatic filaments. (Fig. 2). The nucleus presents an appearance very much like the one described by Platner for the nucleus in the egg of a leech (*Arch. f. Mik. Anat.*, Bd. 33).

This process of pressing on the nuclear substance continues until finally all the solid portion of the nuclear contents is pressed into a flat equatorial "*plate*," while the two systems of the achromatic rays proceeding from the two asters form a spindle-shaped body, the *achromatic spindle*. As is evident from the nature of its origin, the spindle is not a simple structure, but is composed of two cone-shaped groups of fibres meeting with their basis turned towards each other. Each cone is again a part of a more general structure, the aster, which sends out its rays in all directions. The rays which form the spindle are smooth and clear in outline, while those that lie in the matrix of cytoplasm appear slightly irregular.

After the formation of the equatorial plate, comes an important stage, viz.: the separation of the single mother plate into two daughter plates— the metakinesis of Flemming. With the formation of the double plates there appear the interzonal filaments which run between the two daughter plates.

How is this separation of chromatic plates effected? What is the origin of material which forms the substance of the interzonal filaments? Do the interzonal filaments come from the achromatic stroma of the original nucleus, as held by van Beneden, Boveri and others, and increase in size by the

osmotic absorption of the cytoplasmic fluid, as held by Strassburger? Is the separation of the daughter plates due to a chemical process, or due to some mysterious forces emanating from the poles of the spindle, as conjectured by some cytologists? Or is it due to some mechanical causes arising outside of the chromatin bodies themselves? My answer to this part of the problem is an extremely simple one, as may be gathered from the following. With the formation of the equatorial plate there comes a stage of relative quiescence. It may be described, as Boveri observes, as a stage of rest in the course of nuclear division, if we can judge of the activity of a cell by the change in form of its constituents and consider it in a state of rest as long as there is no tangible change. But is this apparent cessation of activity really a state of rest? As the two systems of spindle rays press on the equatorial plate, which is no longer compressible, its more liquid part being already squeezed out, the chromatin plate shows no external marks of change for a time, but nevertheless it is not strictly true to say that the active growing force of the spindle fibre is temporarily suspended. I am inclined to believe that the two opposing filaments from the two spheres come to a standstill by a mutual pressure on the equatorial plate. Soon, however, this stage is over. A group of spindle fibres coming from one pole attach themselves to a certain part of chromatic threads and press forward, pointing in the direction of the opposite pole. A similar group of spindle fibres from the other pole does the same and presses in the contrary direction. Thus at the equator there exist two opposing forces acting on the same plate. Finally the filaments from each group break through those of the other group. Where the two sets interpenetrate they constitute the zone of *interzonal filaments*. The separation of the chromatin plates and the formation of the interzonal filaments are therefore concomitant phenomena—in fact, the results of the same activity. The interzonal filaments are direct continuations of the spindle rays, extending beyond the migrating chromatin plates. The zone of filaments between the two chromatin plates differs from the spindle itself only in the fact that there exists two sets of filaments running in the opposite directions, emanating from the two opposite asters. If one take two brushes, bring the two bristle-covered ends into apposition and press them towards each other, we may be able to bring the two sets of bristles into the condition which obtains in the interzonal region of the achromatic spindle, namely—the two sets of bristles will dovetail into one another and at the same time, with more pressure, they will travel in opposite directions.

We must bear in mind that up to the period of Metakinesis, the nuclear membrane persists, so that the dislocation of the chromatic substance into any other direction than the longitudinal one is impossible. There are, however, several reasons for believing that this persistence of the nuclear membrane is not universal in the animal and vegetable cells that have been studied. Several cases are on record in which the nuclear membrane disappears very early in the history of nuclear division. Certain irregularities observable in the behavior of the chromatin threads during the course of Karyokinesis may be ascribed to the premature disappearance of the limiting membrane, which would otherwise keep the threads in compact shape and expose them to the full influence of the invading achromatic threads.

As a daughter plate approaches the aster, and attains the position beyond which it cannot go, the whole outline becomes rounded off, and completely covered by a nuclear membrane. The chromatin threads are arranged in a radial fashion, following the direction of the achromatic filaments radiating out from the aster. The side which faces the aster shows a slight concavity, while the side away from the aster is smoothly rounded off, exhibiting a broad curve, concentric with the smaller, concave border on the opposite face of the nucleus. The condition with which we started in our description of Karyokinesis is thus again attained. The aster of each daughter nucleus again divides and behaves towards the adjoining nucleus in a similar way as before.

To recapitulate the points emphasized in the preceding:

1.) I hold with van Beneden and Boveri, that the achromatic spindle plays the most important part in the production of the Karyokinetic phenomenon. It is the mechanism by which the chromatic substance of the nucleus is divided among the daughter cells. But unlike the above mentioned authorities, I do not find any evidence of the *contractility* of the achromatic fibrils; on the other hand, I find the achromatic threads are constantly lengthening, stretching, and pushing away from the centres of asters from which they start. I do not consider the passive shortening of

the spindle fibrils due to the mutual pressure at the equator, as an evidence of their *contractility*.

2.) This achromatic spindle in its perfected form consists of two cones with their bases turned toward each other, and interposed between them a sheet, as viewed from the side, of the achromatic substance of the nucleus.

3.) Each cone composing the half of the spindle is a part of a more general system of radiating fibrils forming one of the asters.

4.) The asters in a cell arise from the preceding single aster, as the new nuclei arise from the preceding nucleus. The old aster divides into two, each daughter aster having a granular substance in its centre and around it the achromatic rays extending in all directions.

5.) As the rays from each of the small asters grow longer, the centres of the corresponding asters become separated wider and wider from each other. A small achromatic spindle is formed by the two groups of achromatic rays between the two centres. This spindle lies in the outside of the nucleus, and has no connection with the other mentioned already (1) and (2). The latter is an entirely new structure, although derived from the same source and formed in a similar way.

6.) When the two asters become so widely separated as to interpose between them the whole nucleus, they apparently come to rest and begin their work on the nucleus, by pressing on the more solid portion of the nuclear content.

7.) The formation of the equatorial chromatin plate is solely due to the pressure exerted by the two systems of rays from the opposite sides of the nucleus.

8.) The separation of the equatorial plate into two daughter plates travelling in opposite directions, and the formation of the interzonal filaments are due to the continuation of the same action which has been going on before, viz., the continuous growth of the achromatic fibrils.

9.) When each of two daughter chromatin plates approach the extremities of the spindle a new nuclear membrane is formed around each chromatin plate, each plate thus forming a complete nucleus.

10.) The interzonal filaments ("connective filaments," "Verbindungs-fäden," "filaments réunissants," of authors), are the same substance as the spindle filaments—the actual continuation of the spindle filaments. They do not *unite* in any way the two daughter chromatin plates.

11.) In the interzonal region, therefore, two systems of filaments exist, each running in opposite directions.

Looked at in this way, the whole phenomena of Karyokinetic changes may be connected in one continuous series of activities of the cytoplasmic asters upon the nucleus.

It follows from the above that the rapidity of the cleavage process depends, in a great measure, upon the rapidity with which the cytoplasmic asters can migrate to two opposite poles of the nucleus. The presence, therefore, of the inert, passive yolk-granules embedded in the cell-body of the ovum necessarily interferes with rapid movements of the cytoplasmic asters. Hence in eggs in which the distribution of food-material is not uniform, segmentation does not take place with equal rapidity through all parts of the egg. Such a view of the mechanism of Karyokinesis, therefore, suggests a rationale to the well-known fact in embryology, first definitely formulated by Balfour, viz.: The velocity of cleavage in any part of the ovum is, roughly speaking, directly proportional to the concentration of the protoplasm, or inversely proportional to the quantity of yolk-granules embedded in the protoplasm. (*Quart. Jour. Micro. Science*, Vol. XV, 1875.)

The mechanism involved in the multiple nuclear division can be explained exactly in the same way as in the binary karyokinesis. Instead of starting with the binary division of the aster, we here start with the triple or quadruple division. Each of the daughter asters being settled in three or four equidistant points around the nucleus, act towards the latter in the same way as has already been described in the case of binary division. If the number of asters be three, then the chromatin content of the nucleus will be reduced into a tri-radial body, each process of which corresponds to the equatorial plate of the achromatic spindle. The longitudinal axes of three spindles thus arranged form the sides of an equilateral triangle. Each of the resulting daughter cells of such a division will contain one-third of the total quantity of the chromatin in the original mother nucleus. When the original mother aster divides into four, and the daughter asters occupy equidistant position from each other around the nucleus and behave towards the latter in the same way as before, then the chromatin substance of the nucleus will be pressed into a cross-shaped body as viewed from the

side, each stem of the cross corresponding to the equatorial plate of each of the four achromatic spindles, whose longitudinal axes form the sides of a square.

In the quadruple division of the nucleus, one-fourth of the original chromatic substance goes to each of the daughter cells.

The formation of the daughter chromatin plates in each of the spindles in the multiple karyokinesis is identical with that already described in the case of binary nuclear division. The multi-nuclear division was observed twice in the blastoderm of the squid. If in a given stage of cleavage, say in the eight-cell stage, one blastomere on the right-hand side of the bilateral ovum shows multiple karyokinesis, the corresponding segment on the left half of the ovum shows exactly the same peculiarity. The morphological correspondence of the two sides of the bilateral blastoderm is so close that if in a given stage of cleavage, a certain cell on the one side deviates from the normal method of karyokinesis we can pretty surely predict which cell in the opposite half of the body deviates also from the normal. Although it is easy to identify two corresponding sides of the blastoderm, in a superficial way, the interest becomes still more enhanced when this intimate correspondence in both halves of the body is manifested by the individual cell, and in such a striking way as by the abnormal karyokinesis, when all the rest of the cells are dividing normally.

Baltimore, February, 1890.

On the Development of the Sea Bass (Serranus atrarius.)

By HENRY V. WILSON, Laboratory of the U. S. Fish Commission, Wood's Holl, Mass.

(Preliminary Communication.)

The Sea Bass is one of the important food fish of our Atlantic coast, having a wide distribution, all the way from Cape Cod to Florida, and the facts of its life history may on this account have an additional value. The spawning season at Buzzard's Bay and Vineyard Sound extends from about the middle of May to the last of June, during which time the eggs are found in comparative abundance floating on the surface of the water. The eggs used for the present research were artificially fertilized and reared in the hatchery of the Woods Holl station, during the first days of June, 1889, when the temperature of the water was about 60° F. The time required to hatch them out was 75 hours. The egg is not a difficult one to rear, and the percentage of fish hatched out was very large.
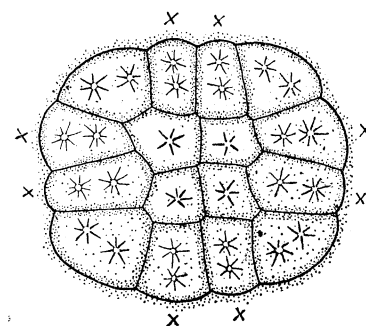

FIG. 3.

The egg of the Bass is a small pelagic egg with one oil globule, imbedded in the yolk at the pole opposite the blastoderm. About a third of the surface of the globule is covered by a thick mass of protoplasm unprovided with a nucleus, and free from the thin layer of protoplasm which surrounds the yolk after the blastoderm is formed.

In almost all the eggs the segmentation is strictly regular and bilateral as far as the sixteen cell stage. In passing from the sixteen to the thirty-two cell stage, about one-half the eggs are strictly bilateral, the cleavage planes being those shown in Fig. 3 (the four central cells divide parallel to the surface of the blastoderm). It is very easy, however, to find an egg in which any one of the eight peripheral cells, marked with a cross in the figure, is dividing in a plane at right angles to the normal plane of cleavage. Usually not more than one of the eight cells, in a single blastoderm, offers

this variation. Where thirty-two cells are formed, a rearrangement takes place and the blastoderm is no longer bilateral.

The formation of the periblast takes place in the manner described by Agassiz and Whitman for Ctenolabrus. The marginal cells of the blastoderm fuse with the periblastic protoplasm, which thus acquires its nuclei. Henneguy (1888) has recently advanced the old notion that cells are formed in the periblast round the margin of the blastoderm, and are added to the blastoderm cells. The work of Agassiz and Whitman thoroughly reflected this idea, and my own observations are a detailed confirmation of theirs.

The lower surface of the blastoderm has hitherto been plane or slightly convex. It now becomes concave—an excentric thinning out takes place, by which means the late phase of the segmentation cavity (so-called germinative cavity of some authors) is inaugurated. The excentric thinning out leaves one portion of the blastoderm thicker than the rest, and it is round a small arc of this portion that the germ ring first begins to form. The germ ring is everywhere formed as an ingrowth of cells from the edge of the blastoderm, in which the superficial or horny layer takes no part. Round the general periphery the ingrowth is thin, not more than two cells thick. At the spot where the ingrowth begins, however (tail end of future embryo), there is formed a thick tongue of cells. The cells of the horny layer are flat except at the extreme edge of the blastoderm, where they project into the small ring-like space which here separates the blastoderm from the periblast. It was probably this feature of the peripheral horny cells which led Kingsley and Conn (1883) to describe the horny layer as involuting to form the germ ring.

When the embryonic shield has reached its full size, the primitive endoderm is composed of two layers of cells, distinctly marked off except in the middle line, where there is a fusion (Fig. 4). The streak of fused cells presently acquires a sharper lateral boundary, and becomes the notochord.
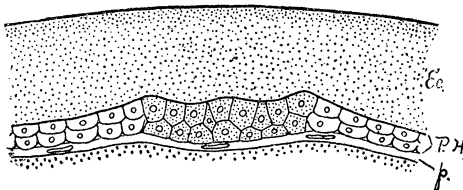

FIG. 4.—Transverse section through middle of embryonic shield just before neural cord begins to form. *Ec.*, ectoderm. *P. H.*, primitive hypoblast. *p*, periblast.

On each side the under of the two lateral layers grows beneath the notochord, and uniting with its fellow, forms the endoderm proper. The upper lateral layer thickens by cell division, and forms on each side the mesoderm plate. In the extreme anterior region beyond the limits of the incipient notochord the whole of the primitive endoderm becomes transformed into the mesoderm of the head.

The neural chord and brain are formed as a simple thickening of the ectoderm, and though there is in early stages a well marked neural groove, it disappears before the neural cavity begins to form. The neural cavity is established by a rearrangement of cells and subsequent separation in the middle line.

The formation of the alimentary canal takes place by a process of folding, essentially akin to that found in the Amniota. The endoderm after the formation of the notochord is a membrane, one cell thick, and in which the cells are uniform. In the trunk region the cells in the median line become thicker than the rest, and along this line the endoderm rises up in a fold, the two sides of which are separated by a slit-like cavity. Anteriorly in the branchial region, as has been known since Peelacher's paper, the alimentary canal is formed in a somewhat different manner, though the underlying principle is the same. Here there is a fold on each side of the median line. The folds grow up and on meeting with the ectoderm, form the embryonic pair of gill slits. The lower edges of the lateral folds approach each other in the middle line, and by their fusion close in the branchial region of the gut. In the trunk region the lower edges of the median fold likewise fuse and close in the gut. The lateral parts of the endoderm lamella are gradually drawn into the walls of the fold, so that by the time the closure takes place they have usually quite disappeared. In the posterior part of the trunk (the tail has not yet begun to form) the endoderm lamella becomes thickened in the middle line, but is not raised up in a fold. The thickening increases and a solid cord is produced, which, when the tail is folded off, comes to lie in it, and forms the greater part of the postoral gut.

The terminal portion of the postoral gut is formed by Kuppfer's vesicle, the history of which I will now give.

Just before the closure of the blastopore, the endoderm cells immediately in front of the tail swelling, in the region where notochord and neural cord are fused, become columnar. This takes place several hours before the endoderm cells in the middle line of the body thicken to form the alimentary canal. Next a narrow cleft appears between the endoderm cells and the periblast. The cleft increases in size and becomes a conspicuous cavity by means of two processes: the lower wall, or the periblast, is pushed down, while at the same time the endoderm at points *a-a* in Fig. 5 begins to fold in towards the middle line, and thus the roof of the cavity is raised. As the folding proceeds the lateral parts of the endoderm, *l. e.* in the figure, are absorbed, by which time the vesicle has grown pretty deep. Its inner outline at this stage is given by the line *2* in the diagram. Three or four hours later the lower edges of the fold, *m*, have met in the middle line, and the vesicle is closed in ventrally. At this time the tail has just begun to to be marked off, and the alimentary canal is a closed tube, except in the region immediately in front of Kuppfer's vesicle. Here it is a solid cord, which together with the vesicle represents the postoral gut. The cavity of the vesicle is very soon obliterated by the proliferation of the cells of its own wall. When the tail is folded off, the postoral gut comes to lie in it, but soon begins to atrophy. The atrophy proceeds from before backwards, and Kuppfer's vesicle may be recognized for several hours, in sections through the posterior part of the tail, as a solid mass of cells.
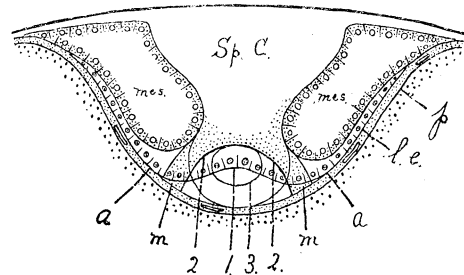

FIG. 5.—Combination of those transverse sections through different stages of Kuppfer's vesicle. *Sp. C.*, spinal cord (with fused notochord). *Mes.*, mesoblast. *p*, periblast. *l. e.*, lateral parts of endoderm. *1*, roof of vesicle in an early stage. *2*, roof of vesicle after lateral endoderm has been absorbed. *3*, inner outline of vesicle after the folds have met ventrally.

It is evident from the foregoing that the formation of Kuppfer's vesicle does not essentially differ from that of the rest of the alimentary canal. The whole tract, excepting the solid part of the postoral gut, is formed as a fold of the endoderm lamella, which fold in the region of Kuppfer's vesicle is unusually wide. The peculiar features of the vesicle are the size and early appearance of the fold, and (in pelagic eggs at least) the fact that the periblast is here pushed down, so that a true cavity exists between the endoderm and periblast before the fold can be said to have begun. The anatomical relations are thus quite clear. In the last two papers dealing with the subject, Henneguy (1888) and Schwarz (1889) give a very different account of the formation of the vesicle. They both state the vesicle arises as a closed cavity in the mass of cells just in front of the tail swelling, which is continuous with the postoral gut. The cavity has a cellular floor, and the periblast at no time helps to enclose it. These authors say that previous observers such as Cunningham, Agassiz and Whitman, who have described the cavity as between endoderm above and periblast below, have mistaken for Kuppfer's vesicle a large but inconstant and insignificant yolk vacuole. This remarkable bit of criticism may be due to the fact that in the trout (Schwarz studied also the pike) the vesicle has an abbreviated development. It seems more likely however that both authors missed the early stages.

Whatever significance Kuppfer's vesicle has for us must depend on our conception of the teleost gastrula. In this matter I am in thorough accord with Zeigler, who first carried out the comparison between the Teleost and amphibian embryos. The Teleost embryo before the invagination of the germ ring answers to the amphibian blastula. The point where the germ ring begins to invaginate (posterior end of embryo) corresponds to the dorsal lip of the amphibian blastopore—the entire edge of the blastoderm bounds the blastopore itself. The tissue of the germ ring (primitive hypoblast) which lies within the limits of the embryonic shield, is homologous with the dorsal hypoblast of the amphibian gastrula. The ventral hypoblast

of this gastrula is represented by the periblast and yolk. The ordrentron thus lies in the teleost embryo between the endoderm proper and the periblast, and the alimentary canal of the first is formed exclusively from the dorsal hypoblast of the gastrula. The ventral hypoblast of the gastrula, which in the amphibia forms the ventral wall of the alimentary canal, has in the fish lost its original function and become a mere food store for the active tissues. With reference to the meaning of what may be called the non-embryonic part of the germ ring, Zeigler is by no means clear, though its interpretation seems to me a mere corollary of the foregoing propositions. In the frog's gastrula there is an ingrowth of cells all around the lip of the blastopore. Dorsally the ingrowth (primitive hypoblast) forms the notochord, dorsal hypoblast, and paired mesoblast. Ventrally the ingrowth is almost exclusively mesoblastic—a very small portion of it appears to form the hypoblast in immediate contact with the ventral lip of the blastopore. The ventral ingrowth of mesoblast grows forwards and is an important part of the mesoderm of the body. Now if a longitudinal section of a fish embryo some little time before the blastopore is closed, be compared with a figure such as Goette's section of the frog (Balfour, Vol. II, p. 105), it will be obvious that the non-embryonic part of the germ ring in the Teleost corresponds with the ventral ingrowth of mesoderm in the amphibian. In the fish, however, just as the ventral hypoblast has lost its function and no longer shares in forming the alimentary canal, so the ventral mesoblast no longer forms the ventral musculature of the body, but remains insignificant in size, and is by the closure of the blastopore absorbed into the tail swelling. It must be looked on as a rudimentary organ of the gastrula. The germ ring (*in toto*) on this view is not a peculiarity of the Teleost. It is a feature which the Teleost gastrula owes to an ancestor more or less like the gastrula of amphibia, but which has gained in the Teleost a distinctive character owing to its appearance all around the lip of the blastopore at a time when the latter is very large.

To return to Kuppfer's vesicle. The archentron of the gastrula is represented by the virtual space between the hypoblast lamella and the periblast. When the true alimentary canal begins to form, its cavity may be looked on as a part of the archentron which is constricted off. In the region of Kuppfer's vesicle, however, before the fold can be said to have begun, a true cavity is produced by the separation of endoderm and periblast, and by the pushing down of the latter. The existence of a real cavity in this place is a satisfactory argument that we are right in interpreting the virtual space between endoderm and periblast as the archentron. To the question as to why a real cavity should appear here, I shall return in a minute. After Kuppfer's vesicle and the rest of the alimentary canal has been folded off from the archentron, the vesicle is obviously homologous with the postoral vesicle of Selachians. In each group the vesicle represents the dilated terminal portion of an alimentary canal which has been folded off from the archentron, and into this terminal dilatation the neurentric canal opens or would open if it existed. Before Kuppfer's vesicle is folded off it represents the terminal dilatation of the archentron itself, and in this phase is to be compared with the dilated posterior extremity of the archentron in certain amphibian gastrulas. In a word, the early condition of Kuppfer's vesicle indicates that at a time when in the ancestors of the Teleosts the archentron was converted bodily into the alimentary canal, the terminal portion of the archentron and, in these or in still earlier ancestors, of the alimentary canal itself, were dilated. And here it is well to recall Balfour's suggestion that in the primitive chordata, the neurentric canal and rectum opened into a dilated cloaca, which itself communicated with the exterior by the terminal blastopore.

A word in regard to the later history of the periblast. The periblast takes no share in forming the alimentary tract, and I have been unable to find the slightest evidence that it gives rise to blood corpuscles. It suffers no change in appearance until the liver has become prominent. The liver arises as a solid outgrowth of the mesenteron and growing down, adheres to the posterior wall of the yolk sac. It is only however when the latter has become very small (third day after hatching) that there seems to be any intimate connection between the liver and the periblast. At this time the periblastic protoplasm is diffused through the yolk, which in consequence takes the stain, and it is difficult to fix upon the exact limits between liver and yolk sac. Whatever be the precise manner, the sections make it evident that it is the liver which finally absorbs the periblast and yolk.

In the development of the somites and body cavity certain peculiarities enter, which cannot be here described, and which are due to the fact that the body of the embryo is so entirely buried in the yolk. At the time of hatching the pericardial cavity is not developed. In the Bass, the intermediate cell mass found in the trout, is not present, but this need not surprise us since it appears to be absent in several other fish, and its nature in the trout is far from clear.

The Wolffian duct arises as a fold of the body cavity. It at no time has any connection with the ectoderm. Brook (1887), who described it as originating from the ectoderm, very probably mistook for the Wolffian duct the lateral line anlage, to which and related structures I now turn.

Two or three hours before the closure of the blastopore, there is formed on each side of what will become the hind brain, an elongated tract of thickened ectoderm. The tract stretches from just behind the eyes nearly to the anterior somite, and forms a very shallow furrow. It is only the cells of the deeper layer which are thickened; the horny layer remains thin and lines the furrow. The groove along its whole length grows deeper, the horny layer no longer lining it, but passing over it as a bridge (in transverse section). But on two points especially the invagination is deep and conspicuous. At the posterior of the two the invagination is the larger, and here the ear sac is formed. The ear in the bass is therefore not formed as it is in the trout, as a solid thickening of the nervous layer which subsequently acquires a cavity. The nervous layer invaginates, and the cavity is there from the start. The sac thus formed is soon constricted off.

The invagination in front of the ear forms a sac, the cavity of which is closed in by the horny layer. This sac becomes the branchial sense organ of the posterior gill slit (the only one present during embryonic life). At first, however, there is no gill slit, and as long as the ear remains an open sac, its cavity communicates with that of the branchial sense organ by means of the intermediate portion of the original linear involution. When the ear is constricted off, this intermediate portion of the original furrow disappears, while at the same time the branchial sense sac acquires a more definite outline. Where the gill slit breaks through, the sense organ lies just at its anterior edge. The cavity of the sac now begins to flatten out, the horny layer still passing over the cavity as a bridge. This continues until the cavity of the sac is obliterated, or is reduced to the slightest of superficial concavities which the horny layer now lines. The columnar epithelium of the wall of the sac has, in the meantime, lost its embryonic character, and developed special sense cells. The sense organ has, by this time, all the characteristics of the well known "segmental" sense organs of larval fishes.

In the earliest stages the anterior end of the sense sac has a very indistinct outline. As the sac acquires the definite shape of later stages, there is found running from it, anteriorly and dorsally, a rod of cells clearly marked off from the surrounding cells of the nervous layer. Near the time of hatching, the cells at the anterior end of the rod (at a spot on the side of the head) have begun to proliferate and form a new sense organ, which, however, does not pass through the condition of a sac. The further multiplication of sense organs probably takes place in the same way, by cell proliferation along a growing cord—a way essentially similar to that recently described by Allis (1889) for Amia calva.

The branchial sense organ just described would seem to be homologous with one of the primitive branchial sense organs described by Beard in the Selachians. There are important differences between the two (nerve supply especially) into which I cannot enter here, but it would seem that the ancestral branchial sense organ, the position of which is indicated in the Selachian embryo by a histologically undifferentiated mass of cells, has really been retained in a functional condition by the Teleost larva, though even here the primitive sac-like condition of the organ has been lost.

After the ear and branchial sense organ have been marked off as deep portions of the elongated involution of the nervous layer, the posterior part of the involution becomes the anlage of the lateral line. This part from the start is narrow compared with its length, and its cavity is of a uniform depth. While the ear remains open, it is in direct connection with the cavity of the lateral line. After the closure of the ear the lateral line forms an elongated sac, nearly circular in cross section and with a narrow slit-like cavity, which is wider at the ends. The sac retains its connection with the surface ectoderm, and over the mouth of the cavity passes the horny layer. When first formed the lateral line anlage lies just in front of the anterior somite. It presently begins to grow back, but while it remains a single sac, is found in the anterior somite region. In this condition it is very apt to part from the surface ectoderm during the processes of imbedding and

sectioning, except at the ends where the attachment is stronger, and then gives one the impression of a free tube lying on the somites. As I have said, it is this sac which Brook has mistaken for the Wolffian duct.

The separate sense organs of the lateral line are formed in the following way. The elongated sac constricts and is divided into two parts, of which the anterior is the smaller. The two portions separate, remaining connected for some little while by a strand of cells which subsequently disappears. The anterior sac runs through the same series of changes described for the branchial sense organ. Its cavity is flattened out, histological modification sets in, and it becomes a "segmental" sense organ, situated in the anterior part of the trunk. The posterior sac shortly after divides in the same way, the anterior portion becoming a sense organ in front of the anus. The posterior portion, by the time the division is over, has lost the character of a sac and is a mere cord of cells, lying behind the anus. On this cord on one, and in some cases on two spots, sense organs are found in process of forming. This is the condition at the time of hatching.

Prof. Whitman, in a recent paper (1889), states he is satisfied that it is not by division that the lateral line organs increase in number. How the increase takes place after hatching I do not know. From the character of the sensory tract in the tail of the just-hatched bass, I should think it probably was effected by local proliferation along a continuously growing linear tract of sensory cells. But before hatching the increase unquestionably takes place by division.

Without going far into theoretical questions, the development of the lateral line and branchial sense organ in the Bass, indicates that the superficial sensory patch, such as is found in larval fish, does not represent the condition of the primitive segmental or branchial sense organ. This sense organ was, in all probability, a sac, as the nose has remained (in fishes), and as the ear is in its early stages. Thus while the development of the Teleost strikingly confirms Beard's hypothesis of the homology between the ear, nose, and branchial sense organs, it leads one to believe that the ear and nose were not surface areas over which were scattered a number of sense organs, but that they were themselves single sense organs.

The lateral line anlage of the Teleost is evidently homologous with that of the Selachian. But while in the Selachian the anlage is a mere thickening, in the Teleost it recalls the primitive condition of the organ. One of the most striking differences between the development of the lateral line in the two groups is the absence of the lateral nerve in the Teleost embryo. At the time of hatching no nerve at all can be made out, supplying the line.

The account I have given of the development of the lateral line organs is radically different from that of Hoffmann (1883), who makes them arise *in situ* by local modification of the ectoderm. It is, on the contrary, similar in many points to Beard's account for the Teleostii, and can easily be harmonized with the accepted account (Van Wighe and Beard) for the Selachians. While Hoffmann's description lends countenance to the homology drawn originally by Eisig between the lateral line organs of fishes and those of certain Annelids, the account given here would seen to be irreconcilable with such a theory.

Wood's Holl, February 10, 1890.

Notes on the Pronephros of Amblystoma punctatum. By J. L. KELLOGG.

The following is a summary of the chief points obtained from observations on Amblystoma embryos in regard to the head kidney or pronephros, the work having been begun in October last.

The segmental duct, which is to connect the gland proper with the cloaca, first makes its appearance, and arises from the somatic mesoblast. The anterior end of the duct becomes constricted off from the peritoneal epithelium—after this has occurred posteriorly—except at two points, where the nephrostomes are to open into the body cavity. A lumen does not appear anywhere in the organ until sometime after it has been thus separated from the peritoneum. The places of attachment which remain, do not have the shape of funnels, a form which they afterward acquire. They are merely thickenings of the peritoneal epithelium, much elongated from above downward, and connected with the pronephros. As the whole organ becomes older, and the openings into the body cavity are acquired, the nephrostomes become more and more funnel-shaped in outline, though they are always elongated somewhat in a dorso-ventral direction.

A series of models has been constructed, showing the development of the gland. They are made of wax, the drawings being taken from cross sections. The most important point to be noticed is the formation of the so-called ventral part of the gland. It has always been described, at least in Amphibia, as being formed from that part of the duct immediately behind the last, or most posterior nephrostome. This is said to grow rapidly, and to be placed on the ventral side of the first formed, or dorsal part of the gland.

In Amblystoma, and also in the frog, this lower portion is formed from the ventral side of the dorsal part of the pronephros, and anterior to the last nephrostome. That is, the head kidney of a comparatively young Amblystoma embryo would consist of a dorsal tube with a nephrostome at each end, and a more or less convoluted ventral portion uniting with it from below. This latter portion is one simple, convoluted tube, extending backward to the cloaca. If the first view were correct, some portion of the gland would show in cross sections behind the most posterior opening into the body cavity, but in some eighty series of cross sections of Amblystoma and many of Rana temporaria, this has never been the case.

The ventral portion of the pronephros, at first with a simple curve backward, grows rapidly in length, and takes on a definite series of convolutions, the first ones appearing anteriorly. When the organ has become fully developed, the many foldings are closely packed together, and the walls of the tube become much thinner, making it relatively much smaller.

The dorsal portion grows in length much more slowly, and the growth is confined, for the most part, to the portions nearest the nephrostomes.

In neither of the forms mentioned do diverticula with blind ends appear until the gland has become very much convoluted.

The nephrostomes are segmentally arranged. The anterior opening in Amblystoma is opposite the anterior end of a somite, and the second opening is opposite the posterior end of the next succeeding segment. In the frog, where there are three nephrostomes, they are found to be opposite three successive segments.

The glomerulus, a vascular protrusion of the peritoneum into the body cavity opposite the openings of the pronephros, appears very late in Amblystoma, compared with that of the frog.

The body cavity is separated from the pericardial much sooner in Amblystoma than in Rana. In the first of these, constrictions appear dorsally, and are either two or three in number. Whether or not the third is added at a period later than those showing but two, I expect to determine presently. These constrictions, shutting off a space opposite each nephrostome, do not extend to the extreme ventral portion of the body cavity.

Models have also been made of the pronephros of the frog, and the same general structure of the gland occurs as is found in Amblystoma.

It is my intention to satisfy myself as to one or two more points in the structure of this gland; and to make, also, a more careful comparison between it and the same organ in the frog.

Baltimore, March 20, 1890.

A Preliminary Note on the Embryology of the Pycnogonids. By T. H. MORGAN.

The position of the Sea Spiders, amongst the Articulates, and the speculations as to the relationship of this to other orders, have been almost entirely based on the anatomy of the adult animal. Kröyer, in 1840, described some embryos, and Dohrn and Hoek have carried this a step further. Owing to the extreme difficulties of technique, nothing is known of the internal changes that take place during development.

A study of the germ layers and their subsequent differentiation into organs ought to throw, I thought, some light on the phylogeny of these most interesting animals. Material for work was collected during the summer of 1889 at Wood's Holl, Mass., and I am under very great obligations to Professor McDonald for the opportunity to collect and study at the laboratory of the Fish Commission Station.

Three species of Pycnogonids are found amongst the sea-weeds and hydroids about Wood's Holl, viz.: Phoxichilidium maxillare, Tanystylum orbiculare, and Pallene empusa. During July and August these carry eggs.

The first two genera have a small, free-swimming, six-legged larval stage. In Pallene the eggs are much larger, resulting in abbreviated development, and the young leave the cluster of eggs, carried by the male, in a practically adult condition.

In Phoxichilidium and Tanystylum the egg undergoes a regular segmentation into 2, 4, 8, 16; all the segments being equal. After one or two more divisions a condition is reached as shown by Fig. 6. Here the segments have the form of pyramids, with the apices together at the centre of the egg. A nucleus is at the outer part of each pyramid. The egg continues to divide, the pyramids becoming smaller, and then each pyramid divides into an outer and an inner part, each part with a single nucleus. See Fig. 7. Here we have a most perfect delamination, resulting in an outer circle of ectodermal cells and an inner mass of cells. Both inner and outer cells continue to divide. Many of the inner cells now break down, as is seen in the endoderm of many Coelenterate planulae, and the cells of the outer circle become smaller; the line of demarcation between inner and outer cells remains sharp and distinct. It is very difficult to follow out the fate of these two cell masses. Many of the inner cells seem to form a yolk-like substance, with a few scattered nuclei, while the outer cells form undoubtedly the ectoderm of the adult. I believe the endoderm to be formed from some of the nuclei of the inner cells, but I cannot be entirely certain of this, nor have I any observations as to the origin of the mesoderm.

In Pallene, on account of the much larger and more manageable eggs, I have been able to carry out in much more detail the origin and fate of the germ layers. The eggs measure .25 mm. in diameter, and have 125 times the volume of the preceding species. The segmentation is quite interesting, and I had the good fortune to be able to follow it quite far along in the living egg.
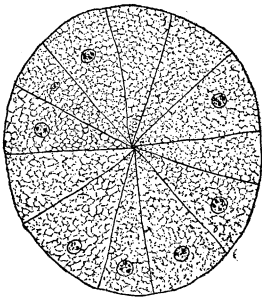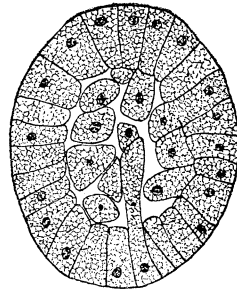

FIG. 6.   FIG. 7.

The first furrow divides the egg into two very unequal parts—a large macromere and a small micromere. The latter is about one-fourth the size of the first. Each segment has a single nucleus. The next furrow—at right angles to last—divides each of the first two into two equal halves. In some cases the larger cell divided five minutes before the smaller, but in other cases the reverse process took place. The furrows dividing the egg into these four parts *nearly* coincide for the micromeres and macromeres. The third furrow divides both micromeres and macromeres into four each, and is at right angles to the first two planes of division. The next furrow is seen to divide the four macromeres into eight, is at right angles to the last two furrows, or is parallel to the first plane of segmentation. At the same time each micromere divides into two, but no definite plane of division is apparent. There are now eight macromeres and eight micromeres. Each of the eight macromeres divides into two in planes at right angles to the last furrow or parallel to the second and third planes of division. This is followed later by a division in the macromere in a plane at right angles to the last or parallel to the first and fourth; but only those cells above the horizontal furrow (fourth) were seen to divide. The micromeres were not seen to keep pace with these last divisions, so that the upper (micromere) pole of the egg is covered with a mass of cells of about the same size. Sections of eggs in this stage show pyramidal figures somewhat similar to Fig. 6, but the upper pyramids are smaller, and some do not run to the centre of the egg. Each pyramid has a nucleus in its outer part, and each nucleus is accompanied by a mass of protoplasm which sends out processes into the surrounding yolk of the cell. Soon after this the formation of yolk pyramids ceases, and the nuclei (and their protoplasm) lie at the periphery of the egg. At the upper pole the nuclei are much more numerous, but smaller than at the lower, and the protoplasm forms a thick covering to the egg. Here,

also, the blastoderm develops rapidly. At the lower pole there are scattered nuclei at the surface of the yolk.

The early separation of the egg into two unequal parts is apparently closely connected with the more rapid development of the embryo in the region of the smaller segment. About the time when the pyramids become lost (and perhaps at that time) each of the peripheral nuclei divides radially into an outer and an inner nucleus—each of course with its cell protoplasm. It takes place first over the upper pole, and not till very late over the lower area. This is undoubtedly the same thing as the delamination in the smaller eggs. The differences are these—that in Pallene the pyramids do not divide themselves each into two cells, but only the nuclei and protoplasm; and this takes place later at the lower than at the upper pole. The delaminated nuclei remain just under the outer cells—ectoderm—and only exceptionally do one or two wander into the yolk. These nuclei form the endoderm of the mid-gut after having devoured the yolk. The protoplasmic layer at the upper pole becomes wider and the nuclei more numerous, each nucleus being the centre of a distinct cell. At one place may be seen from surface veins an opaque area (much like the early stage of the primitive cumulus in spiders), and sections show that here an invagination of ectoderm is forming—the stomodæum. Around its periphery there is a collection of cells which are, no doubt, the beginnings of the mesoderm. The stomodæum increases in depth, and at this time the appendages and their nerve ganglia may be seen on the surface. Above and anterior to the stomodæum are the thickenings of epiblast to form the brain. On *each side* of the invagination the first pair of appendages arises. Behind the stomodæum five pairs of large ganglia appear, and on each side of the three posterior pairs are formed the fourth, fifth and sixth pairs of appendages.

At the "lower" pole of the embryo (at this time the dorsal and posterior) the nuclei are slowly multiplying and cover the surface with thin protoplasm. Now the embryo lengthens in the antero-posterior direction, the appendages and their ganglia become more conspicuous, and there is seen a slight *invagination in the centre of each ventral ganglion*. There are five pairs of these invaginations corresponding with the same number of ganglia, and I shall call them the *ventral organs*.
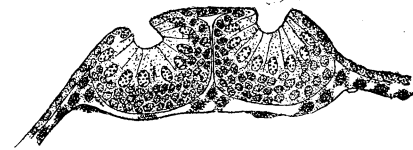

FIG. 8.

Cross sections of embryos show distinctly a wide ingrowth of the surface epithelium into the centre of each ganglion. The cells of its walls are rather high, with a clear outer portion, and with large nuclei. Fig. 8 shows such a cross section. These organs close on their outer surface, and there remains in the centre of each ganglion a cavity, which is rather longer than wide from side to side, and persists till quite late in embryonic life.

I need only refer to similar (?) invaginations in Peripatus, and more especially to a section of a pair of these organs figured by Sedgwick for the ventral organs of the jaws of Peripatus (*Studies Morph. Lab.*, Cambridge, Vol. IV, Pt. I, Plate 10, Fig. 4), which easily suggests a comparison with an *early stage* in the development of the ventral organ of Pallene. Although I have looked very carefully I have not satisfied myself as yet as to whether or not there are any invaginations for the brain of the Sea Spiders. The appendages grow in length, and into each there is pushed an outgrowth from the mesenteron. These outgrowths contain yolk, and this is covered by a layer of endodermal cells. Along the ventral half of the embryo there are scattered mesoderm cells, and these extend into the appendages between the endoderm and ectoderm cells.

In Pallene the second pair of appendages, which are found in other pycnogonids, never appear, and seem to have been completely dropped from the ontogeny. The third pair of appendages (the egg carriers of the male) appear at the time when the young is about to leave the parent. Two pairs of nerve ganglia develop for these appendages, and each ganglion contains a ventral organ. Later the two pairs form a single pair with four ventral organs. The presence of these ventral organs is conclusive proof as to the

erroneousness of Schimkewitsch's hypothesis, that the third pair of append-ages are outgrowths of the second pair. The fourth, fifth and sixth pairs of appendages appear (as already given) simultaneously and quite early. The seventh appears a little before the embryos leave the parent. Like-wise do the ganglia of the seventh appear quite late. There is in addi-tion one pair of ganglia for the rudimentary abdomen. The yolk mass becomes smaller as it is eaten by the endoderm cells, and exceptionally a wandering endoderm cell may be found in the mass of yolk. As the yolk disappears a number of schizocoels appear in the mesoderm between the body walls and the digestive tract. Dorsally the heart appears as a simple tube. The stomodæum communicates with the mesenteron and the proc-todæum forms very late—at the time when the seventh pair of appendages appear. Soon the embryo leaves the parent and no doubt crawls off among the sea weeds and hydroids to shift for itself.

The Phylogeny of the Pycnogonids.

It will be impossible to give here the bearing of these embryological facts upon the phylogeny of the group, and I reserve for the future a fuller discussion.

It seems to me, however, that when all the embryological phenomena are taken together they give quite strong evidence for the relationship of the Pycnogonids to the Arachnids. Dohrn and Hoek have each recently reached independently the belief that the group must be considered an isolated one, with a more or less independent origin from the Annelids. I hesitate before offering an opinion against those who are so well qualified to speak authoritatively on the subject. On the other hand their opinions are based largely on the adult anatomy of the group, as little or nothing has been known concerning the germ layers, &c., of these animals; and it is chiefly on embryological grounds that I believe a comparison with the other groups of Anthropods must be based.

It is generally believed that the adults are in many respects degenerate and adapted to a very special habitat—the abdomen has become lost, or almost so, and all traces of respiratory organs are gone, the general surface of the body functioning as such: also that the group is an old one, and not derivable from any existing groups of Arthropods. So far we are together. Without going into details, it does not seem probable that the group is closely related to the Crustacea, nor very closely to the Insects. Here I can only use the *tout ensemble* of the above facts as evidence for this statement. We are then left to decide between an independent origin for the group and an alliance with the Arachnids. If there are any special reasons for an alliance with the Arachnids, I believe such facts must turn the greater weight of evidence towards such a relationship. Briefly then in this connection these considerations must be given:

1. The process of *multipolar delamination to form the endoderm* is, I believe, common to the two groups. We have it represented in its greatest sim-plicity in the majority of the Pycnogonids, while Pallene furnishes an analogy to the changes which an accumulation of food yolk will cause in this process, and renders a comparison with the Arachnids quite possible. I will refer to Metchnikoff's figures for Chelifer, and to Balfour's embryology for the Spiders (Vol. I, page 119, Sec. Ed.). Here we read: "It appears to me probable that at the time when the superficial layer of protoplasm is segmented off from the yolk below, the nuclei undergo division, and that a nucleus with surrounding protoplasm is left with each yolk column." Com-pare Fig. 6 and 7, and see account of Pallene.

2. The formation of an opaque area (Pallene) at the place where the stomodæal invagination appears.

3. The early formation of *mesoderm at this place*—the primitive cumulus of Spiders. (?)

4. The general mode of appearing of ganglia and appendages.

5. The body cavity of the appendages and the early presence of mesoderm.

6. The formation of *endodermal pouches* from the mid-gut into the append-ages, these pouches *containing yolk in the embryo.* Compare Chelifer and Spiders.

7. The large "upper lip" of Chelifer suggests an homology with the pro-boscis of pycnogonids.

8. The first (Chelate) appendages appear at the sides of the stomodæum and subsequently move forward, and are innervated from part of the supra-oesophageal ganglia (brain). They will in this bear out a close comparison with Chelifer (or with Arachnids.)

9. The lumen of the invagination of the stomodæum is triangular in

outline and remains so in the adult. Schimkewitsch describes a similar triangular invagination in the Spiders, and compares it directly to that of the Pycnogonids.

The full meaning of the ventral organs I cannot discuss now. I have compared them to similar organs of Peripatus. It may be that in this respect the Pycnogonids show a very primitive structure, common to them and to Peripatus, and if so, traces ought to occur most probably in other Arachnids.

The absence of brain invaginations would be a more weighty objection against the relationship of the two groups, and really the only good objec-tion I know from the embryology.

The openings of the reproductive organs of the adult on the legs cannot be fairly urged against my comparison, for we have so far no explanation of the meaning; and on the other hand this gives little better foundation for a relationship with the Annelids.

All the above comparisons are not of equal weight, and some may be wrong; but taken all in all, I must appeal to them to bear out the hypoth-esis of the relationship of the Pycnogonids to the Arachnids.

Baltimore, March 15, 1890.

Notes on the Physiology of Caravella Maxima. By ROBERT PAYNE BIGELOW.

(Preliminary Abstract.)

The Portuguese Man-of-War,—*Caravella Maxima*, Haeckel,—was for a few weeks last summer quite plentiful in the neighborhood of Woods Holl. The author was thus enabled to make some observations on the physiology of this curious animal.

It will be remembered that we have here a large bladder-like float, the pneumatophore, from the under side of which is suspended the large num-ber of fighting, feeding, and reproductive members of the corm. The repro-ductive and the feeding members with the small tentacles are arranged in clusters, cormidia. The chief fighting members, each consisting mainly of a very long tentacle, are attached directly to the float.

As the caravella rests undisturbed on the surface of the water, some of these parts are constantly in motion; lengthening and shortening, or twist-ing about. No rhythm was observed in the motions of the tentacles, but it was seen in the contractions of the cormidia, which were at the rate of about eight per minute and the bouts of contractions would last for from one to four minutes, with somewhat shorter intervals of rest.

Exposure to unfavorable conditions will cause the appendages to break off from the float while still alive. The muscles of the float, in this case, become opaque and contracted, apparently going into rigor mortis, until the float bursts; or else the float simply becomes dry and parchment like.

The food of the caravella consists probably almost entirely of small fish, which are caught by running against the tentacles. The tentacle is imme-diately firmly attached to the fish, probably by the nettle cells, and it is very soon temporarily paralyzed by the poison from them. But before it suc-cumbs, the fish manages to give a pretty vigorous pull on the tentacle. This acts as a stimulus to cause the tentacle to contract, the impulse appa-rently coming from its base. If the fish offers no resistance the tentacle does not contract. By the contraction of the tentacles the fish is brought into contact with the mouths of some of the siphons, the feeding members. These mouths are spread out over the fish until they completely envelop it. It is there finally killed and digested. The products of digestion with undigested fragments are taken into the stomachs of the siphons until they are gorged; then what is left of the fish is allowed to fall. The digestion is completed in the stomachs and the nutrient fluid is conveyed by the hollow pedicels to the rest of the corm.

The presence of food does not act as a stimulus to the tentacles nor to any other parts except the siphons, and then only when the mouth of the siphon is in actual contact with the food. In fact, no difference is notice-able between the behavior of a siphon towards a piece of fish and its beha-vior towards a small stone, until it has been attached for some time. It will fasten itself with equal alacrity to either, but does not remain attached to the latter very long.

Mechanical stimuli applied to any part cause local contraction. Strong stimulation of the float or bases of the cormidia is followed by a general contraction. Wind or rain beating against the float causes its muscles to

contract so as to erect the crest, which normally lies flat on the water. To keep the crest erect seems to be a good deal of an effort, for the muscles usually soon relax and the float rolls over on to its side again. The ordinary drying of the surface of the float seems to produce an effect similar to that of the wind, and it seems probable that the effect of the latter is due to the increased evaporation that it causes.

The animal may be cut into very small pieces and each piece will retain its spontaneity and will respond to stimuli in the usual way. But some parts will be more active than others.

The secretions that I have observed in Caravella are: A mucous secretion on the surface, a gluey substance at the mouth, a digestive fluid, a poison in the nettle cells, and probably the gas in the float.

The nervous system seems to be very poorly developed. There is some indication of a motor centre at the base of each tentacle, and impulses may be transmitted from one part to another. But I found no part that was incapable of originating an impulse, and I could see no correlation of movements. There is no trace of any sense of sight, hearing, or smell; and it is doubtful if there are any special senses. So far as my observations go, the only external stimuli to which the animal normally reacts and the reactions are these: Irritation of the float by fresh water in the shape of rain and by drying of its surface, especially by wind, which is followed by a turning over of the float and the erection of the crest; violent contact with a foreign body, followed by the discharge of nettle cells at the point stimulated; a pull on a tentacle, followed by a total contraction of the tentacle, starting from its base; and attachment of a siphon mouth to a foreign body, followed by the spreading of the mouth out over the object. If the object is digestible, the mouth is widely spread out and remains attached until the siphon is gorged with food; otherwise, the siphon will soon detach itself.

New Observations on the Multiplication of Bryophyllum. By B. W. Barton.

Since the preliminary report on the "Multiplication in Bryophyllum," other experiments which have been made have given results of more or less interest and importance. Only the following need be described at this time.

1. The possible morphological relations of the marginal leaf buds to ovules, suggested the idea of grafting upon the former some of the plant's own pollen, with a view of possibly stimulating such buds to growth upon leaves yet attached to the present plant. The result was nearly negative, but the pollen was of questionable healthfulness. The experiment may be worth repeating under more favorable conditions.

2. Large leaves were torn across the blade and left connected by a narrow strait of tissue to the base of the leaf, and so to the mother plant. Upon the margin of the nearly detached part of the leaves shoots developed freely. The same took place on leaves whose petioles were nearly severed by a knife or on any leaves whose free communication with the parent plant was in any way interrupted, as in the case even of some of the older leaves situated near the base of the plant and which were undergoing the natural process of defoliation.

3. All the foliage except the lowermost pair of simple leaves was removed from a strong young plant. In a short time the buds in the axils of these leaves began their growth but were carefully cut away. Later there appeared other buds successively lower and lower down on the stem from older nodes. These were likewise removed until there remained no axillary buds on the plant. The two leaves were now raised so as to face one another and loosely tied together in a vertical position. By the accidental slipping of the string one of the leaves returned to a partial dorsi-ventral position, but still remained with one lateral margin higher than the other. The second leaf remained standing vertical. From both leaves the marginal buds now developed freely. On the leaf with apex uppermost, the shoots first to appear and to continue largest were those on the apical margin. So also on the leaf which was partly turned on its side, the shoots developed first and continued largest on that part of its margin which was highest. This experiment was repeated with variations as to conditions, but always with essentially the same results.

Comparative experiments were made upon Bryophyllum and other related and unrelated plants. Their behavior when subjected to the influence of the klenostat and to sundry other unusual conditions, causing them to manifest geotropic phenomena, were compared, and the sum of all the results lead to the conclusion that the marginal buds under investigation were most nearly of the nature of axillary buds of the least preferred kind, comparable to the axillary buds on branches of the second or third order in ordinary plants, and that they offer an example of axial growing on foliar structure, and so furnish more evidence of the homology of leaf and stem.

Studies on Thysanuran Anatomy. By H. T. Fernald.

(Preliminary Communication.)

The importance of a complete knowledge of the anatomy of the Thysanura for the intelligent consideration of questions relating to insect phylogeny, has led me to devote considerable attention to this group. A more complete paper, of the original portion of which this is but an abstract, is now ready for publication.

The forms here considered are Anurida maritima, and Lepisma saccharina—the former quite fully, the latter only in a few particulars.

In Anurida the two pairs of mouthparts—mandibles and maxillae—can be protruded from, or drawn into the cavity of the head where they lie in a sort of pocket, ventral to the pharynx. The digestive tract is composed of three chief divisions—fore, mid and hind gut—separated from each other by structures functioning as valves. The fore gut has no longitudinal muscles and the circular fibres have all their nuclei on the middle dorsal line of the gut. In the mid gut no chitinous lining is present, but the free surface of the epithelium is covered by fine hairs which constitute the "Härchensaum" described by Frenzel (Arch. f. Mik. Anat., XXVI, p. 229, 1886). In molting, the nuclei of these cells divide, and the products of this division pass to opposite ends of the cells. The cells themselves now divide, and the portions nearest the lumen are set free. These form a mass which lies in the gut till the cuticula of the exterior of the body is thrown off, when it is expelled.

A structure termed by Sommer (Zeits. w. Zool., XLI, p. 683, 1885) the "exkretionsorgane" in Macrotoma, is present in Anurida, and I regard it as homologous with the fat body of the higher insects. Its connection with the hypodermis, Sommer believes, proves that this is not correct, but my specimens appear to indicate that such a relation is only secondary.

The heart lies on the dorsal middle line, and at its anterior end becomes continuous with the aorta, which passes forward and after a time bends ventrally and rests on the fore gut. Farther forward it entirely surrounds this, and extends below it to the sub-œsophageal ganglion. A little anterior to this point it abruptly ends. The nervous system consists of a brain, a sub-œsophageal ganglion and three ventral ganglia, one in each segment of the thorax. No abdominal ganglia occur, but the metathoracic ganglion appears to have resulted from the fusion of at least two.

In each of the main nerve trunks, near its origin, lies a very large nucleus, more than twice the size of the nuclei of nerve cells, nuclei of the ganglia or brain. I have been unable to ascertain its significance. From the brain are innervated the antennae and eyes; from the sub-œsophageal ganglion, the mouth parts; from the thoracic ganglia, the different parts of the segments in which they are situated; the metathoracic ganglion, in addition innervating the abdomen. One or two small ganglia with two or three nerve cells are present in the more distal joints of the legs. Tactile bristles are scattered over the surface of the body, and are especially abundant in the antennae and around the mouth.

On the terminal joint of each antenna is a small trilobed organ, similar to the bilobed organ described for Campodea by Kingsley (Am. Nat., XVIII, p. 540, 1884.) I have traced its connection with a nerve fibre, and am inclined to look upon it as in some way aiding in the determination of the forms of the objects which it may touch. There are five eyes on each side of the head, each consisting of a nearly spherical mass of protoplasm containing four nuclei, and covered externally by the cuticula which is here smooth, though bearing small protuberances elsewhere. Immediately beneath the protoplasm is a dense layer of pigment. The different eyes of each side are entirely independent, and lie some little distance apart. No structure resembling an ommatideum could be found.

The post-antennal organ described by Laboulbéne is situated between the eyes and the base of the antenna, on each side of the head. It is a rosette like structure, consisting of from seven to nine ovoid bodies radiating from a centre. At the central end of each is a sort of pedicel or stalk joining the

ovoid portion to the head. Both parts of the organ are filled by a pigmented protoplasm continuous with the hypodermis. No nerve connection was observed.

The abdominal vesicle is situated on the ventral surface of the first abdominal segment. It is cleft longitudinally and the hypodermic cells lining this cleft are glandular in appearance, and are larger than on the outer sides of the vesicle. Passing forward from this point on the ventral middle line of the body to a median cleft in the lower lip, is a small tube, in the formation of which both hypodermis and cuticula take part.

In the posterior portion of the head are a pair of glands which resemble salivary glands and which I regard as their homologues here. From these glands a duct leads forward and soon fuses with its fellow, and the median duct thus formed passes along the under surface of the buccal cavity to a median cleft of the under lip, where, instead of emptying into the mouth, it turns downward and joins the ventral tube just described. This remarkable relation of the parts concerned I am unable to explain, although sure that no error of observation was made. Traces of a rudimentary "spring" occur in the fourth abdominal segment. The female reproductive organs are a pair of tubes, the ducts from which join, forming a median duct with a ventral diverticulum—the receptaculum seminis. The structure of these parts and the formation of ova is nearly the same as described by Sommer (*l. c.*) for Macrotoma.

The number and arrangement of the male reproductive organs is the same as in the female except that there is no ventral diverticulum of the median duct. In both sexes the external opening of this duct is on the fifth abdominal segment.

In the immature testis are groups of granules and scattered nuclei. These groups develop, the granules elongate, and form threads lying in bundles. Soon degeneration occurs, resulting in the formation of a quite homogeneous mass, with scattered nuclei, around which protoplasm collects, forming a sort of epithelium in which fat globules and spermatozoa are formed.

Lepisma saccharina is larger than Anurida, and has ten abdominal segments. Three anal cerci and several abdominal appendages are present, and the female has in addition a long ovipositor.

The mouth parts are of the mandibulate type and cannot be retracted into the head. The digestive tract behind the brain gradually enlarges till near the end of the fourth abdominal segment, where it abruptly contracts, and now in its walls six chitinous rods are formed, armed with stout teeth, while the circular muscle layer becomes much developed. Beyond these rods the lumen becomes still smaller and turns dorsally, then suddenly bends ventrally and enters the stomach. This has extending forward from the entrance of the portion just described, six short cæca. It passes back into the seventh segment of the abdomen, where it makes a half circle to the left, and joins the ileum which completes the circle, passing dorsal to the stomach, and continues back about one segment when it enters the rectum. The rectum is a large irregularly shaped chamber with many folds and diverticula. It finally narrows and leads by a very short straight tube to the anus.

The epithelium of the stomach bears a "Härchensaum," and in many places forms small crypts, the function of which is claimed by Oudemans to be the formation of new cells. The crypts are certainly suggestive of a glandular function, however. The stomach has no chitinous intima, but all the other parts of the digestive tract are lined by a cuticle. This is the case in the ileum, from the anterior end of which several Malpighian tubes are given off. The epithelium of the ileum is thrown into six longitudinal folds. This whole portion is supported by mesenteries passing in various directions, accompanied by muscle fibres. The rectal epithelium is columnar and its cell walls stain very faintly. The nuclei lie near the bases of the cells.

Salivary glands of a simple nature and with no noteworthy features, are present. The fat body is apparently of the same structure as in Anurida.

The heart leads forward into an aorta, which in the prothorax bends downward from the dorsal surface of the body till it touches the œsophagus. It does not enclose this but soon becomes lost. The heart at its posterior end is wide open, somewhat like the mouth of a trumpet, anterior to which point it becomes small, and with very muscular walls. About two abdominal segments farther forward it quite rapidly enlarges till its lumen is seven or eight times as large as it was before, while the walls become thinner. The heart between here and the aorta has its usual structure, with ostia, none of which were observed in the muscular portion.

A tracheal system is present, but I failed to ascertain its exact distribution.

The nervous system consists of a brain, sub-œsophageal ganglion, three thoracic and eight abdominal ganglia. Of these the last thoracic and first two abdominal ganglia, and the seventh and eighth abdominal ganglia are partially fused.

The brain is more complicated than in Anurida, calyces being present. No trace of the large nuclei at the origins of the main nerve trunks, was found.

Each eye consists of twelve facets or ommatidea. Each ommatideum consists of a large cornea, beneath which are two corneagen cells. The crystalline cone has the form of a concavo-convex lens, and just lateral to it are the four cells of the vitrella.

The rhabdomere is pyramidal, its base resting against the internal face of the crystalline cone. Surrounding it are four retinulae which are densely pigmented and the proximal ends of which perforate the basement membrane and become optic nerves. The ommatidea on the whole somewhat resemble those of Serolis. Between them are packed pigment cells.

The position and structure of the reproductive organs of both sexes is much the same as in Anurida.

Lepisma seems to represent about the highest grade of differentiation yet attained by the Cinura, while Anurida seems to have undergone a differentiation perhaps even greater, but followed by a degradation, probably correlated with a change of habits and food. The internal anatomy of the Thysanura as a whole appears to confirm the Campodea theory advanced by Braur.

On the Migration of the Retinal Area, and its Relation to the Morphology of the Simple Ocelli and the Compound Eyes of Arthropods. By S. WATASE.

By the term *Ocellus* in the Arthropod I here refer to a visual organ in which the sensory nerve-end cells are segregated into definite groups called retinulæ, a group of retinulæ being again characterized by possessing a single dioptric apparatus in common. Being thus broadly characterized, the term *Ocellus* also includes the visual organs of several other groups of animals, the lateral eyes of Vertebrates for instance, but I confine my remarks at present to the visual organs of Arthropods as exemplified in the ocelli of *Limulus*, Scorpions and the like. There are reasons to believe that in some cases the mosaic arrangement of the nerve-end cells is obliterated more or less completely by secondary modification, and the number of nerve-end cells entering into the formation of a single retinula may vary considerably, ranging from two to five or more, according to the different animals, and even in the different individuals of the same species.

In my previous paper, "*On the Morphology of the Compound Eyes of Arthropods*" (*Stud. Biol. Lab.*, Johns Hopkins Univ., Vol. IV, No. 6), I have pointed out that a single retinula is morphologically a pit-like invagination of the skin. Thus, for the term "segregation" of the retinal cells, each group constituting a single retinula, I substituted the term "invagination," which, besides explaining why the retinal cells appear segregated, explains also the tubular arrangement of the retinal cells, the existence of the radial chitinous rhabdom below the surface of the body, and brings the compound eye of *Limulus* into harmony with the compound eyes of other Arthropods. In the ocelli of Scorpions and *Limulus* we see exactly a similar kind of "segregation" of the retinal cells, and if the facts and arguments brought forward in the interpretation of the retinulæ of the compound eyes be accepted, it is difficult to see how one can escape from the conclusion that the same interpretation holds true also with the retinulæ in the ocelli. Hence I conclude that the retinula with its radial rhabdom in the median ocelli of Scorpions and *Limulus* is morphologically a pit-like invagination of the ectoderm cells. I here purposely leave the consideration of the retinulæ in which the rods are "terminal," since it unnecessarily widens the field of discussion.

It is my purpose to show in the present paper, granting the homology of the retinulæ in both the compound and simple eyes, how a group of such retinular invaginations becomes again invaginated as a whole. In this lies the problem; and the morphology of the ocelli consists, in the main, in the unravelling of these secondary involutions of the sensory areas which are carried out in different degrees in different animals. The simplest example of such a step in the invagination of the sensory area is shown in *Fig.* 10. Such

a condition seems to be partly realized in the lateral ocellus of a Scorpion, according to the result given by Lankester and Bourne, although Parker's account seems to point to a more advanced state of fusion if retinulæ had actually existed as is indicated in the diagram. A chitinous substance being secreted on the external surface of the hypodermis, the invaginated area of the skin in which exists a number of pits, is covered by a single, huge, biconvex lens (*L*). In the case of a compound eye (Fig. 9), each pit or ommatidium secreting the dioptric apparatus of its own, there are formed as many corneal lenses (*C*) as there are invaginations. The compound eye results from the elevation of the retinal area, and the ocellus from its subsidence. The character common to both is the existence of retinulæ, each of which is morphologically a simple pit in the skin. The starting point for both may be assumed to be a group of pit-organs situated on the body surface of the animal. This state is partially realized in the lateral eye of *Limulus*. In the one, this area of pit-organs becomes elevated, the depth of each pit increasing with the height of the elevated area (Fig. 9); in the other the whole area sinks below the level of the body surface, and with it the chitinous covering of the body thickens over the area of subsidence and forms the lens. (Fig. 10.) The question, therefore, of the relation of the ocellus to the compound eye is identical with others of a similar nature, as for instance, the relation of the pulmonary-pocket of *Scorpio* to the gill-book of *Limulus*. If a portion of the embryonic, external tissue make an inward growth into the body of an animal instead of an outward one, and attain maturity beneath the surface of the body, the fate of resulting organs may be different. The
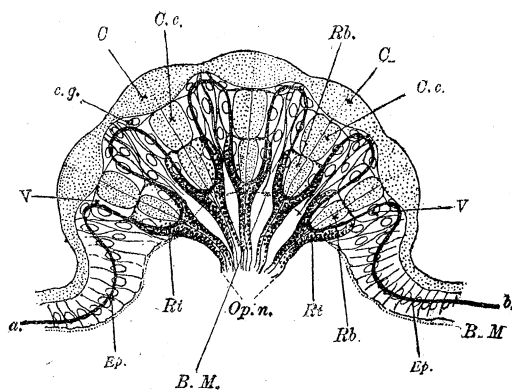
Fɪɢ. 9.

development of the Tœnia-head upon the cyst of the hydatid in such a form as *Tœnia Solium* has already been cited by Lankester as an instance in which an inward growth of a tissue takes the place of an outward one. To this I may add the origin of the remarkable pouch in *Actinotrocha*, or the invagination of the enamel organ in the formation of a mammalian tooth for further illustrations.

Turning back to our subject, we may say that an area of primitive pit-organs which probably occupied the level of the general body surface, may migrate, under certain circumstances, inward into the body in an embryonic stage, as the same does migrate outward under another. Since a slight difference in the level of the sense area in reference to the level of the surroundings, introduces a fundamental difference in the formation of the dioptric mechanism in an Arthropod, and therefore presumably a difference in functions, two organs serving different purposes may easily be formed out of the common type from comparatively an insignificant circumstance at first. Once the differences thus started, between the two, it is easy to imagine how each be further elaborated in its respective line of differentiation. The formation of the complex compound eye out of a simple pit-organ, I have given in my former paper already referred to. It is easy to indicate in a general way how the complex triplostichous ocellus has been derived from the simple monostichous one. It happens that the lens which was formed by the secretion of the whole submerged area and its periphery comes to be secreted by the special layer of the skin developed at the periphery of the retinal area alone. This lentigenous layer of the eye afterwards becomes duplicated over the retinal layer, and finally completely severed from the latter, thus constituting the *lentigen* of Mark. The lentigen of the ocellus must not be confounded with the *corneagen* (Patten) of the com-

pound eye. The lentigen, the "vitreous layer" of authors, is essentially a new structure and has no homologue in the compound eye. I therefore restrict the use of the term to the ocellus alone. The differentiation of functions in the two adjacent parts of the skin, the one secreting the lens alone, and the other giving rise to the retinulæ alone, becomes more and more specialized; and, due no doubt to secondary causes, the sensory area of the eye originates in one part of the body, and the lentigenous part of the same is supplied from another, strikingly simulating the well-known process which prevails in the formation of the lateral eyes in Vertebrates. This composite mode of origin of the visual organ in Arthropods culminates in that remarkable phenomenon of migration of the sensory area of the eye through the whole thickness of the body, first observed by Brooks and Bruce in the larva of *Limulus* (*Johns Hopkins Univ. Circulars*, Vol. V, No. 43, Oct., 1885). The migration of the sensory area has been repeatedly observed in other Arachnids since. There are several forms which may be considered as of being intermediate between the types as represented in Fig. 10, and the other extreme as represented in the median ocelli of *Limulus*. I have personally observed the migration of the sensory area in the case of *Limulus*.

The view of the relation between the ocellus and the compound eye which has just been given, throws further light on the significance of the postero-lateral folds in the lateral eyes of the *Limulus* larva, which become stretched out later in life, and which I have designated as the *partial optic invagination* (*Johns Hopkins Univ. Circulars*, Vol. VIII, No. 70, March, 1889), in distinction from the *complete optic invagination* of the median ocelli.
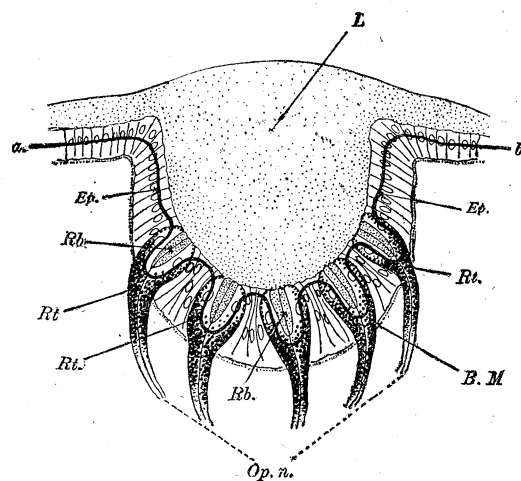

Fɪɢ. 10.

More strictly speaking, *the early stage of the lateral eye of Limulus represents a structure which is neither a compound eye nor a simple eye, but the initial stage for both*. If, as it actually happens, the postero-lateral folds in the *Limulus* larva become stretched out in the level of the body surface, becoming slightly more protuberant later, a compound eye in its primitive form is formed; if, on the other hand, the retinal area be pushed inside as far as the width of the folds permits, and the chitinous substance be allowed to accumulate in the space thus formed in the depression, a simple ocellus with a huge lens will be formed. This view, therefore, lends no support to the theories which derive the compound eye from the simple eye. Both types of eyes are considered as to have been derived from the common source, viz.: a group of pit-organs arranged on the level of the body surface. They are the differentiations into two different directions from a common starting point, the initiating factor for this divergence being the relative differences of the levels of the primitive sense areas, in reference to the level of the surrounding skin.

In conclusion, I must add that my interpretation of the homology of the retinulæ in the ocellus to those of the compound eye in the Arthropod, thence to the open pit like that of *Limulus*, is based mostly on the study of the adult structures. Although the evidence from the comparative anatomy of the adult eyes on this point appears to me to be very strong, the ontogenetic evidence of the pit formation in a more complex form of eye are imperfect. Instead, for instance, of the cells forming the retinula by the

actual involution of the ectoderm cells for each case and then fusing with each other to form a retinula bundle, they come to assume the form of a bundle at once, the actual open pit stage of each retinula being thus skipped over, a phenomenon not uncommon in the ontogeny of a complex structure. I am aware that my view may meet objection from that standpoint. It appears to me evident, however, that on the acquisition of some method of eliminating the unnecessary ancestral stages from the curriculum of individual development, is due the possibility of elaborating a highly complex structure from a simple "anlage" in the shortest possible period assigned to the development of the embryo in a given species. As to the exact method adopted by the embryo, in skipping over the unnecessary phylogenetic stages, opinions may differ. It is imaginable that some methods of qualitative distribution of the chromatin substances of the nuclii were acquired in the embryonic cells, by which a series of phylogenetic stages may be represented in a few acts of cell division, so that, although the cells as a group do not go through the series of phylogenetic stages of ancestral forms, the contents of their nuclii may. Hence in a short interval of embryonic growth and cell division, a given group of cells may attain the adult morphological structures of the organ comparatively suddenly, without categorically reproducing all the antecedent stages of forms.

Notes on the Body-cavity Liquid of Sipunculus Gouldii, Pourtalès. By E. A. ANDREWS.

The following observations upon this common Sipunculoid were made in July, 1889, at Wood's Holl, Mass., while enjoying the privileges of the U. S. Fish Commission Laboratory, kindly granted me by Professor M. McDonald.

The original name has been used in the sense adopted by Selenka, though the animal is more commonly known as *Phascolosoma Gouldii*, Diesing.

A specimen of the average size contains about one cubic centimetre of a saline liquid with a slight odor and larger percentage of sodium chlorid than sea-water, and which is rendered turbid and reddish by the presence of definite solids. These are red corpuscles, white corpuscles, giant corpuscles, spermatozoa in the male, and eggs in the female. The red corpuscle is a nucleated, pink, biconvex disk, about 3μ thick and 3 to 24μ wide. The white corpuscles are smaller, nucleated, granular, amoeboid cells, presenting a larger coarse-grained and a smaller fine-grained variety. The giant corpuscles have the shape of the red ones, are colorless, multinucleate, as much as five times as wide, and of unknown significance.

Estimates of the number of solid elements in a cubic millimeter of the liquid were made with the hæmacytometer, but the results are of little absolute value, owing to the clotting of the liquid and other sources of error. As thus indicated there are 90,000 red, 3,000 white, and fewer giant corpuscles in the above unit of volume. In the male the immense number of spermatazoa is well shown by an average of estimates giving 1,700,000 in a cubic millimeter; the female has, apparently, not many more eggs than white corpuscles.

The liquid quickly clots when removed from the body, agreeing thus with the body-cavity liquid of many species of Phascolosoma, one Phymosoma and one Aspidosiphon, as observed by Selenka and differing from Ph. elongatum and Sipunculus nudus. The clot is a loose jelly shrinking from a large amount of serum and varying in its firmness and rapidity of formation. Under the cover-glass the clotting is accompanied by the formation of elastic fibrils that stain dark with haematoxylin and which are often aggregated about disintegrating white corpuscles of the larger, coarse-grained kind. No meshworks of pseudo-podia were observed sufficient to account for the clot without the formation of extra-cellular fibers. The arrangement of these near the disintegrating cells suggested a causal connection between them and the definite large granules of the larger white corpuscle.

The shrunken clot washed free from serum and corpuscles resembles vertebrate fibrin in appearance and in the chief of the following reactions. It dissolves completely in nitric acid as a yellow liquid turning orange on addition of ammonia; it dissolves in strong hydrochloric acid, but does not swell up in two per cent.; it dissolves in glacial acetic acid and very slowly in one per cent; it dissolves in potassium hydrate; it does not dissolve in distilled water, sea-water, or in ten per cent. potassium nitrate.

When received directly into saturated solutions of ammonium sulphate,

magnesium sulphate, sodium sulphate, or sodium chlorid, the body-cavity liquid forms, in part, a gelatinous clot containing fibrils, and which in the last salt is exceedingly ropy and tenacious. In each case a precipitate is formed on standing. The dense yellow one formed in ammonium sulphate is complete after twenty-four hours; no proteid remaining in solution. But with magnesium sulphate, as well as with the two other salts, repeated saturations failed to throw down all the proteid. Hence it appears that both native albumins and globulins are present in the body-cavity liquid.

The serum separating from the clot may be obtained clear and pure after long standing. It then contains several proteids, which, however, are present in such small amounts in the pure serum, free from corpuscles and spermatozoa, that the separation of them by the ordinary heating·method proved very unsatisfactory. The results of many trials indicate for the less pure serum coagulation points at 65°, 70°–72°, and 75°–79°.

In the liquid of S. Nudus (which did not clot) Krukenberg found coagulation points at 63°–65° and at 75°–79°, with an increase of opalescence at 72°, whence it seems that very similar proteids are found in the body-cavity liquid in these two animals.

The coloring matter of the red corpuscles, Hæmerythrin, is readily dissolved in distilled water (with difficulty in S. nudus, Krukenberg), and gives a pink, opalescent liquid. On standing, the solution turns yellow, greenish yellow, except at the surface in contact with the air. On shaking with air the pink color returns, to be again replaced by yellow on standing, and so on at will even after putrefactive changes have been in progress in the solution for some days. Dilute solutions after a few days lose this respiratory power and precipitate a white residue. The liquid still contains proteid, however, with coagulation point at 55°.

The pink hæmerythrin solution heated, after slight acidification with one per cent. acetic acid, loses color at 42°, turning yellow and then darker. Precipitates are formed at 50°, 54°–56°, and 58°–59°, the last complete. The yellow color disappears with the second precipitate. All three proteids are precipitated completely by ammonium sulphate, but not completely by the other salts above mentioned. Repeated saturations with magnesium sulphate throw down all the red pigment but leave a yellow.

It thus appears probable that of the three proteids in the red corpuscles, one, hæmerythrin, is a globulin, that turns red in taking up oxygen, and probably colorless when reduced, while another is yellow, though commonly masked by the red color of the hæmerythrin.

That iron is contained in the red corpuscles, and thus probably in association with the hæmerythrin was indicated by the following: The solution of corpuscle proteids in water, with eggs or spermatozoa present, gives a bright blue reaction with dilute hydrochloric acid and potassium ferrocyanide, but when serum with eggs or spermatozoa was used, the blue reaction was very faint (as in the distilled water here used).

The presence of large amounts of proteids and saline material in the body-cavity liquid is connected with its use as the only nutrient internal medium as well as the chief and ultimate respiratory liquid. The special respiratory liquid of the branchial and blood sac contains red corpuscles almost identical with those of the body-cavity, with which it can communicate only by osmosis through the walls of the thin blood sac.

The presence of iron in the corpuscles would, perhaps, indicate a genetic connection between hæmerythrin and the hæmoglobin of Phoronis and the Echiuridæ (Lankester), while the other characters of these two pigmented proteids would point to the remoteness of this connection and favor the separation of the Sipunculoids from the other members of the "Gephyrea.'

The Marginal Sense Organs in the Pelagidae. By ROBERT PAYNE BIGELOW.

[This is a preliminary account of work done upon material obtained at the U. S. Fish Commission Station in Wood's Holl, Mass.]

At the meeting of the National Academy in May of last year Dr. Brooks pointed out that in the *Pelagidae* we have a complete gradation of form from Ephyra to Dactylometra, and that as far at least as the more striking external characters go, each species in course of its ontogeny recapitulates in successive stages the forms of all the simpler genera of the family. At the suggestion of Dr. Brooks, I have undertaken the study of the sense organs in this group with the view of discovering whether there exist the same

relations between these organs in the different genera as exists in them between the forms of the umbrella margin, the arrangements of the tentacles, and the like. My work has been on the sense organs in *Pelagia cyanella* P. & L., in the large *Chrysaora* of the Chesapeake allied to *C. Mediterranea*, and in *Dactylometra quinquecirra* L. Ag., chiefly the second, as the material in this one was the most abundant.

Among the stages in the development of Chrysaora that were studied the four principal ones are: the Ephyra, the eight tentacle stage (Pelagia stage), the beginning of the Chrysaora stage, and the fully adult condition. The only part of the sensory apparatus present in the Ephyra is the *Rhopalium* of Haeckel (Sinneskolbe, Randkoerper). The general form and position of this organ is familiar to every one who has given any attention to the Discomedusae. The very much flattened general ectoderm of the body in this stage passes abruptly at the base of the Rhopalium into a very much thicker layer which covers all of that organ, except the distal crystal bearing part where the ectoderm is extremely thin. This thick layer is made up of closely packed ciliated columnar cells with their nuclei arranged in two or more rather irregular rows. Between the cell bodies and the supporting membrane a thin layer of nerve fibres can be made out. The supporting membrane is directly continuous with the umbrellar jelly. The gastric pouch which penetrates the Ephyra arm ends in a small canal which extends a short distance into the Rhopalium and there ends blindly. There are no diverticulae at this stage into the marginal lobes nor into the hood. The single layered entoderm is considerably thickened in the lumen of the Rhopalium and passes distally into a mass of large cells, the visceral mesoderm of Lendenfeld, each of which contains a calcarious concretion. The concretion is prismatic in shape, hardly twice as long as broad, and it seems to contain an organic core. When the concretion has been dissolved away, the protoplasmic part of the cell is seen to be reduced to a thin capsule with a nucleus embedded in its wall.

In the eight tentacle, or Pelagia stage, there is present besides the Rhopalium, a pair of ectodermal thickenings at its base. These extend on to the proximal wall of the little niche, the *sensory niche*, which surrounds the sense organ. The ectodermal and entodermal layers of the Rhopalium have in this stage twice the thickness that they had in the Ephyra. The nerve fibre layer has become well marked and shows a slight U-shaped thickening. The loop of the U lies in the upper distal part of the layer, and the limbs of the U extend thence along the upper lateral parts of the Rhopalium and are continued beyond its base as two bands of nerve fibres, one of which goes to each of the ectodermal thickenings. The ectoderm of these structures is composed of cuboidal cells with long cilia, and is folded inward and towards the axis of the Rhopalium, the fold lying in the plane of the entodermal lamella. Besides this main one there are a few slight secondary folds.

With the second set of tentacles there appear rudiments of all the structures characteristic of the adult sense organ. The ectodermal and entodermal layers of the Rhopalium, including the nerve fibre layer, have continued to thicken, and the lumen of the Rhopalium now occupies an increased proportion of its length. The ectodermal folds are but little changed. The new structures which now appear are a thickening of the ectoderm around the base of the Rhopalium and the so-called olfactory groove. The latter, for which the name *Dorsal Sensory Groove*, given by Lendenfeld, is better although less convenient, appears on the upper surface of the hood immediately above the base of the Rhopalium. It is a saucer-shaped pit lined with a columnar epithelium.

The size of the body and the thickness of the jelly and of the cell layers has increased very much when the jelly-fish has arrived at maturity, and similar changes have occurred in the sense organs. The whole ectoderm of the Rhopalium has increased still more in depth, the nerve fibre layer and especially the U-shaped thickening is now very prominent, and beneath the latter the supporting layer has become thickened. Extending perpendicularly through the nerve fibre layer are plainly visible the processes of the supporting cells described by Schewiakoff. The ectodermal thickening which in the last stage extended a short distance around the base of the Rhopalium has now spread so as to line the entire sensory niche, reaching proximally to the edge of the band of muscle fibres. The cells in this area have become deeply columnar, are apparently sensory, and form, I suppose, what Haeckel has briefly described as the "'Tastplatte' (?)". The paired ectodermal folds which were feebly developed in the last stage are now highly developed, and the description of them given by Claus as "cone-shaped swollen thickenings of the ectoderm containing a layer of ganglion

cells and nerve fibres," does not give one an exact idea of their structure. Before opening into the large gastric pouch, the rhopalial canal extends for some distance along the roof of the sensory niche through what we may call the *radial ridge*. Except for a short area next the Rhopalium, the convex under surface of this ridge is clothed with the common columnar epithelium of the niche. The sides of the ridge, on the other hand, are concave, and in each of the lateral grooves thus formed there runs a band of nerve fibres continuous with the thickening in the Rhopalium. Each nerve fibre band is covered by the cuboidal ciliated epithelium already described, but which is now very much folded and pitted, and nerve fibres extend outward between the cell layers that form the walls of the numerous secondary folds. These two structures meet across the ridge at the base of the Rhopalium, and each extends proximally into and lines a deep pocket-shaped cavity in the jelly which lies in the deepest part of the sensory niche in the plane of the entodermal lamella and extends centrally somewhat beyond the mouth of the rhopalial canal.

Only two other features of the adult stage need be noticed here. Relatively to the length of the Rhopalium the length of its lumen has continued to increase, so that it now extends some distance into the crystal-bearing part, and the mass of crystals, instead of being globular as before, is now cup-shaped. The dorsal sensory groove has become deeply funnel-shaped, the axis of the funnel being directed obliquely downward and outward. The epithelium lining the groove resembles that which lines the sensory niche, and beneath it there is a thin layer of nerve fibres. Throughout the general ectoderm and entoderm of the animal, but especially abundant in the epithelium lining the sensory niche, the olfactory groove, and the rhopalial canal are mucous gland cells like those described by Wilson in *Manicena*.

In Pelagia I have only studied the adult sense organ, which is very much simpler than in Chrysaora. Little is to be added to the description already given by the Hertwigs, except to notice the presence of a dorsal groove and of a band of columnar epithelium which runs some distance proximally from the base of the Rhopalium along the under surface of the slightly raised radial ridge. Aside from the latter there is no sensory apparatus in the niche but the Rhopalium. The entodermal lamella extends along the sides of the rhopalial canal and nearly to the extremity of the small pocket into the hood. The dorsal sensory groove is funnel-shaped, but much smaller and shallower than in Chrysaora, and is broadly V shaped in vertical section.

The adult Dactylometra differs, on the other hand, from Chrysaora, in that the features of the sense organ characteristic of the latter are exaggerated in the former, but nothing new is added. The rhopalia are essentially the same in both. The chief differences between the two species are in the folded ectoderm lying along the sides of the radial ridge and in the shape of the dorsal groove. The radial ridge is of a somewhat different shape from the ridge in Chrysaora, and is almost entirely covered by the nerve fibre layer. The ciliated pits seem deeper and more closely packed both here and in the pockets, which in turn are somewhat larger in proportion. The dorsal groove is like that in Chrysaora except that it is much deeper and much longer radially. In a tangential vertical section of the umbrella this groove appears deeply U-shaped.

My material for the study of the development of Dactylometra is very scanty. In a specimen which I suppose to be the Ephyra of this species the sense organ is like the one in the Ephyra of Chrysaora. The next older specimen was one three millimeters in diameter, just passing into the Pelagia stage. In this there is hardly any change; the first traces of the paired thickenings of the ectoderm may possibly be present. As in Chrysaora, so here, the second set of tentacles and the dorsal groove appear simultaneously. The latter is at first merely a thickening in the exumbrellar ectoderm. Later it forms a saucer-shaped depression, as in the same stage in Chrysaora. While the rhopalia in the two species are also similar at this stage, the other structures in the sensory niche are more advanced in Dactylometra. At the time when the dorsal sensory epithelium forms a flat plate we have a very short radial ridge soon passing into the posterior wall of the shallow niche. At this point there open into the niche two pockets situated as in the adult. The ciliated epithelium which with its nerve fibre layer completely covers the radial ridge, is continued into these pockets and lines the floor of the outer half and all of the inner half of each pocket, the outer half of the roof being lined by the ordinary flattened epithelium of the niche. The in-pittings of the pocket epithelium

at this stage seem to be in the process of formation, for they are very shallow and broad compared to the adult form. The principal mass of nerve fibres lies along the lower lip of the pocket.

Comparing now, as far as our material will allow, the corresponding stages in the different species studied, we find in the adult *Pelagia cyanella* a well-marked dorsal sensory groove, but no trace of the paired folds of ectoderm in the sensory niche, while in the Pelagia stage of the *Chrysaora* the rudiments of these folds have appeared, and in this species and *Dactylemetra quinquecirra* the dorsal groove does not appear until the second set of tentacles begins to form. The Chrysaora stage in the Dactylometra has the paired folds more developed than in the beginning of the adult condition in the Chrysaora and different from what they are in the fully formed adult of that species. Of the adult sense organs in the three species, those in the Dactylometra are the most highly developed. As an answer, therefore, to the problem with which we started, we may say that from the material studied it would appear that in *Pelagia, Chrysaora,* and *Dactylometra,* with increased complexity of general characters, there is both phylogenetically and ontogenetically an increase in complexity of the sense organs, but the steps in the ontogeny of these organs are not strictly identical with the condition at the corresponding points in the phylogeny of the species.

The Development of the American Lobster: Homarus Americanus. By F. H. Herrick.

These observations on the development of the lobster were undertaken last summer while enjoying the facilities generously afforded by the Laboratory of the U. S. Fish Commission at Woods Holl, Mass. Through the aid of the Commission I was enabled to obtain an abundance of material which otherwise would not have been easily accessible.

The embryology of the crustacea has occupied my attention for some time past, and more particularly that of Alpheus, a genus of prawns with which we should expect the development of the lobster to agree in all significant points. This expectation is confirmed, although in the lobster there are some interesting variations in the earlier stages. From the egg-nauplius period, onward the two forms run closely parallel. The following notes will be chiefly confined to the points in which Homarus differs from Alpheus.

The spawning season of the lobster does not seem to be confined to any season of the year, although it is probable that they are most prolific during the summer months. The eggs, which are carried on the abdominal appendages of the female, are of large size, having an average diameter of about 1.6 mm. They are invariably of a deep olive-green color. The period of hatching at Woods Holl in the summer (July–Sept.) is not far from 100 days. The fully developed egg-nauplius embryo is about 15 days old, and at the 30th day eye pigment makes its appearance.

The initial stages of segmentation were not obtained. At the earliest which we found, the surface of the egg is divided into thirty to fifty round, convex segments, which are most numerous over one half of the egg. Sections show that the typical yolk-pyramid structure is not present, but that the entire egg is divided into a large number of subspherical segments of irregular size. Nuclei appear both within and between the segments at the surface of the egg. The nuclei are multiplying rapidly and often occur in clusters. Each nucleus, with the small amount of cytoplasm which surrounds it, is to be regarded as an independent cell. It may turn out that the condition of the yolk at this stage is not strictly normal. It is quite probable however that the segmentation of the lobster's egg has been considerably modified from the usual type.

At the next stage which was examined, the surface of the egg was composed of several hundred small polygonal segments. Each segment corresponds to a yolk pyramid. It is separated from adjoining segments by cleavage planes, which pass into the yolk, and is also truncated, or constricted off from the central yolk not far from the surface. Near the convex base of the segment there is a single nucleus or often nests of 4–8 nuclei. The protoplasm has nearly reached the surface of the egg, but there is still a thin layer of yolk between the latter and the cell-nucleus. Cell division is indirect at all stages of development, and nuclear figures show that the division of cells at the surface is always tangential. The great mass of the egg consists of yolk, which is divided into irregular segments,

for the most part destitute of protoplasm. At this stage, very few cells (less than a dozen in some eggs) occur below the surface, which shows that there has been a continuous migration of protoplasm from the central toward the peripheral parts of the egg, since the stage first described, supposing that to represent a normal condition. The yolk cells have the common amoeboid appearance, and in their movements among the yolk spheres the nucleus conforms to the requirements of the cell. The superficial cells are now about evenly distributed, but in an egg 16 hours older, the cells become more numerous over one half of the egg, and the protoplasm of the outer segments is strictly superficial.

Twenty-seven hours later a small patch of cells, in the midst of which there is a slight depression, makes its appearance on the side of the egg where the cells are thickest. This is the beginning of the egg-gastrula phase, and the minute circular depression (which may be called the blastopore), marks the point where numerous cells at the surface pass into the yolk and spread out on all sides. At the time of gastrulation the great central yolk mass is destitute of protoplasm, unlike Alpheus in which there is a migration of cells from the surface into the yolk before gastrulation begins. In forms like the lobster, gastrulation has evidently lost its former significance. The establishment of the germinal layers is deferred until a later period, but what is accomplished is simply the rapid introduction of a number of cells below the surface at a certain point on the surface of the egg.

Twenty-nine hours later the position of the blastopore is marked by a solid, deeply-staining core of cells, from which, at the surface, the cells gradually thin out on all sides. The anterior side of this cell mass which we will call the *keel*, is marked by the more crowded condition of the cell-nuclei. This forms the proper embryonic area, as in it the egg-nauplius is subsequently outlined. Both the invaginated and superficial cells multiply rapidly, and nuclear nests or clusters are frequently met with.

After a period of 88 hours, the keel appears at the surface as a cell-plate or disc, having a central opaque area, distinguished from a clearer portion which shades off into the embryonic area and over the rest of the egg. Sections show plainly how the keel is produced. The cells included at gastrulation multiply rapidly, and gradually build up a large cell-mass. The cells as they multiply extend downward into the yolk, but mainly backward, that is behind the blastopore and future embryo, in the form of a wedge or keel. The cytoplasm seems to be distributed in thin layers around the yolk corpuscles, so that we see little but the nuclei. Yolk spherules are intermingled with the cells of the keel as well as closely associated with the cell-protoplasm over the surface of the egg, and it is probable that the digestion of the yolk is effected by an intracellular process.

One egg at this stage presented an interesting anomaly or variation, the keel having been hollowed out so as to contain a distinct cavity or rather several communicating cavities. The wall of the chamber has an even surface, and is thickly studded with nuclei, some of which are passing into the yolk. Many of these nuclei are degenerating or breaking up into a residue of chromatin-containing masses. Other series taken from a similar stage, show how this condition has been reached. In this case the cell-mass or " keel " is flask-shaped, the solid neck of which extends down from the surface near the point which corresponds to the blastopore. The body of the flask consists of an irregular tier of cells which encloses yolk and degenerating nuclei. The yolk is perhaps in some cases rapidly absorbed, and there is left a cavity like that described. It is probably not constant and has no morphological significance.

In an egg older than the last by 27 hours, the superficial dimensions of the keel have considerably increased. It has the appearance of a circular, oval or horseshoe-shaped plate, and either blends with the surrounding cells or is sharply marked off from them, in cases where in section we find the flask-shaped cell-mass. There is a slight depression at the centre of the plate (corresponding to the blastopore in position), and there is considerable variation both in its shape and structure which cannot be described in this abstract.

The naupliar appendages appear nearly simultaneously in the embryonic area at a considerable distance in front of the keel, the first pair of antennæ lagging a little behind the others. The appendages are at first widely separated, but after a short interval the embryo undergoes a marked contraction. The optic discs are represented by a single tier of columnar cells. The anterior portion of the keel enters into the abdominal plate, or thoracic-abdominal process. The invagination of the stomadæum occurs at a point on line between the 1st and 2d pairs of antennæ. Soon the labrum begins

to grow down over the mouth, the proctodæum is established as an ingrowth of ectoblast on the surface of the thoracic-abdominal process, and the thoracic-abdominal fold can soon be distinguished. By this time the second pair of antennæ are biramous. When the egg-nauplius is sectioned we find that the keel of previous stages is represented largely by sheets or trains of cells, which extend throughout a large part of the egg, and also by a portion of the cells which enter into the thoracic-abdominal process. These central cells enclose more or less completely large masses of yolk, which come to have a different appearance from the surrounding portions. The stomadæum is a tube, which is bent forward and flattened antero-posteriorly. Its wall consists of a single layer of cells. It is surrounded by degenerating nuclei, yolk and cells derived either from the abdominal plate or from the epiblast. The appendages are filled principally with yolk which is not absorbed until a comparatively late period. The optic discs from which the eye originates, become thickened, apparently by the usual process of cell-division and emigration.

The spores form a marked characteristic of the early stages of the lobster, and throw light on similar bodies which I have observed in Alpheus and other crustacea. They are small, deeply-staining masses of chromatin, and correspond to the granules or nucleoli of ordinary embryonic cells. I formerly thought that these bodies which resemble spores, might be produced by a normal process of endogenous cell division, and might themselves develop into cells. This is certainly not true so far as the majority of these bodies is concerned. The study of the lobster shows that we have to do primarily with a remarkable case of cell-degeneration. The cells go to pieces, for some unknown reason, and the chromatin particles are gradually degraded into a substance resembling yolk. Possibly these dissolving cells act as yolk-digesters.

At a later stage, when 5–6 pairs of appendages are formed, "spores" have almost entirely disappeared. This is also true of the central yolk cells, which are now mostly confined to the peripheral parts of the egg, and occur in some numbers near the proximal end of the hind-gut, where the latter joins the mesenteron. The heart is represented by space between the proximal end of the hind-gut and the body wall. This is filled with plasma, blood-corpuscles, and mesoderm-cells, derived from the thoracic abdominal process. Blood corpuscles also occur in various parts of the embryo, these are probably derived from the yolk cells and from the mesoblast cells of the abdominal plate.

In embryos of this stage there is a conspicuous circular patch of cells back of the heart at a distance of about 90° measured on the surface of the egg from the centre of the embryo. This probably represents one of the structures described under the name of "dorsal organ." The surface cells become columnar at this point, and the layer undergoes a slight thickening.

When 8–10 pairs of appendages are present, the entoblast appears as a definite layer, next the body wall on either side of the bend made by the thoracic-abdominal fold. Its cells are derived from the yolk. It is thus at a comparatively late period that the germinal layers are established. At the egg-gastrula stage we distinguish an outer and an inner cell-layer. The inner layer gives rise to a keel, the cells of which represent mesoblast, probably also entoblast, and cells which take no part in the embryonic tissues. In the egg nauplius we recognize an external layer or ectoblast, and an internal layer, consisting of yolk cells, poliferated ectoblast and cells derived from the abdominal plate, or mesoblast. The two latter classes cannot certainly be distinghished. The yolk cells play a more subordinate part in the formation of the ultimate germinal layers than in Alpheus. The keel probably represents the endodermal disc or eminence in the Cray-fish. In the latter this is directly invaginated, while in the lobster it sinks gradually into the yolk. The structure and development of the nervous systems, the fore and hind guts and various other organs, so far as they have been studied, have been found to agree essentially with Alpheus.

April 2, 1890.

NOTE.—EXPLANATION OF FIGURES IN MR. WATASE'S PAPERS.

On Karyokinesis and the Cleavage of the Ovum. See page 55.

FIGS. 1 and 2.—*Two Stages of Karyokinesis.*

    A. R. Achromatic rays of asters.   *C. A.* Centres of asters.
    S. P. Space from which the nuclear substance has been displaced by the achromatic rays which form the rudiment of the future spindle. This space is filled by a clear fluid.
    N. Nucleus.   *Ctp.* Cytoplasm.

On the Migration of the Retinal Area. See page 64.

FIG. 9.—*Diagram of a compound eye.*   FIG. 10.—*Diagram of a simple eye.*

    a–b, indicates the amount of folding undergone by the ectoderm to form four ommatidia.   *B. M.* Basement membrane.
    C. c. Crystalline cone.   *C.* Corneal facet.   *c. g.* Corneagen.
    Ep. Ectoderm.   *L.* Lens.   *Op. n.* Optic nerve fibres.
    Rb. Rhabdomere.   *Rt.* Retinula.   *V.* Vitrella.

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