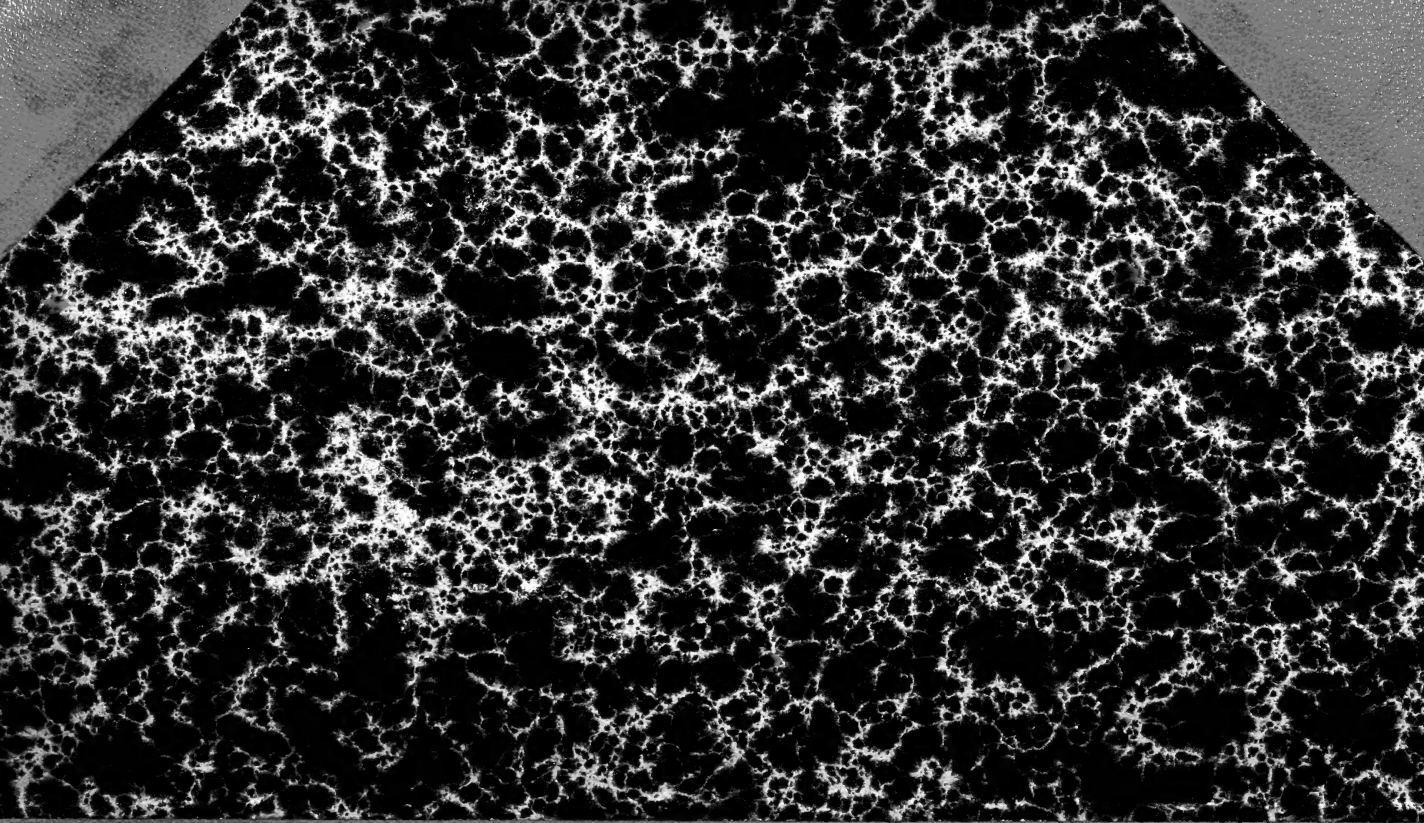


JOURNAL

OF

MORPHOLOGY

FOUNDED BY C. O. WHITMAN

VOLUME 36
DECEMBER, 1921,
MARCH, JUNE, SEPTEMBER, 1922

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

PHILADELPHIA

L21710

CONTENTS

No. 1. DECEMBER, 1921

No. 2. MARCH, 1922

No. 3. JUNE, 1922

No. 4. SEPTEMBER, 1922

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, OCTOBER 17

THE DEVELOPMENT OF PARACOPIDOSOMOPSIS[1]

J. T. PATTERSON

University of Texas, Department of Zoology

TWO TEXT FIGURES AND TWELVE PLATES (NINETY FIGURES)

CONTENTS

1. INTRODUCTION

The object of the present paper is to give a rather complete account of the development of a polyembryonic insect. We owe a great deal to Marchal and Silvestri for their pioneer studies in this field. They have given us a good outline of the general course of events in the development of some three or four species. Nevertheless, there are many important points in the embry-

[1] Contribution no. 145.

ology of these insects which are as yet obscure, and there is still to be written a complete account of a single representative species. It is highly to be desired that a description be given of the development of a typical species in each of the three families of parasitic hymenoptera in which polyembryony has been reported. The evidence, although fragmentary in many instances, clearly indicates that there are significant differences in the type of development in each of the three families.

This paper is an attempt to give an account of each of the more important steps in the embryology of the egg up to the point at which the sexual embryos are formed. For this purpose I have selected a representative of the family Chalcididae. The species used is Paracopidosomopsis floridanus. Ashmead. It was chosen primarily because of the ease with which it can be secured in this vicinity[2] and because it can be reared readily under laboratory conditions. In studying the development of parasitic forms, the latter advantage cannot be over emphasized; for if the work is to be free from suspicion, one must scrupulously avoid the possibility of the host eggs becoming infected with foreign parasites. This can only be done when the material used is reared under laboratory conditions. Another reason for studying Paracopidosomopsis is the fact that non-viable larvae appear in the polygerm stages of this species. These are similar to, if not identical with, the asexual larvae of Litomastix.

I shall first give a brief résumé of the life-history of Paracopidosomopsis, as it will then be easier to follow the account of the development of the egg. The parasite lays its egg in the egg of the common cabbage looper, Autographra brassicae. The two eggs develop simultaneously, and, as is the case in several other species, there is one generation of the parasite for each generation of the moth. The moth reaches its complete development several days before the parasites, but is not ready to lay its eggs until the parasites emerge. I have not been able to determine the number of generations per year for the Austin region. The looper is

[2] I am deeply indebted to Mr. Thomas H. Jones, of Baton Rouge, La., for sending me parasitized carcasses at times when they could not be found here at Austin.

found on various Cruciferae throughout the summer months, but the main generation appears about the 1st of October, when the fall crop of cabbage is a month or six weeks old. There is of course a considerable overlapping of generations. From October 1st until late in December I have been able to rear two complete generations of the parasite in the laboratory. October and November are therefore the best months in which to collect material.

The parasite will deposit its eggs at any time during the embryonic period of the host, which lasts from seventy to eighty hours. After the young caterpillar hatches, the parasite will no longer parasitize it. There are many interesting points in the behavior of these insects, especially in connection with egg laying. They are positively heliotropic and move with great rapidity toward the light, which fact makes it easy to handle them in the laboratory.

The individuals in one carcass all emerge at about the same time, and the females whether fertilized or not are ready to lay. If males are present, they at once mate with the females. One male will mate with several females. If a single female be introduced into a vessel containing a brood of males, which have become quiet, the entire blood immediately becomes active.

In crawling over the surface of the table or leaf, the parasite feels its way along by means of the antennae. This is the method used by the female to find the host egg on the leaves of the cabbage. Once an egg is located, the female examines its surface by the tips of the antennae, which vibrate with great rapidity. If the egg happens to belong to an insect other than the Autographra moth, the female leaves it in a little while and continues her search for the desired egg.

In preparing for oviposition the female mounts the egg and clasps it with the second and third pairs of legs. The first pair of legs are either held free or placed on the surface of the leaf. The tip of the abdomen is then bent down until it comes in contact with the egg. She then braces herself and forces the ovipositor into the egg. During the act of laying, the female remains perfectly motionless, with the head and antennae bent

down and backward. With the aid of the binocular microscope and strong transmitted light one can easily observe the egg passing down the lumen of the ovipositor. The egg is forced out by rhythmic pulsations of the abdomen. As soon as the egg is deposited, she withdraws the ovipositor, remains motionless for a second or two, rubs the pair of hind legs together and then proceeds to search for another egg. The act of oviposition varies in time from two to as long as fifteen minutes. If only a few eggs are present on the leaf, she may in time return and lay again in the same egg. The number of eggs deposited at one oviposition is either one or two. My records show that in about two times out of three two eggs are deposited.

The parasitic egg may be deposited in any part of the host egg or embryo, but only those which become included in the tissues of the embryo are able to complete development. The egg develops whether fertilized or not. Eggs laid by virgin females always produce male broods, but broods arising from eggs laid by fertilized females are nearly always mixed.

The process of maturation is completed in one and one-half hours after the egg is deposited. Cleavage then follows, and the polygerm stage is reached in about seventy-two hours. The polygerm is composed of a number of primary masses, each of which consists of a group of embryonic cells surrounded by a nucleated membrane. The primary masses multiply, by constrictions of this membrane, to produce secondary masses, and these in turn divide to form tertiary masses. Further divisions follow and the entire polygerm becomes a very complex structure. The tertiary mass stage is reached in from seven to nine days.

The tertiary divisions produce what I have called components. These become scattered throughout the body cavity of the caterpillar through the dissociation or fragmentation of the polygerm. The tertiary components form centers for further multiplication, or for the formation of groups of sexual embryos. The sexual embryos begin to form on the fifteenth day and reach the free larval stage sometime between the twenty-second and twenty-fourth days. Pupation occurs on the twenty-eighth day, and the adult insects emerge on the forty-seventh day.

The time given above is for stages reared in the laboratory during the months of October to December. Out in the field in the sun development occurs much more rapidly. Under such conditions, the entire life-cycle is completed in about thirty days. There is also considerable variation in the rate of devel-

TABLE 1

STAGE	TIME	FIGURES
First maturation	25 to 30 mins.	3, 34
Second maturation	60 to 65 mins.	4
Pronuclei	1 hr. 30 mins.	6, 7
Fertilization	1 hr. 50 mins.	8, 9, 36
First cleavage spindle	2 hrs. 30 mins.	11, 12
2-celled stage	3 hrs.	15
Second cleavage spindle	3 hrs. 30 mins.	16
4-celled stage	4 to 5 hrs.	17, 18
8-celled stage	7 hrs.	19
14-celled stage	8 hrs.	20, 21
28-celled stage	9 hrs.	43, 44
50 to 60 cells	19 hrs.	45, 46
70 cells	26 hrs.	47
135 cells	40 hrs.	48
220 to 225 cells	45 to 50 hrs.	50
Young polygerm	70 to 72 hrs.	52, 54
Completed polygerm	75 to 80 hrs.	53, 55–60
Division of primary masses	77 hrs.	60
Division of secondary masses	4 to 7 days	62
Division of tertiary masses	7 to 10 days	65
Beginning of sexual embryos	14 to 15 days	76–77
Free larval stage	22 to 27 days	
Pupation	28 days	
Adult insects	47 days	

opment in different eggs, irrespective of temperature. In order to avoid referring repeatedly to the age of different stages, I have compiled in table 1 the average time for each of the more important stages. The data are taken from material reared in the laboratory and the time is determined from oviposition.

2. STRUCTURE, MATURATION, AND FERTILIZATION OF THE EGG

a. The freshly laid egg

The freshly laid egg is a pear-shaped cell, surrounded by a very thin but tough membrane, which is probably a true chorion (fig. 1). The broad or posterior end of the egg corresponds to the vegetative pole of other eggs. It is from this region that the true embryonic cells are formed by the process of cleavage. The anterior end of the egg gradually narrows down and finally terminates in a finger-like process, which is characteristic of the eggs of many parasitic hymenoptera. As development progresses the content of this process is gradually taken into the egg proper, so that in later stages it is no longer seen.

As a matter of fact, the different eggs vary greatly both in shape and in size (figs. 1 to 13). The average unfixed egg measures about 155 μ by 60 μ in its major axes. Its content consists of a very finely granular protoplasm, in which are found a few yolk or oil spherules (fig. 34). In the fertilized egg there are found three distinct bodies, the oocytic nucleus, the sperm, and the so-called nucleolus.

In the freshly deposited egg the nucleus is an oval-shaped body in which the chromatin appears as elongated threads running more or less parallel with the long axis of the nucleus (figs. 1, 2). It is always situated well toward the anterior end of the egg, and usually near the base of the finger-like process. Martin ('14) and Hegner ('14, '15) have traced out the origin of the nucleus in two species of polyembryonic hymenoptera (Ageniaspis fuscicollis, and Copidosoma gelechiae). According to the accounts of these writers, it has a rather remarkable history. In Copidosoma the young oocyte in its nurse chamber has a very large nucleus, in which is found an irregular, deeply staining mass of chromatin. The cytoplasm forms a very thin layer about the nucleus. During the growth period the cytoplasm increases rapidly, while the nucleus enlarges but very little. Later the chromatin loosens up and forms a spireme, which finally breaks up to form thin, irregular-shaped chromosomes.

The chromosomes then become shorter and thicker, and appear to unite near their ends—a process somewhat resembling synapsis. The pairs of chromosomes straighten out and become arranged in a parallel series, with the points of union lying at the equator. According to Hegner, the number of rods thus arranged is eleven or twelve, but in reality there should be only ten, for the cytological studies of two of my students have demonstrated clearly that the diploid number of chromosomes in Copidosoma gelechiae is twenty. At first the parallel pairs of chromosomes are scattered, but the entire mitotic figure soon undergoes condensation, by which the chromosomes become shorter and thicker and are brought close together. Finally, there is produced a homogeneous mass of chromatin, and all trace of individual rods is lost.

Martin's account of the origin of the nucleus in Ageniaspis, although differing in details, is in agreement with that just given for Copidosoma. The entire process is peculiar, especially in the light of the maturation divisions, which are soon to be described. It may be, as Hegner ('14) suggests, that this precocious, mitotic-like figure is comparable to the disappearing or aborting spindle which has been observed in the eggs of turbellarians and polyclads. The important point is that the chromatin body found at the anterior end of the freshly laid egg of these parasites is a derivative of the germinal vesicle, and hence is the true oocytic nucleus.

In fertilization the entire spermatozoon enters the egg. The head of the sperm is frequently sickle-shaped, with a long tapering tail attached (figs. 1, 3). Apparently, the sperm may enter the egg at any point on the surface of the posterior region. This conclusion is based on a study of many fertilized eggs which had just been deposited. Both Marchal ('04) and Martin ('14) believe that they can demonstrate the presence of a micropyle on the surface of the anterior process of the egg of Ageniaspis, which would indicate that the entrance of the sperm was restricted to that point.

The most remarkable body present in the cytoplasm is the so-called nucleolus. It was first described by Silvestri, who thought

that it came from the oocytic nucleus, and hence its name. This structure has given rise to a great deal of discussion, and no less than five different theories have been advanced to explain its genesis. It was not observed by Marchal ('04) for the egg of Ageniaspis, but later Silvestri ('08) and Martin ('14) both demonstrated its presence in the egg of this species. Martin, who gives a very clear account of the history of this body, first demonstrated that it arises outside of the germinal vesicle before the nuclear wall breaks down, and hence could not be regarded as a true nucleolus. He showed that it first appears in a young oocyte as a collection of small deeply staining bodies, among a cloud of very fine granules, situated near the posterior end of the cell. It gradually increases in size and becomes fully formed at about the same time the egg attains its full size.

In the meantime, Hegner ('14) also showed that in the egg of Copidosoma gelechiae the nucleolus was not a plasmosome coming from the germinal vesicle. However, he reached the untenable conclusion, based on a study of an incomplete series of sections, that the matured egg was a composite structure, produced by the fusion of two oocytes. He thus derived the nucleus of the egg from the germinal vesicle of one of the oocytes and the nucleolus from that of the other oocyte.

Silvestri ('14) replied almost immediately in an article dealing with the development of Copidosoma buyssoni. In this paper he admits his error in deriving the nucleolus from the germinal vesicle of the oocyte, and suggests the possibility that it may arise from a group of granules lying near the posterior side of the nucleus. He offers the term oosoma in lieu of nucleolus. He also points out that what Hegner regarded as a composite structure in sections is in reality only the anterior and posterior ends of the same oocyte—a correction which Hegner ('15) accepts.

The chief interest in the nucleolus lies in the fact that a very important function has been assigned to it by Silvestri and Hegner. Silvestri ('06) showed that it is distributed to a single blastomere of the four-celled stage in Litomastix, and suggested that this cell may be the progenitor of all the germ cells of the

sexual larvae. Hegner ('14) has elaborated this idea, and classifies the nucleolus as a germ-line determinant. I have elsewhere ('17 a) pointed out the difficulties which stand in the way of accepting this interpretation, especially as regards its application to the origin of the asexual larvae in Litomastix and Paracopidosomopsis.

I have gone into the subject of the origin and function of the nucleolus rather fully with the hope of showing how necessary it is that a reinvestigation of its genesis and fate should be made. Perhaps this could best be done by the methods employed in the study of mitochondria.

b. Maturation

I have elewhere ('18) described maturation and fertilization, and the account given here may be confined to a brief statement of the principal points of interest.

1. The first maturation. The process of maturation is identical in fertilized and unfertilized eggs. As is the case in many other hymenoptera, the maturation divisions involve only the chromatin, and consequently distinct polar bodies are not formed. The first maturation spindle is formed about fifteen minutes after the egg is laid, and within the next ten or fifteen minutes the chromosomes have reached the late anaphase stage (fig. 3). The long axis of the spindle is not quite parallel with that of the egg, but it meets the latter at a slightly oblique angle. This brings the outer end of the spindle near to the surface of the egg at the base of the anterior process. The chromatin of the first polar body is therefore found in this region.

The first maturation division results in reducing the number of chromosomes from sixteen to eight (figs. 26 to 28). In certain cases one can easily count eight chromosomes in the first polar body and in the second oocyte (e.g., fig. 27).

2. The second maturation. The second maturation follows almost immediately after the first is completed, without the reorganization of a nucleus. Likewise the first polar body chromatin forms a spindle and divides without forming a nucleus. These two divisions may occur simultaneously (fig. 29), or the

first polar body division may either precede (fig. 28) or follow (fig. 4) the second maturation division. Consequently, there is no close correlation between the two divisions. This is exactly the condition in Ageniaspis as reported by Martin.

Each of the two divisions is equational in character. In figure 28 is a remarkably clear case of the late anaphase stage of the first polar body division. At each pole of the spindle are eight distinct chromosomes (A_1, A_2). In figure 29 the ootid (B_2) shows eight chromosomes, and in the second body (B_1) seven are visible. Doubtless one of the chromosomes is hidden by some of the other seven, for in other figures one can count eight in the second polar body (fig. 31, B_1). The result of these two divisions is the formation of four groups of chromosomes, of which three are polar bodies (fig. 29, A_1, A_2, B_1) and one the ootid (B_2). The latter forms the female pronucleus.

3. The formation of the polar nucleus. At this point we shall describe the formation of the polar nucleus, which is destined to play an important rôle in the development of the polygerm. This body was first described, under the term paranucleus, in the egg of Ageniaspis by Marchal ('04), who failed to observe its formation, but who gave a very good account of its later history.

It was next described by Silvestri for the egg of Litomastix. The process of maturation in this species is identical with that of Paracopidosomopsis. Consequently, at the close of maturation the egg of Litomastix contains, in addition to the nucleolus and the female pronucleus, three masses of chromatin lying close together, but distinct from one another. These are the three polar bodies. In connection with his account of the first and second cleavages, he makes the following brief statements concerning the origin of the nucleolus from the polar bodies: That the three polar nuclei, "which during such a period are close together, fuse together to form a single mass of chromatin, a nucleus without membrane and with the chromosomes condensed" ('06, p. 14); and later, "During this stage the chromatin mass of the polar bodies is formed into a complete nucleus with membrane and reticulum very distinct, and is always found

in the anterior part of the egg" (p. 15). Silvestri also gives a very clear description of the fate of the polar nucleus.

In a paper published two years later, Silvestri ('08) described the formation of the polar bodies in the egg of Ageniaspis. The polar bodies are formed exactly as in the egg of Litomastix, but their subsequent history is somewhat different. The three masses of chromatin usually become reconstituted, each with a reticulum and membrane, and all three lying more or less on top of one another. In some eggs, however, the three polar nuclei fuse to form a single mass, while in other eggs the second polar body and the inner nucleus of the first polar body (or only one of them) divide irregularly into parts, thus producing in all some four or five nuclei. In the period between the third and fourth cleavages, the polar nuclei lose their membranes, and their chromatin becomes scattered in the form of minute granules. This entire structure is now recognized as the paranucleus of Marchal, and the polar protoplasm surrounding the embryonic cells in which it lies is his trophamnios.

Martin ('14) has since reinvestigated the early development of Ageniaspis, with the express purpose of studying the origin of the paranucleus. He also finds that three polar bodies are formed, but his account of their exact origin and position does not seem to me to be entirely consistent. He states that the two chromatin masses which lie toward the center of the egg are both derived from the first polar body, while the third mass situated at the extreme anterior end of the polar region is the second polar body. He bases his conclusion on certain stages in which the second maturation division precedes that of the first polar body. It is very difficult to understand how a chromatin mass, such as that of the second polar body, could reach the position assigned to it by Martin in his figure 16. Furthermore, he admits that the time relation may be just the reverse of that seen in this figure. I believe it is practically certain that the three chromatin masses shown in his figures 17 and 18 are incorrectly labeled. In each figure I should interpret both the anterior and middle masses as derivatives of the first polar body, and the posterior mass, not the anterior, as the second polar body. As to the sub-

sequent history of the polar bodies, Martin in the main is in agreement with Silvestri. His account of the organization of the paranucleus and trophamnios is especially clear.

I have given the subject of the formation of the polar nucleus, which is the homologue of the paranucleus, very careful study, and the conclusion at which I have arrived is based on an examination of several hundred eggs, both in sections and in whole mounts. This conclusion is slightly at variance with that reached by Silvestri in his studies on the egg of Litomastix. So far as the formation of the polar bodies is concerned, I am in complete agreement with Silvestri; but according to my observations on Paracopidosomopsis, the second polar body and the inner nucleus of the first body fuse to form the polar nucleus, and this occurs irrespective of the time relations between the second maturation and the polar body division.

At the close of maturation the female pronucleus moves toward the sperm, which is situated at the posterior end of the egg, leaving the three polar bodies at the anterior end. The polar bodies are almost invariably arranged in a row (figs. 5, A_1, A_2, B_1). At this stage each polar body consists of a number of delicate chromatin threads or rods surrounded by a clear space. In later stages the two posterior polar bodies come to lie close together, in a single clear space, and somewhat apart from the third or anterior group of chromatin (figs. 8, 11, 13, 14, 31). In some eggs one can still recognize the individual chromosomes (figs. 31, A_2, B_1), but from now on their individuality gradually disappears, and the single body thus formed consists of a coarse reticulum of chromatin (figs. 10, 23, P). This body is of course the formative polar nucleus, and by the time the four-celled stage is reached it is completely organized and appears as a conspicuous figure lying at the base of the anterior process (figs. 17, 18, 22, P).

In the meantime the outer nucleus of the first polar body undergoes certain changes, the most important of which is the condensation of its chromatin into a single mass (fig. 23, 31, A_1). This body very quickly dissolves and disappears. The changes here recorded have been observed in a large number of

eggs, and there can be no doubt as to the manner in which the polar nucleus is organized in this species. However, it is only fair to state that occasionally one finds eggs in which all three polar bodies would appear to fuse, or at least only a single group of chromatin threads or rods can be detected (figs. 16, 24). This appearance is perhaps more apparent than real and is probably due to one of two causes. Either the polar body, A_1, has already disintegrated or else it is hidden beneath the forming polar nucleus.

The real proof that only two polar bodies enter into the production of the polar nuclei is seen in certain eggs in which the disintegration of the third polar body chromatin has been delayed until the nuclear membrane is completely formed. Such a condition is shown in figures 22 and 36.

Another line of evidence which supports our conclusion is obtained in studying the mitotic figures of the dividing polar nuclei. I have shown above that each polar body received eight chromosomes. Therefore, if three polar bodies enter the polar nucleus, its subsequent divisions should reveal twenty-four chromosomes, or the triploid number. I have succeeded in finding three clear metaphase plates, and in each case the diploid number of sixteen chromosomes is present (fig. 25).

c. Fertilization

The egg is inseminated by a single sperm, which penetrates the surface at any point on the posterior half. Polyspermy never occurs. The entire spermatozoon enters (figs. 1, 3, 5, *S*), but the tail disappears and only the head is transformed into the male pronucleus. After maturation is completed, the ootid group of chromatin forms the female pronucleus and at the same time moves toward the sperm, which now lies at the posterior end. The two pronuclei thus come to lie close together (fig. 6). Both nuclei then expand, come in contact with each other (fig. 7), and finally fuse (fig. 8) to form a single large conjugated or cleavage nucleus (fig. 9, *F.N.*), which can always be distinguished from the smaller cleavage nucleus of the unfertilized egg (fig. 10, *C.N.*).

During the process of fertilization or in the corresponding period of the unfertilized egg, the nucleolus gradually moves down from its original position near the middle of the egg (figs. 1 to 5, *No.*) to the side of the cleavage nucleus (fig. 10).

3. THE CLEAVAGE STAGES

a. The first division

In respect to cleavage, the egg of polyembryonic insects differs from that of the typical insect egg, in that the cleavage nuclei are from the first accompanied by cytoplasmic segmentation. Another point of interest is the fact that the course of development is in nowise modified by fertilization. The history of cleavage, as well as that of the polygerm, is the same in fertilized and unfertilized eggs. At least, one can detect no difference. The first segmentation spindle, which is organized about the cleavage nucleus, is devoid of asters at its poles (fig. 11). It takes a position at the extreme posterior end of the egg, with its long axis approximately at right angles to the long axis of the egg (fig. 12). The chromatin divides in a typical manner. The chromosomes soon move to the opposite ends of the spindle, and remain connected for some time by a series of curved interzonal fibers (fig. 13). The daughter nuclei are then reorganized and move in opposite directions, finally coming to rest just inside the cell membrane (fig. 14). The cytoplasmic division follows. It starts as a furrow extending around the posterior end and in a plane practically coinciding with the median longitudinal plane of the egg. Each end of the furrow passes upward for a distance equivalent to a third or a fourth of the length of the egg proper, and then curves to the right and to the left, each branch finally reaching the side of the egg at a point near its middle (figs. 15, 16, 37).

By this manner of division the egg protoplasm is divided into three parts, of which the two at the posterior end are the true embryonic cells or blastomeres. The third, or anterior part, is the polar region or cap, and this contains the polar nucleus. It includes slightly more than one-third the volume of the entire

egg. The study of many two-celled stages reveals the fact that the two blastomeres are not always of the same size. Their disproportion in size may be accentuated by the position the plastic egg happens to take on the slide.

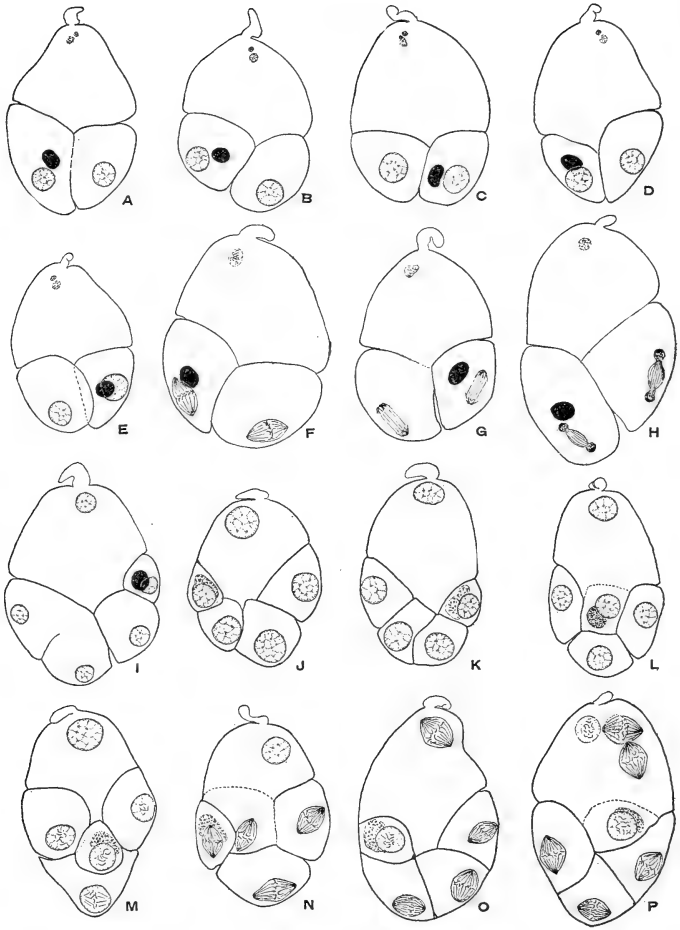
The nucleolus becomes associated with one of the daughter nuclei (fig. 14), and is thus included in the blastomere formed about that nucleus (fig. 15). It passes into the cell unchanged (text fig. 1, *A–H*).

b. The second division

In preparation for the second division, each mitotic spindle is arranged so that the angle formed by its major axis and the long axis of the egg is less than a right angle, and with this axis lying more or less parallel with the outside margin of the cell (fig. 16 and text-fig. 1, *F–H*). When the divisions are completed, the four-celled stage consists of two cells forming the base of the egg and two lying above these, one on each side (fig. 18 and text fig. 1, *I*, *J*, *K*, *O*). This is the typical arrangement; but there are variations from this typical figure in which only one of the blastomeres forms the base of the egg (text fig. 1, *L*, *M*, *N*, *P*). Such variations may be due to one of two causes, either the blastomeres shift after they are formed or, what is more probable, the direction of the mitotic spindle in one or both blastomeres varies from that seen in such figures as 16.

The nucleolus, which, as we have seen, enters one of the first two blastomeres, again passes unchanged into a single cell (text fig. 1, *I*). The nucleolus is thus invariably inherited by one of the first four blastomeres. Very shortly after the second division is completed, this peculiar body breaks up and forms a granular area lying about one side of the nucleus (figs. 17, 18 and text fig. 1, *J–P*).

It would be interesting to know whether it is always received by a definite cell; that is to say, whether in all four-celled stages the blastomeres inheriting the nucleolus are homologous. While this point is difficult to determine, nevertheless, after examining many two- and four-celled stages, I have reached the conclusion that its distribution is a matter of chance. In the first place, if



Text fig. 1, *A to P* A series of camera lucida outline drawings of two- and four-celled stages.

there is an appreciable difference in the size of the two blastomeres, it is seen to pass with about equal frequency into the large (fig. 15 and text fig. 1, *A*, *H*). and smaller cells (fig. 16 and text fig. 1, *D*, *G*). It is found with equal frequency in the right- and left-hand blastomeres as they lie on the slide; but this would not disprove homology any more than would size differences, for it is evident that the position of an egg on the slide is a matter of accident, so that what appears from above to be the right side in one egg may correspond to the left side in another egg. The point raised above cannot, therefore, be decided from a study of two-celled stages.

In the four-celled stage the typical condition shows the nucleolar cell to be one of the two upper cells, which is invariably smaller than any of the other three cells (text fig. 1, *I–K*, *O*, *P*). This condition is found with very great frequency, and were it not for certain variations, might easily lead one to conclude that the nucleolus is handed on to a definite blastomere of the four-celled stage. The most significant of these variations is the one showing the nucleolus in one of the lower cells (fig. 15). It is impossible to homologize the nucleolar cell in figure 18 with that in text figure 1, *K*.

The reason why the nucleolus is found so often in one of the upper cells is not to be explained on the basis of homology, but on entirely different grounds. I have already pointed out that as the female pronucleus moves to the posterior end of the egg it is followed by the nucleolus, and by the time the cleavage nucleus is organized it comes to lie close to this nucleus, usually to one side and above, rarely below. In the two-celled stage the nucleolus occupies this same relative position with reference to the nucleus of its blastomere (figs. 14, 15). The cytoplasmic division which produces the four-celled stage will result in placing the nucleolus in an upper cell. If the nucleolus sinks below the level of the equatorial plate (fig. 16), the resulting division will produce a condition like that seen in figure 18. A study of many four-celled stages shows that any one of the four cells may inherit the nucleolus, but that it goes into one of the upper cells much more frequently than into one of the lower cells.

c. The third division

The third set of divisions produces the eight-celled stage, all four cells dividing about at the same time. There is nothing constant about the arrangement of the spindles in preparation for this division, and hence we find a great variety of cleavage figures in eight-celled stages.

Some time before the division is completed, the polar nucleus becomes active and undergoes two divisions. It forms a large spindle which lies at right angles to the major axis of the egg (text fig. 1, *O*). The two nuclei produced by this division are shown in figure 39, *P*. These two polar nuclei quickly divide to produce four, which in turn form spindles. A case of this kind is seen in text figure 1, *P*, in which two of the spindles are in side view and two in polar view. The first two polar-nuclear divisions and the formation of the spindles for the third all occur before the eight-celled stage is reached. During the latter stage the eight polar nuclei, produced by the third division, very soon divide again to form sixteen nuclei (fig. 19).

It is during the eight-celled stage that another remarkable change also takes place in the polar cap. The protoplasm of this region gradually moves down along the sides of the eight embryonic cells (fig. 19), and finally encloses them by a thin layer (fig. 20). The layer thus surrounding the embryonic cells is destined to play an important rôle in the formation of the polygerm. It is in a way comparable to the trophamnios of Ageniaspis, but we shall refer to it as the polar region or membrane. In later stages the polar nuclei from the anterior portion of the polar region also move down alongside the embryonic cells, so that the polar membrane becomes nucleated.

d. The fourth division

In the fourth division all of the embryonic cells divide, except the two which contain the nucleolar substance. The result is the production of a fourteen- instead of the typical sixteen-celled stage (fig. 21). In the four-celled stage the nucleolus breaks down and its content spreads around the nucleus (fig.

39), and gradually becomes scattered throughout the cytoplasm of the two daughter cells (fig. 40). In the fourteen-celled stage these two blastomeres are recognized easily, owing to the presence of the nucleolar granules, which cause the cytoplasm to take a deeper stain (figs. 41, 42). They lie well toward the top of the group of enclosed embryonic cells.

It is clear that the presence of the nucleolus or its material exerts a retarding influence on the divisions of the cells which happen to inherit it. The inhibitory influence is sometimes shown in the formation of the eight-celled stage from the four. In some eggs (text fig. 1, *O, P*) while the nucleus of the nucleolar blastomere is still in the prophase condition, the spindles of the other three cells have reached the metaphase stage.

The fact that the nucleolar substance retards divisions has been noted by other investigators. Silvestri ('06, '08) observed this phenomena in the eggs of Litomastix and Ageniaspis, and Martin ('14) has shown in the egg of the latter species that in the two-celled stage the nucleolar blastomere does not divide so quickly as the sister cell. There is thus produced a typical three-celled stage. At this point of development the nucleolus breaks down and cannot be traced further.

e. The fifth division

In the fifth division all of the blastomeres, including the two which inherited the nucleolus material, divide, thus producing twenty-eight cells. In this stage one can still recognize the four descendants of the original nucleolar cell by the fact that the granules in their cytoplasm cause them to take a deeper stain than the other embryonic cells. In figure 43 three of these cells are clearly seen; the other lies in an adjacent section. The egg from which the figure is drawn has twenty-seven cells, but one of the blastomeres is dividing to produce the typical twenty-eight-celled stage. The cells do not form a solid mass, for owing to their rounded condition many interstices are found.

The polar region has formed a definite and complete membrane around the blastomeres through the gradual movement of its

protoplasm toward the posterior end. This results in transforming the elongated, pear-shaped egg into a figure more or less circular in outline. The number of polar nuclei at this stage is sixteen. In one egg all sixteen polar nuclei are dividing simultaneously (fig. 44). After this period these divisions become irregular, as indicated by the fact that one frequently finds single nuclei in mitosis.

f. The morula stage

After the twenty-eight-celled stage all synchrony in division is lost, and one may find from one to several blastomeres undergoing division in any egg. Consequently a typical fifty-six- or 112-celled stage is not seen. We may therefore consider together several eggs which represent steps leading up to the formation of a solid-ball stage, or what may be called the morula stage.

Figure 45 is a section through a fifty-two-celled stage. There are present four nucleolar cells, showing that these cells have not further divided. Figure 46 represents a sixty-celled stage. The polar membrane is remarkably clear and of almost equal thickness around the entire egg. This stage represents a condition characteristic of this period of development, viz., a tendency in certain cells for the cytoplasm to become drawn out into an elongated process. Such cells often become spindle-shaped.

Figure 47 is a median section through an egg composed of about seventy cells. It is of peculiar interest in that it represents the most advanced stage in which one can recognize the descendants of the nucleolar blastomere. In the section drawn five of these are clearly visible, and a sixth lies in an adjacent section. It is clear that an irregularity in the division of these cells has already set in, which is further evidenced in other eggs showing but one of the four nucleolar cells undergoing division. The nucleolar cells therefore follow the rule of loss of synchrony in divisions as do the other cells.

Figure 48 is a median section of a 135-celled stage, which has to a remarkable degree retained the original pear-like shape of the egg. At the anterior end there exists a rather interesting condition, which has been noted in some other eggs. A single

large cell (marked *X*) is embedded in the polar cap. In some eggs two or even more such cells may be found. The first impression one gains on examining such preparations is that these have been organized about polar nuclei from the polar protoplasm. But a detailed study of several eggs showing a similar condition has convinced me that these cells have separated from the main mass of embryonic cells and have pressed up into the plastic polar region. A very clear case, which supports this interpretation, is shown in figure 49. The two large cells (*X*) have their upper portions embedded in the polar cap, while their under surfaces are still connected with the other embryonic cells by means of protoplasmic strands.

In stages younger than this one it is not unusual to find several large blastomeres lying in contact with the under surface of the polar cap (figs. 45, 47). Silvestri ('06) has noted a similar group of cells in the egg of Litomastix, and attributes to them an important significance; but it seems more reasonable to suppose that they owe their large size to their proximity to the polar cap which undoubtedly serves as a nutritive organ to the growing embryonic cells.

Figure 50 is the final morula-like stage that we need consider. It has 221 cells which form a solid spherical mass. Some of the cells are spindle-shaped, others are polygonal. The latter class is frequently grouped together (fig. 50, *Y*). In one region of the egg a group of polygonal cells has become transformed into a nest or cyst, in which the core consists of several cells surrounded by a layer formed by the fusion of spindle-shaped cells. The central group is made up of the true or definitive embryonic cells (fig. 51, *D.E.C.*). The outer layer becomes syncytial in character (fig. 51, *I.M.C.*), and finally forms the inner membrane of the primary mass and their derivatives in the polygerm.

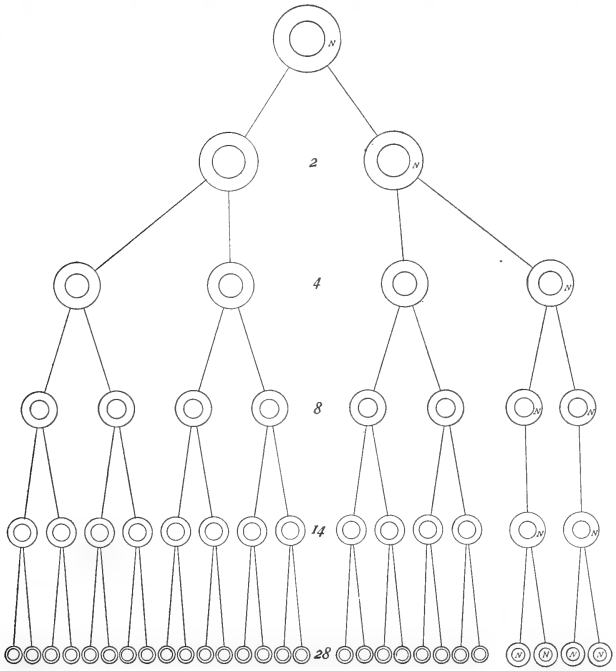
In this stage the polar membrane is of equal thickness about the entire embryonic mass, and its nuclei are fairly evenly distributed. At certain points mesenchyme cells, derived from the host tissue, adhere to its outer surface (figs. 49, 50, *M.C.*). In most eggs these mesenchyme cells are isolated, although in one case they formed a membrane over about half the circumference.

However, they never form a complete membrane, as Marchal ('04) observed in the egg of Ageniaspis.

We shall conclude this section by a statement concerning the fate of the cells which inherit the nucleolar materials, as we shall have no further occasion to refer in detail to that subject. In text figure 2 I have outlined in diagram form the history of the distribution of that body up to and including the twenty-eight-celled stage. In certain instances the descendants of the four nucleolar cells of the twenty-eight-celled stage may be recognized (fig. 47, *No.* C.), but beyond the seventy-celled stage one can no longer follow their history, at least in preparations made by the usual methods of technique. There is nothing in the subsequent history of the egg to show that these cells have been set aside for special function or that their behavior is different from that of the descendants of the other three blastomeres. It is true Silvestri has formulated the very attractive hypothesis that the nucleolar cells may become the primordial germ cells for the sexual embryos which later develop. He has apparently strengthened this hypothesis by his studies on the development of the monembryonic egg of certain parasitic species (Silvestri, '08) in which he was able to show that a similar nucleolar-like body is included in the primordial germ cell, and thus may be regarded as a germ-line determinant.

Aside from the failure to trace these so-called germ cells to the separate embryos, there are two other objections which are fatal to his hypothesis. In the first place, it is impossible to conceive of a mechanism which could operate in such a manner as to parcel out exactly predestined germ cells to the several hundred embryos. It would seem that some embryos might receive too many germ cells, while others might receive none at all. To be sure, his corollary hypothesis, that the asexual larvae owe their asexuality to the absence of germ cells, would account for the latter slip in the mechanism; but I have elsewhere ('17 a) pointed out that these non-viable larvae are probably the result of an entirely different cause. In the second place, I hope to show that in some cases an embryo is derived from a single cell during the late history of the polygerm. If this can be established as a

fact, then obviously a given embryo does not originate from two kinds of cells, one of which is derived from predestined germ cells. The best that can be said for the similarity in the distribution of the nucleolus in the monembryonic and polyembryonic eggs



Text fig. 2 Diagram showing the distribution of the nucleolus up to the twenty-eight-celled stage.

is, that while the latter has inherited this condition from the former, the nucleolus has ceased to function as a germ-line determinant, owing to the increase in complexity of development in the polyembryonic egg.

4. FORMATION OF THE POLYGERM

The first steps leading to the organization of the polygerm can be observed as early as the 220- to 225-celled stage (fig. 50). As we have already noted, the initial step consists in the differentiation of the embryonic cells into two classes. Certain blastomeres become transformed into spindle-shaped cells, while others, retaining their polygonal shape, become arranged into groups. The latter constitute the definitive or true embryonic cells. The spindle-shaped cells become drawn out into long processes, which assist in dividing up the egg into its primary divisions. The cells adjacent to the true embryonic cells tend to fuse together. Their intervening walls soon disappear, and thus there is formed about the group of embryonic cells a nucleated membrane (fig. 51, *I.M.C.*). The entire structure thus formed constitutes a primary mass of the polygerm.

These changes occur between forty and fifty hours after the egg is deposited. During this time, and for the next few hours, both kinds of cells multiply rapidly. By the time 500 cells are produced the polygerm is well advanced in its organization. Such a stage is shown in figure 54. In this preparation the primary masses are not especially well defined, for many of the embryonic cells are shrunken and loosely arranged, due in part to poor fixation. In some places the cells adjacent to the true embryonic cells have already formed a nucleated membrane (fig. 54, *I.M.C.*). Protoplasmic strands from these cells are seen extending throughout the egg, in between the formative primary masses (fig. 54, *P.S.*).

The next change which takes place in the organization of the polygerm is the lengthening of the egg along its major axis (fig. 55). There also occurs at the same time a change in the staining reaction of the true embryonic cells. They take a very much deeper stain than do the adjacent nuclei and their cytoplasm (fig. 55, *I.M.C.*).

Figure 52 is a detailed drawing of an oblique section of a young polygerm seventy-two and one-half hours old. The details of structure are remarkably clear, making the matter of interpre-

tation comparatively easy. The polygerm is surrounded by the polar membrane, from the inner surface of which processes extend in toward the center (fig. 52, *P.M.*). In more advanced stages these processes are invaded by the polar nuclei, and the membrane thus formed eventually surrounds each primary mass, becoming what we shall call the outer envelope or membrane of the mass.

Another point of interest in this preparation is the condition of the inner membrane nuclei. These nuclei and their accompanying cytoplasm are in the act of forming the inner envelope of the primary masses. Both stain very lightly (fig. 52, *I.M.C.*). Various stages in the formation of the inner envelope are seen in the preparation. In the upper part of the figure the nuclei lie free in the cytoplasm which surrounds the dark embryonic cells. On the lower side of the formative primary mass lying on the right, a portion of the membrane is fairly well organized.

This account of the development of the inner and outer envelopes of the primary embryonic mass differs somewhat from that given in an earlier paper, from which I may quote: "About seventy hours after oviposition, the nucleated membrane begins to invade the embryonic cells by the formation of trabeculae, which divide the embryonic cells into several groups, or primary masses. During the formation of these masses, or very shortly thereafter, the young polygerm elongates in the direction of the long axis of the egg. In addition to the nucleated membrane, each primary embryonic mass develops a second envelope, which lies just inside the nucleated membrane. Apparently this inner envelope is formed from the peripheral layer of cells of the embryonic mass" (Patterson, '18, p. 365). As a matter of fact, a more extensive study of a completed series of sections shows that the initial steps in the formation of the inner envelope precedes the development of the outer membrane.

The true embryonic cells stand out in sharp contrast to all other structures in the polygerm (fig. 52, *D.E.C.*). They have become spherical in shape. In the section four primary masses are seen. Two of these are practically completed, while two are

only forming. The number of embryonic cells included in a primary mass is extremely variable. I have seen cases where not more than four or five cells were present in a single primary mass; in other cases I have counted as many as fifty. The number of cells included in a primary mass is not a matter of any great importance. The embryonic cells are constantly dividing, so that a primary mass with a few cells would soon have that number increased. Furthermore, the primary masses themselves soon divide, especially those which possess a large number of cells.

Figure 56 represents a further advance in the development of the polygerm. Several of the primary masses are already completed (*Pr.M.*). One of these is differentiating into an asexual embryo (*As.E.*). This is the youngest stage in which one can recognize the asexual embryos. A more advanced stage is illustrated in figure 57. The primary masses are practically all completed. There are fifteen of these masses, in addition to a large conspicuous asexual embryo (*As.E.*). The polar membrane already shows signs of constrictions, which will result eventually in forming an outer envelope around each mass.

A completed polygerm is one in which all of the primary masses are found. Such a stage is shown in figure 58. This one has a large asexual embryo and about twenty primary masses. The asexual embryo has both the outer and inner envelopes completely formed, which has resulted in cutting it off from the rest of the polygerm. Each primary mass has as yet only the inner envelope completed. It consists of a distinct, rather thick membrane containing a large number of nuclei (fig. 58, *I.M.C.*). The cavity contains a variable number of true embryonic cells, loosely arranged and spherical in shape.

Figure 59 represents a stage slightly more advanced than the preceding. The section contains no asexual embryos, but a very young one is found in one of the lateral sections of the series. However, asexual embryos are not found in all polygerms of this age. The polygerm illustrated in figure 60 has no asexual embryo. The significance of this will be discussed in connection with the history of the asexual larvae, given in a later section of the paper.

5. HISTORY OF THE POLYGERM

a. Multiplication of the primary masses

In the completed polygerm each primary mass consists of several embryonic cells surrounded by a relatively thick inner membrane, and the various primary masses are more or less separated from one another by ingrowths from the polar membrane (fig. 58). Soon after the polygerm is formed, the primary masses begin to multiply by fission. The division is initiated by a constriction of the inner membrane, followed by a corresponding constriction or ingrowth of the polar membrane. In figure 59 some of the primary masses are beginning to divide, and in figure 60 the one on the left is in the act of dividing.

In the description of these stages we shall refer to the products of division of the primary masses as secondary masses, and when these in turn divide their products will be referred to as tertiary masses. In later stages the tertiary masses also divide a number of times, but such products will be called components, whenever they can be distinguished from the ordinary tertiary masses. During the late history of the polygerm it is not always easy to determine whether a given mass is secondary or tertiary. Their general structure is the same and both kinds are frequently present in the same polygerm, owing to the fact that the divisions do not occur simultaneously. However, one can usually distinguish the two kinds of masses by their difference in size.

Figure 61 is a detailed drawing of a section passing through two secondary masses that have recently been formed. The mass on the left has received a single embryonic cell, while the one on the right has received three such cells. The general rule is for a secondary mass to have several embryonic cells, but occasionally only a single cell is included.

Figure 62 passes through the middle of a polygerm in which several of the primary masses are dividing. Some of them have already completed the division (fig. 62, *S.M.*). At the points marked *X*, *Y*, *Z*, are three primary masses in different stages of division. In all these cases the division has been accomplished

entirely by the inner membrane or envelope. The constriction or ingrowth of the polar membrane does not take place until somewhat later.

The method of division in the formation of tertiary masses is exactly similar to that just described for the secondaries. No further details are therefore necessary. The formation of the tertiaries may begin as early as the end of the fourth day (fig. 73), and continues through the sixth day. From the seventh to the tenth day the multiplication of the tertiary masses and their components goes on with great rapidity, and by the eleventh day they form a very complex structure, which is sometimes surrounded by adipose tissue developed from the host cells (fig. 72).

Figures 63 to 68 represent a series of tertiary masses which have been set free into the body cavity of the caterpillar. They show the various steps in the multiplication of the components of a tertiary mass. Figure 63 is a mass containing a single embryonic cell. It has just been set free from the main body of the polygerm. Figure 64 is a slightly later stage in which the embryonic cells are multiplying. In figure 65 a tertiary has recently divided, and the component on the right has two cells which have not yet completely separated. Figure 66 is a tertiary component with four cells. Figure 67 is of interest in that it shows how the embryonic cells are being isolated. Ingrowths from the inner membrane have separated the embryonic cells into groups. In some instances only a single cell is thus separated, but usually there are two or more cells in each group. The formation of these groups is then followed by constrictions of the inner and outer membranes, which results in producing many new components of the tertiary masses (fig. 68). The components may later completely separate from each other, becoming scattered throughout the body cavity of the host and forming new centers of proliferation. The rate of their distribution to various parts of the body cavity to a very great extent depends upon their relation to the host tissues. If the polygerm is embedded in adipose or other tissue, the scattering of the tertiary masses and their compo-

nents may be greatly delayed (fig. 72). On the other hand, if the polygerm happens to lie free within the body cavity, the dispersal of its components may begin very early, even as early as the primary mass stage.

b. Relation of parasitic egg to host tissues

To understand fully the account of the distribution of the products of the polygerm, it is necessary to call attention to the relation of the parasitic egg to the host tissues. The question is one of the greatest interest, for it can be demonstrated, by the means of a very simple experiment, that the development of the parasitic egg is dependent upon the development of the host egg. Not only is this true for the late stages, but also for the initial steps in development.

Marchal ('04), Silvestri ('06), and Martin ('14) have all made note of certain points on the relationship of the parasitic egg to the host. In the case of Ageniaspis, Marchal states that in order for the egg to develop completely it is essential that it be placed within the embryonic region of the developing caterpillar (Hyponomentus). In all of his preparations showing the egg of Ageniaspis, he always found it in the body cavity of the embryo, where it normally develops. However, he states that his preparations are not numerous enough to determine definitely whether or not some eggs are lost or die if placed in an unfavorable position, such as the intestine or yolk. He implies that some are thus lost, since it does not seem probable that the parasite could find, by means of its probe, the most favorable region in which to place the egg. In later stages he discovered that the epithelial layer which at first forms a cyst about the developing egg, and then gives rise to the elongated tube of the chain of embryos, is the product of the host tissue. In the case of Polygnotus minutus, Marchal discovered the interesting fact that the egg is lodged in the gastric pouch or stomach of the host (Cecidomyia), and there, curiously enough, undergoes its development.

According to Silvestri's observations, the egg of Litomastix may be laid in any part of the host embryo (Plusia), or even in the yolk outside the embryo. The egg is destroyed if laid either in the intestine or yolk. In late stages the germ mass may be found in any part of the young caterpillar, except the intestine or anterior part of the head. It is most frequently found in the thorax, either above or below the oesophagus. He also found the polygerm in the nerve ganglia, especially the brain ganglia.

In Ageniaspis Martin believes that the frequent occurrence of the egg in the thoracic ganglia of the caterpillar is to be correlated with the laying time of the parasite. He finds that the egg clings to the ganglion in such a manner that the typical shape of the ganglion is preserved. In late stages of development he could no longer find the polygerm connected with a ganglion, which fact leads him to conclude that on account of its growth the polygerm is forced out of the ganglion.

My own observations on Paracopidosomopsis very closely parallel those of Silvestri on Litomastix. The egg may be deposited in any part of the host egg, but disintegrates if it happens to be placed in the yolk or intestine. In the newly hatched caterpillar the egg may be found in any part of the body cavity or embedded in the tissues adjacent thereto. There are two kinds of tissues in which it is frequently found, namely, nervous and adipose.

Both the cephalic and ventral ganglia often contain parasitic eggs. In my preparations I have counted no less than sixty-three cases of infected ganglia, distributed as follows: one egg in ventral ganglion, 40 cases; two eggs in ventral ganglion, 3 cases; one egg in supra- or suboesophageal ganglion, 18 cases; two eggs in brain ganglia, 2 cases. The egg may be deposited directly in the ganglion. Figure 70 is a portion of a suboesophageal ganglion containing a fertilized egg undergoing maturation. Several similar cases have been observed. Figure 69 is a longitudinal section through the third ventral ganglion containing a well-developed polygerm. Figure 71 is a similar section through the fourth ventral ganglion. It contains a large asexual larvae, several secondary masses, and a small group of

tertiary masses. Numerous cases similar to these have been observed. In late stages the embryonic masses break out from the ganglion and become scattered throughout the body cavity.

I have noticed from my records that most of the cases of ganglionic infection, especially in the head region, arise in host eggs that were parasitized during the late embryonic period, just before the young caterpillar hatches. This is probably to be explained by the position of the host embryo in the egg. At the time of hatching the head of the caterpillar is situated at the apex of the dome-shaped egg, and it is at or near this point that the female parasite inserts her ovipositor at the time of laying.

The polygerm is often surrounded or embedded in fat tissue developed from the host cells. The fat tissue probably starts to develop from mesenchyme cells such as are shown in figures 49 and 50. The adipose tissue not only serves as a source of nutriment for the growing polygerm, but it also holds the embryonic masses together (figs. 72 to 75), and thus delays their dispersal.

The relation of the host tissues to the parasitic egg is all important in the development of the latter. It can be shown that the development of the parasitic egg is dependent upon the growth of the host embryo. This has been demonstrated in the following way. A batch of eggs laid in the laboratory by a virgin female moth were exposed for an hour to a brood of female parasites. Several parasitic eggs were deposited in each host egg. Two days later these eggs were fixed and sectioned. The moth eggs of course did not develop, and an examination of the sections revealed the fact that not a single parasitic egg developed. Under similar conditions, but with fertilized moth eggs, all of the parasitic eggs would have been in late cleavage stages.

c. Dissociation of the polygerm

I have already stated that at some period in its history the polygerm undergoes fragmentation or dissociation. The point at which dissociation occurs varies greatly in different cases.

It may take place as early as the fourth day, or it may be delayed until the eleventh day. Indeed, in some few cases the polygerm does not completely break up until the larvae are on the point of being set free. The fragmentation is largely controlled by the relation of the polygerm to the host tissue. If it lies free in the body cavity or in loose tissue, dissociation will occur very early; but if it is embedded in rather dense tissue, such as the ganglion or fat, the dispersal of the embryonic masses may be greatly delayed.

The primary masses are organized toward the end of the third day (figs. 52, 53), and are completed during the early part of the fourth day. If the young polygerm of this period happens to be free from host tissue, the separation of the primary masses may set in. Figure 53 is a polygerm seventy-seven hours old, and signs of breaking up are apparent. The asexual embryo has already become completely separated from the other primary masses. In the same preparation there are several polygerms from which one or more primary masses have broken away and lie some distance from the main body of the polygerm.

The usual time for dissociation to occur is during the period in which secondary and tertiary masses are being formed, that is, from the end of the fourth to about the tenth day. Figure 69 is a ganglion containing a polygerm ninety-five and one-half hours old. The secondary masses are beginning to dissociate.

Figure 73 is a polygerm ninety-five hours old and composed of secondary and tertiary masses. It is undergoing dissociation. The adipose tissue has nearly all been absorbed and the embryonic masses are beginning to scatter.

Figures 74 and 75 are portions of the same polygerm, showing groups of asexual embryos and tertiary masses, respectively. The polygerm is nine days and twenty-three hours old. A large number of tertiary components are found in the body cavity of the caterpillar, scattered throughout its entire extent.

If bound together by nervous or fat tissue, the embryonic masses may remain connected until the eleventh day or even later. Figure 71 is a ganglion containing a seven-day polygerm, which shows no signs of fragmentation. The polygerm shown

in figure 72 is eleven days old, and only a few masses, on the left, are beginning to break away. In stages still older one may find a considerable portion of the polygerm, at the original seat of infection, still intact.

d. Origin and history of the sexual embryos

The multiplication of the embryoic masses, from the primary stage to the formation of the tertiary components, is a continuous process. As already stated, the distribution of the tertiary masses and their components to various parts of the caterpillar follows the dissociation of the polygerm. The components thus distributed became the centers for the formation of groups of sexual embryos, either directly or after further multiplication, depending upon the stage at which the scattering occurs. Since one cannot follow the history of a single tertiary component, it is not easy to determine at just what point multiplication of components ceases and embryo formation begins. However, one can meet this difficulty by studying stages fifteen or sixteen days old with well-developed sexual embryos, and tracing their origin back through a series of younger stages.

As the multiplication of embryonic masses progresses the number of embryonic cells included in each mass naturally becomes smaller and smaller. This occurs notwithstanding the fact that the embryonic cells are also multiplying, because the rate of division of the embryonic cells does not keep pace with the increase in number of the embryonic masses.

In the tertiary divisions, and more particularly in those of the components, it is not uncommon for a single embryonic cell to be separated out into an embryonic mass (figs. 63, 65). The method of division in the tertiary masses is slightly different from that found in the case of primary and secondary masses. Both in the primary and in the secondary masses the division is effected by a simple constriction of the inner membrane (figs. 60, 62). In the case of tertiary divisions there first grows in from the inner membrane a number of protoplasmic processes which divide the embryonic cells into several groups, each con-

taining one or more of the embryonic cells. The inner membranes then completely form, and thus separate the groups from one another (figs. 67, 68). This method of division becomes more accentuated in the formation of components and the sexual embryos.

During the thirteenth and fourteenth days the multiplication of the tertiary masses occurs in the manner just described. At the end of the fourteenth day tertiary components begin to form embryonic masses, each of which will produce a single sexual embryo. Figure 76 is a section of a tertiary component lying free in the body cavity, and in which the formation of sexual embryos is in progress. The section shows six masses, in at least four of which further divisions will occur. In many places in the series single embryonic cells are being isolated to form, in all probability, a sexual embryo (fig. 76, *X*). Owing to the fact that a cell may divide immediately after it is isolated, and usually before the inner membrane is completely organized about it, it is difficult to establish this point. Nevertheless, the evidence revealed in an intensive study of this period of development points to the conclusion that each sexual embryo arises from a single embryonic cell. Just why components continue to multiply up to a certain point and then suddenly cease to divide before producing embryos, is not easy to answer. However, that this point of departure varies in different cases is evidenced by the great variation in the number of individual parasites arising from different eggs.

By the end of the fifteenth day no further divisions of components are seen. Each mass represents an individual embryo. Figure 77 shows the typical condition of this period. Each embryo consists of several cells, closely pressed together and surrounded by a well-formed inner membrane. Later, the embryonic cells form a typical morula stage. From the sixteenth to the eighteenth day the embryos become well organized. The inner and outer membranes thin out to form a double-walled, transparent envelope about each embryo (fig. 78).

On opening up infected caterpillars from the fifteenth to the eighteenth day, one finds floating in the fluid of the body cavity,

or among the tissues therein, a large number of groups of sexual embryos. Each group results from the fact that the embryos arising from a single component tend to stick together. The groups vary in size and shape. Sometimes they form flat or plate-like structures (figs. 78 to 80). More frequently they are spherical in shape, which has given rise to the term 'ball' stage in my notes. The size of the group is determined by the number of embryos present, and these vary from two to as high as seventy. In one lot of seventeen groups I counted the following numbers: 2 (two), 6, 10 (two), 12 (two), 15, 16, 18 (two) 20 (two), 22 (two), 25, 70.

The embryos develop rapidly from the eighteenth day on, and sometimes between twenty-second and twenty-fourth days reach the early larval stage. They then escape from their capsules into the body cavity of the caterpillar. Once free, the larvae proceed to devour the contents of the host, first eating the fatty tissue, and finally devouring the various internal organs. The last of these to disappear are the nervous system and the intestine. In destroying the internal organs, the larvae consume such portions as are dissolved by the action of their salivary secretions. The undissolved parts consist largely of the chitin of the tracheae. They also destroy all of the body wall except the superficial layer of chitin.

The larvae pupate on about the twenty-eighth day. During pupation the non-digested content of the caterpillar hardens and forms the thin-walled, oval chambers in which the parasitic larvae lie and in which they undergo their transformation into pupae. According to some observers, a thin cuticular layer from the larvae forms an inner lining to the chamber and se ves as a sort of puparium. The layer of chitin of the caterpillar is perfectly transparent and at first is very flexible. Later, as drying takes place, it shrinks in on the walls of the chambers and becomes hard and rigid, the whole thus forming the typical mummified carcass, characteristic of polyembryonic parasites. Under laboratory conditions the parasites emerge from the pupae on the forty-seventh day.

e. Origin and history of the asexual embryos

I have already given an account of the history of the asexual larvae (Patterson, '18), and shall quote rather freely from that paper in this section.

The appearance of non-viable, asexual larvae in polyembryonic hymenoptera was first observed by Silvestri ('06) in Litomastix. Briefly summarized, his account is as follows. The polygerm of Litomastix, soon after the polar membrane is established, begins to show differentiation into two distinct regions. The anterior part of the egg is made up of large and small cells, while the posterior part is composed of small cells only. A constriction develops in the polar membrane, which finally separates these two regions. Silvestri calls the anterior region the massa germinigena, and the posterior the massa monembrionale. The posterior part subsequently differentiates into a single asexual larva. In the course of further development, the massa germinigena gives rise to a few secondary monembryonal masses, which develop into asexual larvae, and to a large number of other masses. This is accomplished by constrictions in the polygerm. The masses continue to multiply by constrictions, and from time to time may produce a few asexual embryos, but a large majority of them develop into sexual embryos. In one case Silvestri counted 100 asexual larvae arising from one egg; in a second case he counted about 1700 sexual embryos and 220 asexual larvae. In structure the asexual larva differs from the sexual larva in that it has no reproductive, respiratory, or circulatory system, and no malpighian tubules.

Silvestri has suggested that the asexual larvae may owe their asexuality to the absence of germ cells. He bases his suggestion on the fact that the so-called nucleolus, which in certain monembryonic eggs seems to serve as a 'keimbahn-determinant,' is not inherited by all of the embryonic cells. According to Silvestri's suggestion, an embryo arising from cells all of which are deficient in nucleolar material would be asexual; while one receiving one or more of these potential germ cells would be sexual. Aside from the mechanical difficulty (to which I have already referred)

standing in the way of the full acceptance of this hypothesis, there is the further objection that it does not explain the absence of organs other than those of reproduction, nor does it take into account the fact, established by experimentation, that secondary sexual characters in insects, as well as certain primary organs, such as those of copulation and oviposition, do not depend upon the presence of gonads for their development.

In Paracopidosomopsis I have found similar larvae, which never undergo metamorphosis and are non-viable. In this species the asexual embryos can be recognized in young polygerms seventy to seventy-two hours old. Figure 56 shows the youngest stage that I have found. The young asexual embryo is distinguished from the other embryonic masses by two features of its organization. It has a larger number of cells and the inner membrane is relatively thicker than in the primary masses. The embryonic cells multiply very rapidly and soon form a solid spherical mass (fig. 57, *As.E.*). At the seventy-two hour stage the asexual embryo gives evidence of differentiation, and is surrounded by completed inner and outer membranes (figs. 53, 58, *As.E.*). It is frequently separated from the rest of the polygerm. A single asexual embryo may frequently arise during the primary mass stage of the polygerm, but it is not the universal rule. In some polygerms of this stage no asexual embryo is present. Furthermore, it frequently happens that two or more asexual embryos may arise in a single polygerm at this early period. Figure 72 shows two young asexual larvae that must have started their development during the primary mass stage. The polygerm shown in figure 73 has four asexual embryos, all in the same stage of development, but situated at different points in the polygerm. In figure 56 the asexual embryo has arisen at the side of the polygerm; in figure 57, at the anterior end, and in figure 58, at the posterior end. All of these facts show that in Paracopidosomopsis an asexual embryo may arise at any point in the young polygerm, and not habitually from the posterior region of the egg, as reported for Litomastix by Silvestri.

While some polygerms produce asexual embryos at a very early stage, nevertheless the majority of such embryos do not appear until after dissociation has taken place. Their production in a given polygerm is not confined to a single period of development, but is a continuous process, extending from the third to about the fifteenth day. They arise during both the secondary- and tertiary-mass stages. Sections of practically every polygerm from twelve to fourteen days old will show asexual individuals in various stages of development, from young embryos to fully developed larvae.

During the secondary mass stage one is struck by the frequence with which they are found in groups. In some groups there may be as high as ten or twelve individuals. Figure 74 shows one of these groups embedded in fat. A group of tertiary mass from this same polygerm is seen in figure 75.

The frequent appearance of asexual embryos or larvae in groups suggests that, like the sexual embryos, the individuals of a group have a common origin, probably arising through the division of a single secondary or tertiary mass.

Single asexual embryos also develop, in conjunction with a group of sexual embryos. In one case I found an asexual embryo joined to a single sexual embryo, which is still in the morula stage. In figure 81 is a group of ten sexual embryos and one asexual embryo all held together by their membranes. In figure 83 is a fully developed asexual larvae, freed from its capsule, but still connected by the head to a group of sexual embryos.

In developing into a larvae the asexual embryo becomes bent upon its long axis, with the ventral surface forming the concave side (fig. 72, *As.E.*). Just before escaping from the capsule, the larva has a characteristic shape, like the letter C (fig. 82). Once set free, the larvae present various figures, such as are seen in figures 83 to 86.

The asexual larvae invariably degenerate, apparently they do not live over three days as free larvae. The first free larvae appear on the twelfth day, and degenerating specimens are found on the fifteenth day. The last larvae escape from their capsules on the sixteenth day and none are found after the

eighteenth day. The beginning of degeneration is marked by a foreshortening and twisting of the body. The larva becomes immobile and soon disintegrates (figs. 87 to 90). These larvae apparently perform no function, for there is no evidence that they break down the tissues of the host preparatory to assimilation by the sexual larvae. They disappear at least a week before the sexual larvae are set free from their envelopes.

As one cannot follow the course of development of a single egg, but must depend upon series of sections and dissections, it is impossible to determine whether every polygerm eventually produces asexual larvae. It is possible that some do not. For the same reason, it is difficult to determine the exact number of asexual larvae produced by a given egg. This in part is due to the fact that these larvae are formed continuously from the third to the fifteenth day, and those first developed degenerate before the last ones appear. The largest number of larvae found in a single case is fifteen. The data collected from dissecting a large number of infected caterpillars, reared in the laboratory, indicate that no more than twelve or fifteen such larvae are produced in a single egg.

In conclusion, I should like to point out some of the more important problems which need further study. These are: 1) The exact origin and the late history of the nucleolus; 2) the morphology of the sexual larvae, with especial reference to the origin of germ cells; 3) the morphology of the asexual larvae, which should be compared with that of the sexual larvae; 4) the causes underlying the origin of mixed broods and asexual larvae.

6. SUMMARY

1. There is one generation of Paracopidosomopsis for each generation of the Autographa moth, at least for the fall months.

2. The parasite will deposit its egg in the host egg at any time, but does not parasitize the young caterpillar after hatching. It lays one or two eggs at each oviposition. In about two times out of three two eggs are deposited.

3. The egg may be placed in any part of the host egg, but does not develop unless embedded in the tissues of the host embryo or larva.

4. The freshly laid egg is pear-shaped, and contains, in addition to the nucleus, a large nucleolus. The broad end of the egg is posterior and the narrower end anterior.

5. In fertilization only a single sperm enters. Polyspermy never occurs.

6. The maturation divisions are typical, and result in reducing the number of chromosomes from sixteen to eight. The polar body chromosomes do not form nuclei, and hence are not accompanied by cytoplasmic segmentation. Two of the three groups of polar body chromosomes fuse to form a polar nucleus; the third disintegrates.

7. The egg develops whether fertilized or not. If unfertilized it produces a brood of males. Eggs laid by a fertilized female produce mixed broods.

8. The cleavage nuclei are from the first accompanied by cytoplasmic segmentation. Cleavage is confined to the posterior end of the egg, and eventually results in producing a morula-like stage.

9. The nucleolus is inherited by only one of the first four blastomeres. Its history can be traced accurately to the twenty-eight-celled stage, in which its materials are distributed to four cells. There is no evidence indicating that the descendants of these four cells become the germ cells of the sexual embryos.

10. The polar nucleus divides, forming several nuclei. These with the cytoplasm of the anterior third of the egg flow down and surround the embryonic cells or blastomeres, finally forming a nucleated membrane or envelope.

11. The morula develops into a polygerm. which consists of a number (fifteen to twenty) of primary masses. Each primary mass consists of a group of definitive embryonic cells, surrounded by an inner membrane. This membrane is formed from certain blastomeres during the development of the polygerm.

12. The primary masses multiply by constrictions of the inner membrane, followed by constrictions or ingrowths from the

polar membrane. The products of these divisions are known as secondary masses, which in turn multiply by similar constrictions to form tertiary masses. The tertiary masses later divide to produce components.

13. At some time during the period of multiplication of the masses the polygerm undergoes fragmentation or dissociation. The masses become scattered throughout the body cavity of the caterpillar, and form new centers either for further divisions or for the production of sexual embryos.

14. The sexual embryos arise from tertiary components. In some cases one can trace the origin of an embryo to a single embryonic cell.

15. Asexual embryos may arise as early as the primary-mass stage of the polygerm, but the greater number of them develop during the secondary and tertiary stages. These embryos produce non-viable larvae, which do not live over three days in the body cavity of the caterpillar. Not over twelve to fifteen such larvae arise from one polygerm.

Austin, Texas,
September 17, 1920

7. BIBLIOGRAPHY

BRANDES, G. 1898 Germinogonie, eine neue art der ungeschlechtlichen Fortpflanzung. Zeitschr. Naturw., Bd. 70, S. 420–423.

BUGNION, E. 1891 Recherchés sur le developpement postembryonnaire, l'anatomie et les moeure de l'Encyrtus fuscicollis. Recueil Zool. Suisse, T. 5, pp. 435–536.

1906 La polyembryonie et le déterminisme Sexuel. Résumé des observations de P. Marshal. Bull. de la Soc. Vaudoise des Sci. Natureles, T. 42, pp. 95–112.

GANIN, M. 1869 Beiträge zur Erkenntnis des Entwicklungsgeschichte bei den Insecten. Zeit. f. Wiss. Zool., Bd. 19, S. 381–451.

GATENBY, J. BRONTE 1918 Polyembryony in parasitic hymenoptera: A review. Quart. Jour. Mic. Sci., N. S., vol. 63, pp. 175–196.

GIARD, ALFRED 1898 Sur le developpement de Litomastix truncatellus. Bull. Soc. Ent., France, pp. 127–129.

HEGNER, R. W. 1914 Studies on germ cells. III. The origin of the Keimbahn-Determinants in a parasitic hymenopteron, Copidosoma. Anat. Anz. Bd. 46, S. 51–69.

1915 Studies on germ cells. IV. Protoplasmic differentiation in the oocytes of certain hymenoptera. Jour. Morph., vol. 26, pp. 495–561.

HENNEGUY, L. F. 1891 Contribution a l'embryogenie des Chalcidiens. Bull. Soc. Philom., vol. 3, pp. 164–167.

HOWARD, L. O. 1882 On some curous methods of Chalcid pupation. Amer. Nat., vol. 16, pp. 60–62 and 149–151.

1891 The methods of pupation among the Chalcididae. Insect Life, vol. 4, pp. 193–196.

1892 The biology of the hymenopterous insects of the family Chalcididae. Proc. of U. S. Nat. Museum, vol. 14, pp. 567–588.

1906 Polyembryony and the fixing of sex. Science, vol. 24, pp. 810–818.

1919 Two new instances of polyembryony among the Encyrtidae. Science, vol. 49, pp. 43–44.

KORNHAUSER, SIDNEY I. 1919 The sexual characteristics of the Membracid, Thelia bimaculata. I. External changes induced by Aphelopus theliae. Jour. Morph., vol. 32, pp. 531–636.

KULAGIN, N. 1898 Beiträge zur Kenntnis Entwicklungsgeschichte, von Platygaster. Zeit. f. wiss. Zool., Bd. 63, S. 195–235.

MARCHAL, PAUL 1897 a Les Cecidomyies des cereales et leurs parasites. Ann. de la Soc. Ent. de France, T. 66, pp. 1–105.

1897 b Contribution à l'etude du developpement embryonnaire des Hymenopteres parasites, Platygaster. C. R. Soc. Biol., T. 4, pp. 1084–1086.

1898 a La dissociation de l'oeuf en un grand nombre d'individus distincts et le cycle evolutif de l'Encyrtus fusicollis. C. R. S. Sc., T. 126, pp. 662–664.

1898 b A new method of asexual reproduction in hymenopterous insects. Nat. Soc. London, vol. 12, pp. 316–318.

MARCHAL, PAUL 1898 c Un exemple de dissociation de l'oeuf de cycle de l'Encyrtus fuscicollis. C. R. Soc. Biol., T. 10, pp. 238–240.

1898 d Le cycle evolutif de l'Encyrtus fuscicollis. Bull. Soc. Ent. France, pp. 109–111.

1899 Comparison entre le developpement des Hymenopteres parasites a developpement monoembryonnaire. C. R. Soc. Biol. T. 1, pp. 711–713.

1902 Observations sur la Biologie des Hyponomeutes. Bull. Soc. d'études et de vulgarisation di la Zool. Agricole de Bordeaux, T. 1, fasc. 4, pp. 13–26.

1903 Le cycle evolutif du Polygnotus minutus. Bull. Soc. Ent. France, pp. 90–93.

1904 a Le déterminisme de la Polyembryonie specifique et la déterminisme du sexe dans la polyembryone specifique des Hymenopteres. C. R. Soc. Biol., T. 56, pp. 468–470.

1904 b Sur la formation de l'intestin moyen chez les Platygasters. C. R. Soc. Biol., T. 56, p. 1091.

1904 c Recherches sur la biologie et le developpement des hymenopteres parasites. I. La polyembryonie specifique ou germinogonie. Arch. d. Zool. Exp. et Gen., T. 2, ser. 4, pp. 257–335.

1906 Recherches sur la biologie et le developpement des hymenopters parasites. II. Les platygasters. Arch. de Zool. Exp. et Gen., T. 4, ser. 4, pp. 485–640.

MARTIN, F. 1914 Zur Entwicklungsgeschichte des polyembryonalen Chalcidiers Ageniaspis (Encyrtus) fuscicollis. Zeit. f. Wiss. Zool., Bd. 110, S. 419–479.

PATTERSON, J. T. 1915 Observations on the development of Copidosoma gelechiae. Biol. Bull., vol. 29, pp. 333–373.

1917 a Studies on the biology of Paracopidosomopsis. I. Data on sexes. Biol. Bull., vol. 32, pp. 291–305.

1917 b Studies on the biology of Paracopidosomopsis. III. Maturation and fertilization. Biol. Bull., vol. 33, pp. 57–67.

1918 Studies on the biology of Paracopidosomopsis. IV. The asexual larvae. Biol. Bull., vol. 35, pp. 362–377.

1919 Polyembryony and sex. Jour. Heredity, vol. 10, pp. 344–352.

PATTERSON, J. T., AND PORTER, LELIA T. 1917 Studies on the biology of Paracopidosomopsis. II. Spermatogenesis of males reared from unfertilized eggs. Biol. Bull., vol. 33, pp. 38–51.

PERRIER, E., ET GRAVIER C. 1902 La tachygenese ou acceleration embryogenique. Ann. Sc. Nat. Zool., 8 ser., T. 16, pp. 133–371.

RILEY, C. V. 1869–70 First annual report of the State Entomologist of Missouri.

1883 Annual Report of the Entomologist, U. S. Dept. of Agriculture, pp. 99–180.

RILEY, W. A. 1907 Polyembryony and sex-determination. Science, vol. 25, p. 106.

SACKEN, BARON OSTEN 1863 Lasioptera reared from a gall on the goldenrod. Proc. of Ent. Sco. of Phila., vol. 1, pp. 368–370.

SARRA, RAFFAELLE 1915 Osservazioni biologiche sull' Anarsia lineatella Z. dannosa al frutto del mandorlo. Boll. Lab. Gen. Agr. Zool. Sup. Scul. Agr., Portica, vol. 10, pp. 51–55.

1918 La variegana (Oelthreutes variegana Hb., Lepidottero Tortricide) Ed I Soui Parassiti. Boll. del Lab. di Zool. gen. e agraria della R. Scoula sup. d'Agri. in Portici, vol. 12, pp. 175–187.

SILVESTRI, F. 1905 Un nuovo interessantissimo caso di germinogonia (poliembrionia specifica) in un Imenottero parassita endofago con particolare destino dei globuli polari e dimorfismo larvale. Atti Accad. Lincei Rend. (5), vol. 14, sem. 2, pp. 534–542.

1906 Contribuzioni alla conoscenza biologica degli Imenotteri parassiti. I. Biologia del Litomastix Truncatellus. Ann. R. Scuola Agr. Portici, vol. 6, pp. 1–51.

1908 Contribuzioni alla conoscenza biologica degli Imenotteri parasiti. II. Sviluppo dell' Ageniaspis fuscicollis. Ann. della Regia Scuola Superiore di Agricoltura di Portici, ser. 2, vol. 8, pp. 1–27.

1910 Notizie preliminari sullo sviluppo del Copidosoma buyssoni. Monitore Zool. Ital. anno, vol. 21, pp. 296–298.

1914 Prime fasi di sviluppo del Copidosoma Buyssoni. Imenottero Chalcidide. Anat. Anz., Bd. 47, S. 45–56.

1915 Struttura dell'ovo e prime fasi di alcuni Imenotteri parassiti. Boll. del Lab. di Zool. gen. e agraria della R. Scuola sup. 'Agricoltura in Portici, vol. 10, pp. 66–88.

1916 Sulla maturazione Dell'ovo. Fecondazione E. formazione E. del Trophamnios nel Platygaster Dryomyiae. Tipografia della R. Accademia dei Lincei, vol. 25, pp. 121–128.

WHEELER, W. M. 1910 The effects of parasitic and other kinds of castration in insects. Jour. Exp. Zoöl., vol. 8, pp. 377–438.

8. DESCRIPTION OF PLATES

ABBREVIATIONS

A, first polar body nucleus
A_1, outer nucleus of first polar body
A_2, inner nucleus of first polar body
As. E., asexual embryo
A. T., adipose or fat tissue
B, second polar body nucleus
B_1, second polar body nucleus
B_2, female pronucleus
D.E.C., definitive or true embryonic cells
E.B., embryonic cell or blastomere
F.N., fertilization or cleavage nucleus
I.M., outer membrane
I.M.C., inner membrane cells or nuclei
M.C., mesenchyme cells of host
No., nucleolus
No.C., blastomere receiving nucleolus
Nu., egg or oöcytic nucleus
O.M., outer membrane
P., Polar nucleus or nuclei
P.M., polar membrane
Pr.M., primary mass
P.S., protoplasmic strand
S., sperm or sperm nucleus
S.M., secondary mass
T.M., tertiary mass
1st M., first maturation spindle
2nd M., second maturation spindle

All figures in plates 1 to 11 were drawn with aid of the camera lucida.

PLATE 1

EXPLANATION OF FIGURES

1 to 25 are all drawn from whole mounts made by the smear method.

1 Freshly deposited fertilized egg, showing the sperm, oöcytic nucleus, and nucleolus. × 620.

2 Freshly deposited unfertilized egg, showing the oöcytic nucleus and nucleolus. × 620.

3 Fertilized egg taken twenty-five minutes after oviposition.

The first maturation spindle is in the late anaphase stage. × 620.

4 to 10 are all drawn from the same preparation.

4 The second maturation spindle is in the late anaphase stage. The first polar body chromatin is seen at *A*. The egg is unfertilized. × 620.

5 Fertilized egg in which the second maturation is completed. The female pronucleus (B_2) is migrating toward the sperm. × 620.

6 Fertilized egg with two pronuclei close together. The polar nucleus (*P*) is in the process of formation. × 620.

7 A similar egg showing the two pronuclei in contact. × 620.

8 A stage showing the two pronuclei conjugating. × 620.

9 An egg showing the fertilized or first cleavage nucleus. × 620.

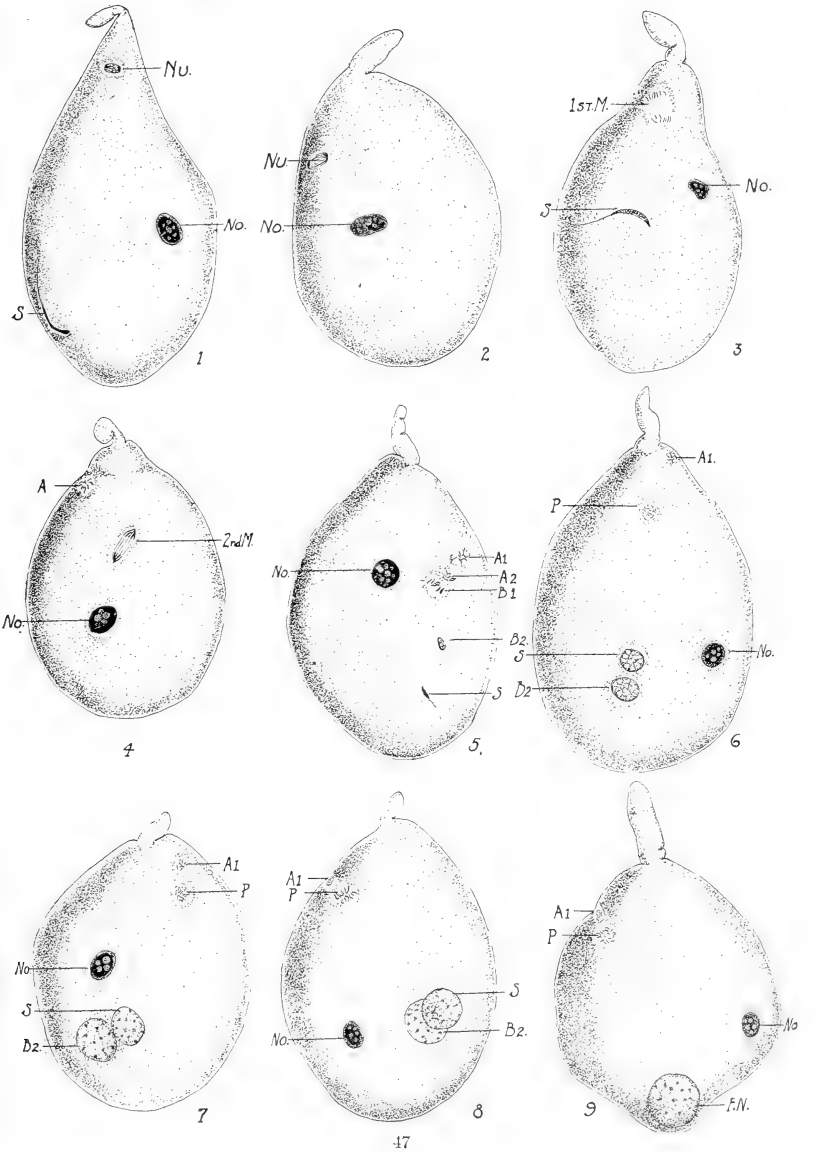

Nu.
No.
S
1
Nu.
No.
2
1st M.
No.
S
3
A
2nd M.
No.
4
No.
A1
A2
B1
B2
S
S
5
A1.
P
S
No.
B2
6
A1
P
No.
S
B2
7
A1
P
No.
S
B2.
8
A1
P
No
F.N.
9

PLATE 2

EXPLANATION OF FIGURES

10 Unfertilized egg showing the female pronucleus and the nucleolus at the posterior end. This nucleus will form the first cleavage spindle. × 620.

11 Egg showing first cleavage spindle in metaphase. × 620.

12 Egg showing first cleavage spindle in anaphase. × 620.

13 Egg showing first cleavage spindle in late anaphase. × 620.

14 In this egg the two cleavage nuclei are reorganized and the cytoplasm is beginning to divide. The nucleolus is closely associated with one of the nuclei. × 620.

15 The two-celled stage. Note that the nucleolus is included in one blastomere. × 620.

16 A two-celled stage, in which the spindles are formed in preparation for the four-celled stage. × 620.

17 The four-celled stage. Only one of the four blastomeres receives the nucleolar material (*No.C*). The polar nucleus is completely formed. × 620.

18 Another four-celled stage. × 620.

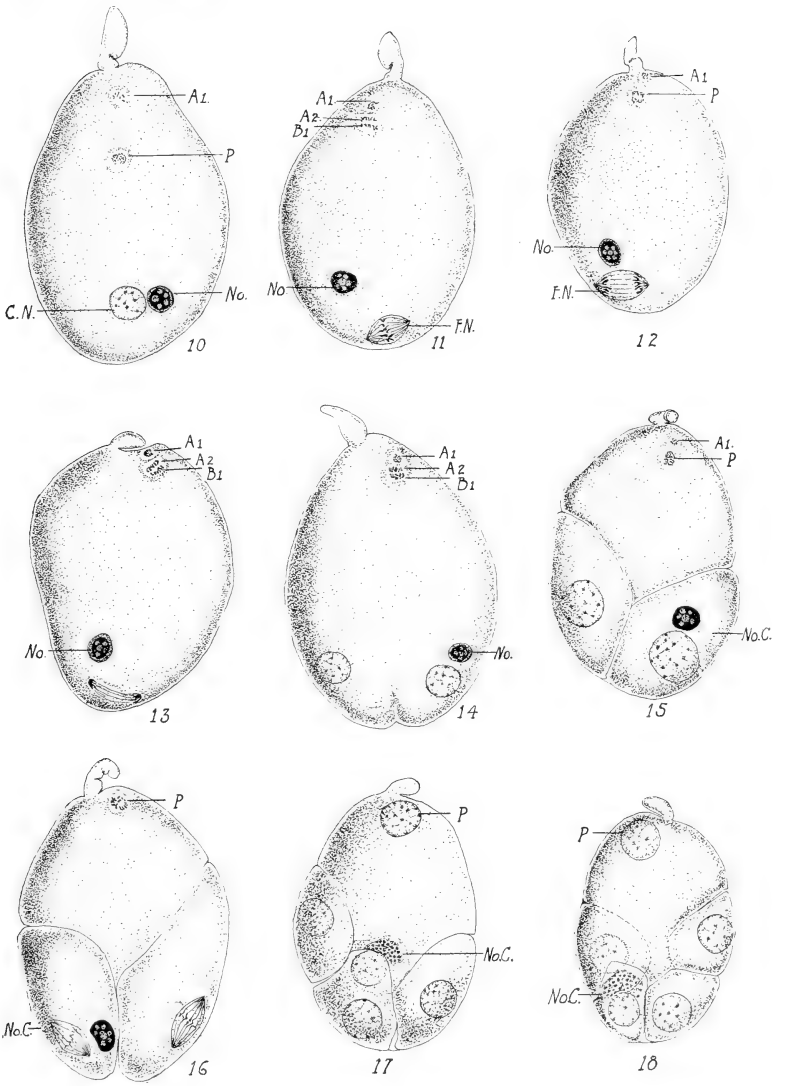

A1.
P
C.N.
No.
10
A1
A2
B1
No
F.N.
11
A1
P
No.
F.N.
12
A1
A2
B1
No
13
A1
A2
B1
No.
14
A1.
P
No.C.
15
P
No.C.
16
P
No.C.
17
P
No.C.
18

PLATE 3

EXPLANATION OF FIGURES

19 The eight-celled stage. The nucleolar material is distributed to two blastomeres (*No.C*). The polar nucleus has undergone rapid division producing thirteen nuclei. Note that the protoplasm containing the polar nuclei is gradually flowing around the embryonic cells. × 620.

20 A stage showing thirteen cells, two of which contain nucleolar material. Usually there are fourteen cells, but in this case one of the blastomeres of the eight-celled stage has been delayed in its division. The polar nuclei are all dividing. × 620.

21 A typical fourteen-celled stage. It is much flattened on the slide. × 620.

22 to 24 Upper or anterior ends of three eggs showing the polar nucleus. × 620.

25 Metaphase plate of a polar nucleus, showing the diploid number of chromosomes. × 1827.

26 First maturation spindle. × 1827.

27 First polar body chromatin (*A*) and the second oocytic chromatin (*B*). There are eight chromosomes in each group. × 1827.

28 The first polar body dividing (A_1 and A_2) and the chromatin of the second oocyte. Each group has eight chromosomes. × 1827.

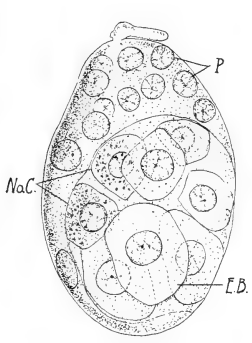
29 First polar body spindle and second maturation spindle. × 1827.

30 First and second polar bodies. × 1827.

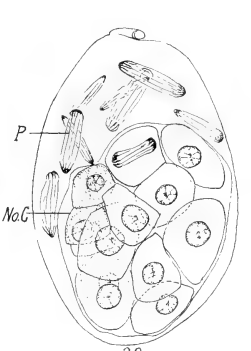
31 A similar stage. × 1827.

32 Side view of a cleavage spindle of one of the first four blastomeres. × 1827.

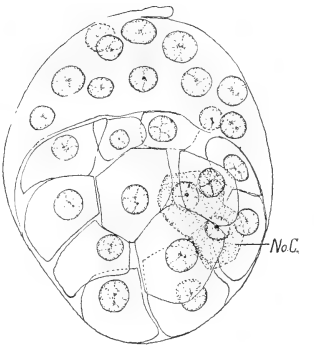
33 Polar view of the first cleavage spindle. × 1827.



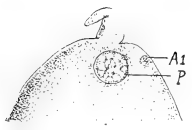
19



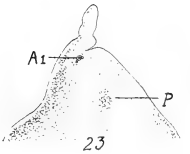
20



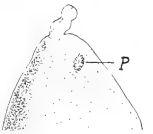
21



22

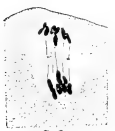


23

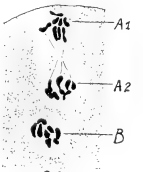


24

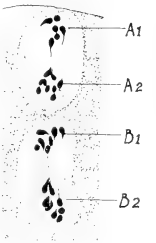

25


26

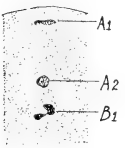
27



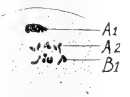
28



29



30



31


32

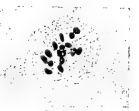

33

PLATE 4

EXPLANATION OF FIGURES

It is practically impossible to obtain a median section showing all of the details of structure. It has therefore been necessary in certain figures to draw in one or more structures (e.g., nucleolus or polar body nucleus) from adjacent sections. This was done in figures 34 to 39.

34 A median section showing the first maturation spindle, nucleolus, and sperm. The latter was taken from the first section on the left. × 1167.

35 A later stage, showing the three polar bodies and the female pronucleus. × 1167.

36 This section shows the cleavage nucleus (*F.N.*), the outer chromatin mass of the first polar body division (A_1), and the polar nucleus (*P*), which has been formed by a fusion of the inner chromosome group of the first polar body and the second polar body. × 1167.

37 A median section of the two-celled stage. × 1167.

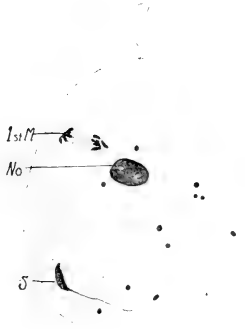
38 Median section of a four-celled stage. × 1167.

39 A similar section of a slightly later stage. The nucleolus has broken up and nearly surrounds the nucleus. The polar nucleus has undergone one division. × 1167.

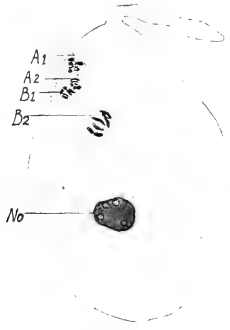
40 The eight-celled stage. × 1167.

41 Median section of a fourteen-celled stage. The two blastomeres which have received the nucleolar material lie at the top of the group of embryonic cells. × 1167.

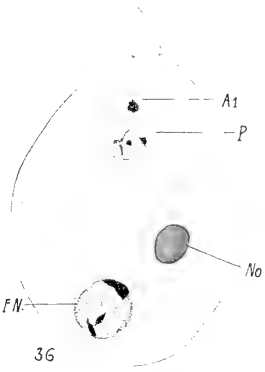
42 A transverse section of a similar stage. × 1167.



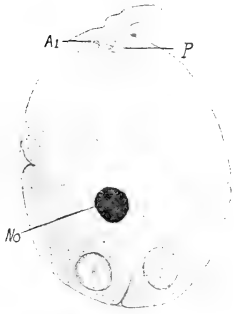
34



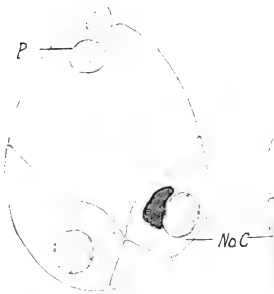
35



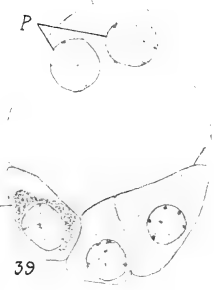
36



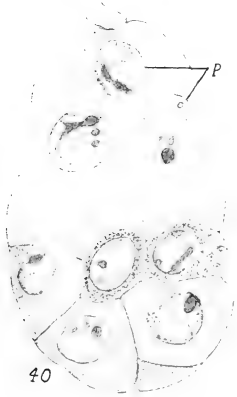
37



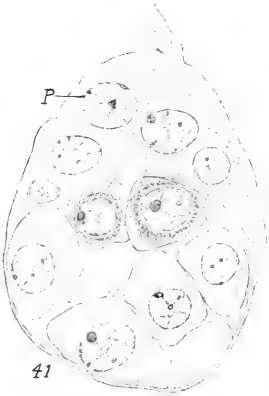
38



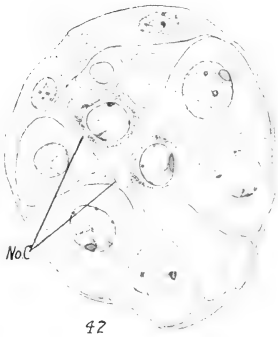
39



40



41



42

PLATE 5

EXPLANATION OF FIGURES

43 Slightly oblique section of a twenty-seven-celled stage, showing three of the four descendants of the original nucleolar blastomere. Nine hours. × 1260.

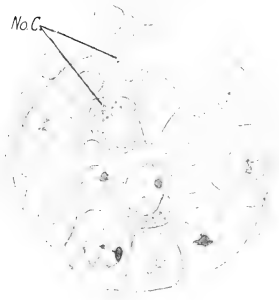
44 Upper end of a section through a twenty-eight-celled stage, with two of the sixteen polar nuclei all in mitosis. Nine hours. × 1260.

45 Median section of a fifty-two-celled stage. The four nucleolar cells are all shown in the section. Nineteen hours. × 1200.

46 Median section of a sixty-celled stage. Nineteen hours. × 1121.

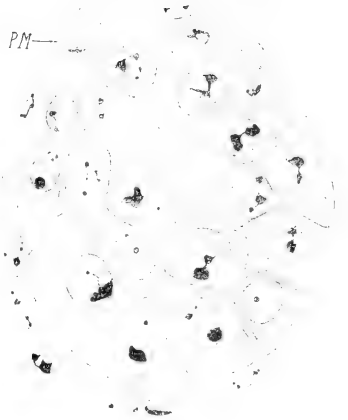
47 Oblique section of a seventy celled stage. It shows five of the six nucleolar blastomeres present in the egg. Twenty six hours. × 1153.

48 Median section of a 135 celled stage. This egg has retained to a remarkable degree the original pear shape. Forty hours. × 1035.

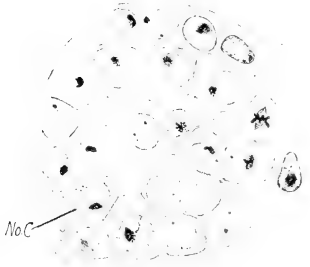


43

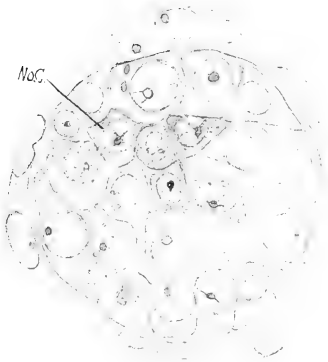
44



46



45



47

48

PLATE 6

EXPLANATION OF FIGURES

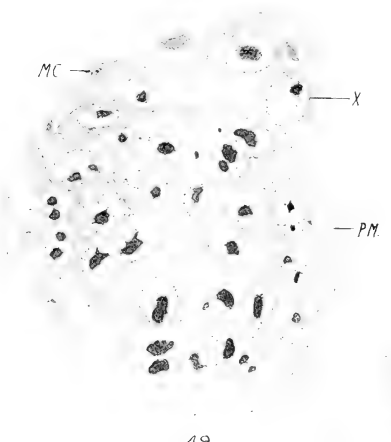
49 Median section of a 169 celled stage. × 1134.

50 Section through a 221 celled stage. Note that some of the cells have become elongated and spindle shaped. Forty hours. × 1134.

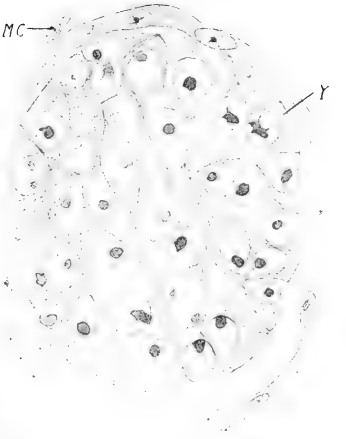
51 Part of a lateral section from the same series, showing certain blastomeres arranged in the form of a nest. × 1167.

52 Oblique section through a young polygerm. The embryonic cells have already differentiated into two kinds; 1) the definitive or true embryonic cells (*D.E.C.*) and 2) the inner membrane nuclei (*I.M.C.*). The true embryonic cells, which take a deeper stain, are in the process of forming primary masses. × 1052.

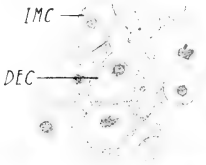
53 Section through a completed polygerm, showing a single large asexual embryo (*As.E.*) and several primary masses (*Pr.M.*). × 612.



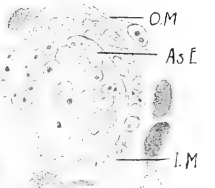
49

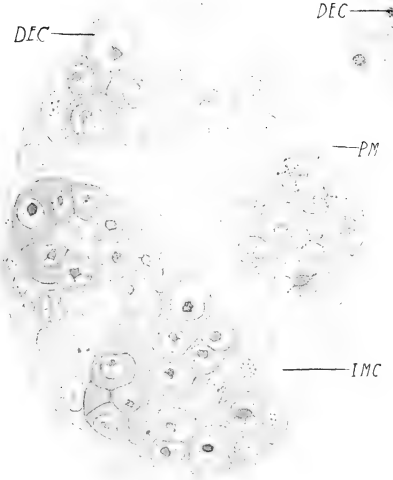


50

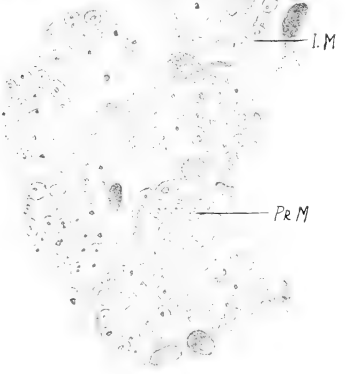


51





52



53

PLATE 7

EXPLANATION OF FIGURES

54 One half of a section through a very young polygerm showing formation of the primary masses. × 1040.

55 Median section of a young polygerm. × 626.

56 Median section of an almost completed polygerm, showing the youngest stage at which an asexual embryo can be recognized (*As.E.*). × 626.

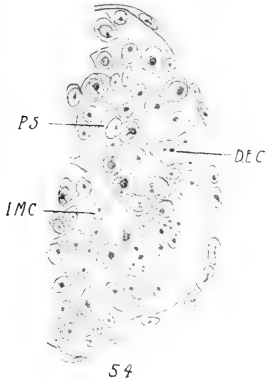
57 A completed polygerm with an asexual embryo at the upper end. × 626.

58 A later stage with an asexual embryo at the lower end. × 626.

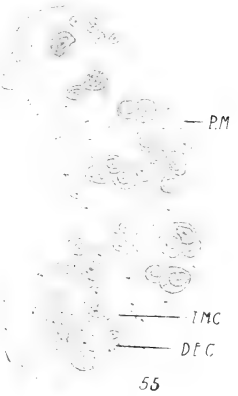
59 Median section of a completed polygerm. × 626.

60 Transverse section of a polygerm showing a primary mass undergoing division (on the left). × 626.

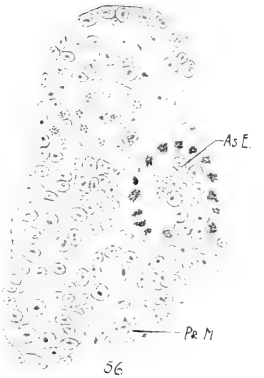
61 Detailed drawing showing the completed division of a primary mass. × 1167.



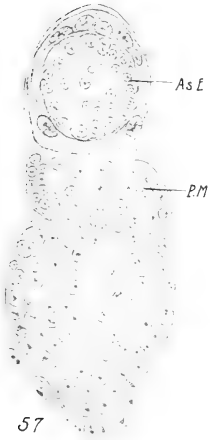
54



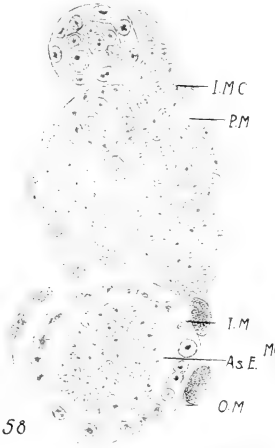
55



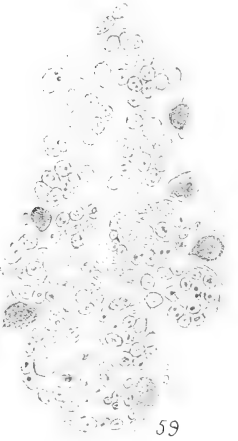
56



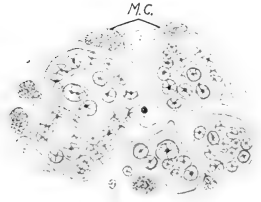
57



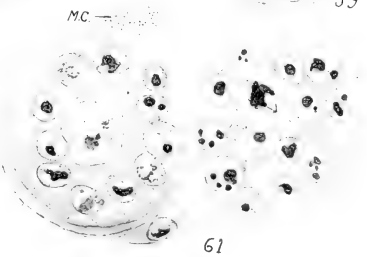
58



59



60



61

PLATE 8

EXPLANATION OF FIGURES

62 Section of a polygerm showing divisions of the primary masses to form secondary masses. *X*, *Y*, and *Z*, primary masses dividing. × 767

63 Tertiary mass containing a single embryonic cell. The mass lies free in the body cavity of the host. × 1267.

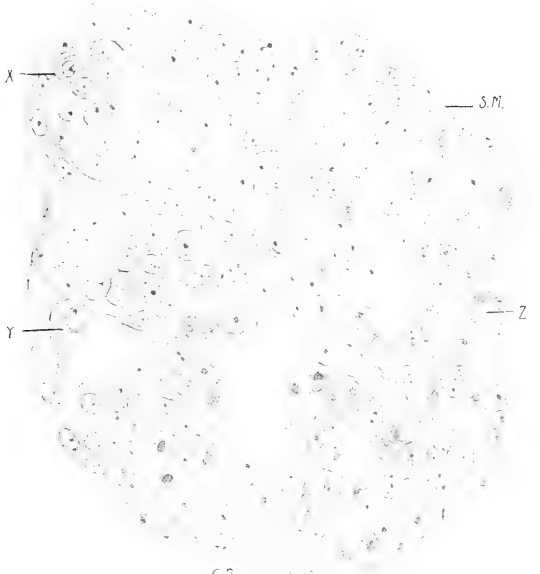
64 Tertiary mass showing division of embryonic cell. Some of the nuclei of the inner membrane are also dividing. × 1267.

65 Tertiary mass which has recently divided. The component on the left has a single embryonic cell; the one on the right has two which have not as yet separated. × 1267.

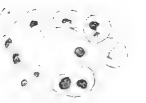
66 Tertiary mass showing the multiplication of embryonic cells. × 1267.

67 Tertiary mass preparing for division. The embryonic cells are being divided up into groups by the activity of the inner membrane. × 1267.

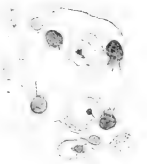
68 Tertiary mass which has divided to produce three components. The one on the right is preparing for another division. × 1267.



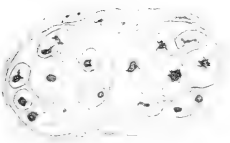
62



63

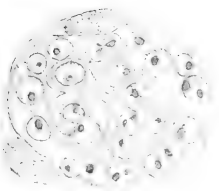


64

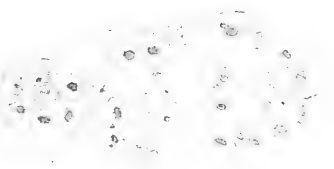


65

66



67



68

PLATE 9

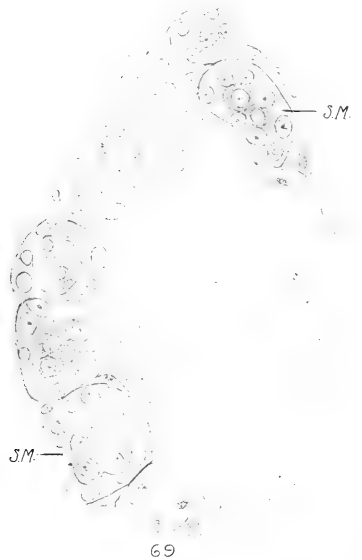
EXPLANATION OF FIGURES

69 Longitudinal section through the third ganglion, which contains a number of secondary masses. × 636.

70 A portion of the brain ganglion containing a fertilized egg in maturation. × 636.

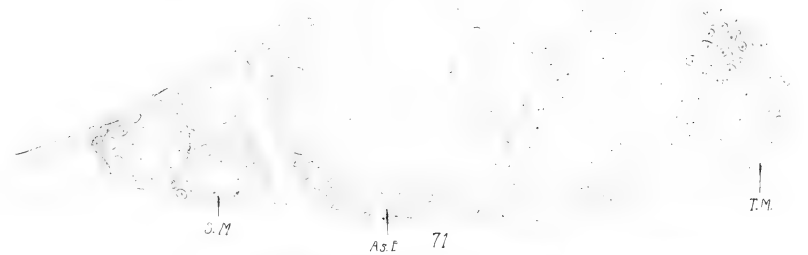
71 Longitudinal section of the fourth ganglion containing a large asexual embryo, several secondary masses, and a few tertiary masses. × 636.

72 Longitudinal section of an advanced polygerm removed from a 12 mm. caterpillar. The polygerm, which is surrounded by adipose tissue of the host, contains many tertiary masses and also two advanced asexual embryos. Some of the masses are dividing (at *X*). × 260.

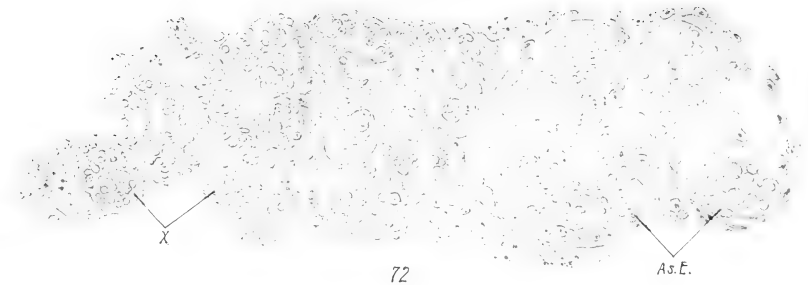


69

70



71



72

PLATE 10

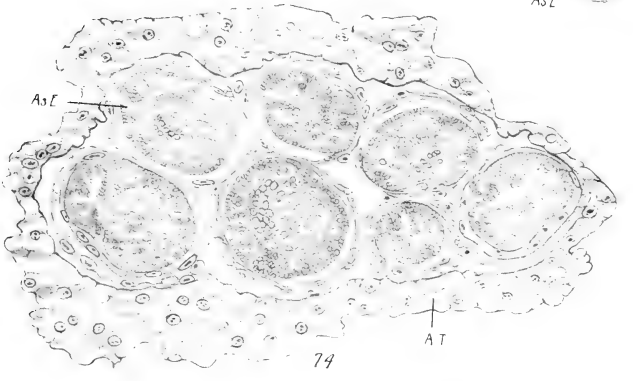
EXPLANATION OF FIGURES

73 Part of a longitudinal section of a polygerm in the body cavity of a 5.5 mm. caterpillar. The polygerm has undergone almost complete dissociation. One of the four asexual embryos found in the series is seen on the right. × 387.

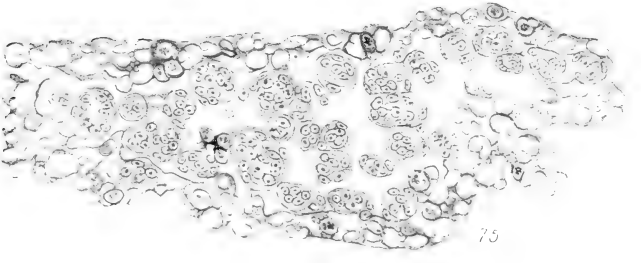
74 Part of a dissociated polygerm, showing a group of seven asexual embryos surrounded by fat tissue. The polygerm is nine days and twenty three hours old. *A.T.*, adipose tissue. × 208.

75 A portion of the same polygerm, showing teritary masses. × 208.

73



74



75

PLATE 11

EXPLANATION OF FIGURES

76 Section of a tertiary mass which has divided up into a number of components. In some of the components the embryonic cells are being isolated through the activity of the inner membrane. Such cells will produce sexual embryos. × 700.

77 A late stage in the development of a tertiary mass, showing early stages in the formation of sexual embryos. × 700.

78 An advanced stage of the development of the sexual embryos. × 110.

76

77

78

PLATE 12

EXPLANATION OF FIGURES

79, 80, 83, 86 to 90 are from eggs laid by an unfertilized female. The specimens were taken from the body cavity of a half grown caterpillar fifteen days after the eggs had been deposited. The body cavity contained four normal and four degenerate asexual larvae, in addition to many masses of sexual embryos. Figures 81, 82, 84, and 85 are from eggs laid by a fertilized female. The specimens were removed from the body cavity of a 20 mm. caterpillar fourteen days after the eggs had been deposited. There were found in the body cavity six free asexual larvae and four larvae still enclosed in their membranes, besides many masses of sexual embryos.

79 and 80 Two masses of sexual embryos. × 100.

81 Mass of sexual embryos with sexual embryo in capsule. × 100.

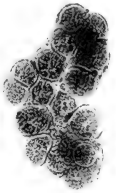
82 Asexual larvae in capsule. × 100.

83 Asexual larvae free from capsule, but still adhering to mass of sexual embryos. × 88.

84 to 86 Three asexual larvae. × 88.

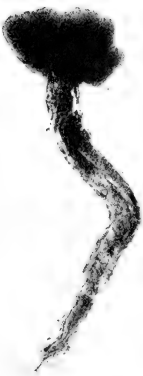
87 to 90 Four degeneration asexual larvae. × 88.


79

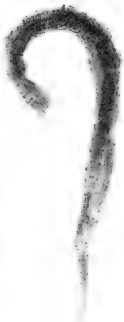

80


81


82


83


84


85


86


87


88


89


90

Resumen por el autor, Caswell Grave.

Amaroucium constellatum (Verrill).

II. La estructura y organización de la larva "renacuajo."

Los resultados del presente trabajo que ofrecen novedad son: 1. Una periodicidad en la liberación de las larvas de la colonia parental. Las colonias numerosas dejan escapar los renacuajos en bandas durante la aurora o próximamente a esta hora, pero de vez en cuando una larva escapa durante otras horas del dia. 2. Unas sesenta vesículas multicelulares, semejantes a blástulas se invaginan en el manto durante los últimos estados del desarrollo embrionario, permaneciendo aisladas del cuerpo en la substancia de la túnica durante todo el periodo de natación libre de la larva. 3. Los cristalinos del ojo no son células retinales modificadas sino productos que se depositan dentro de las células gangliónicas, y son de naturaleza semilíquida o gelatinosa. 4. Los bastones visuales, diferenciados en las células retinales del ojo, se proyectan a través de la zona pigmentaria y terminan en la superficie interna de la copa pigmentaria. 5. Las pruebas estructurales y fisiológicas indican que el ojo funciona en las respuestas de orientación del renacuajo a la luz. 6. El estatolito se forma dentro de una vacuola de la célula estatolítica sensorial y está formado de una substancia dura que no se disuelve en los ácidos fuertes. 7. El autor describe un nervio visceral que se origina en el ganglio visceral distribuyéndose en la región del endostilo. 8. El cordón nervioso no ocupa posición dorsal, sino que a causa de un giro permanente de la cola de noventa grados hacia la izquierda viene a situarse al lado izquierdo del notocordio. 9. Las dos series de células musculares situadas en los lados ventral y dorsal del notocordio, según indican las pruebas morfológicas y fisiológicas, funcionan como unidades de tal modo que producen un movimiento rotatorio del cuerpo durante la locomoción.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 7

AMAROUCIUM CONSTELLATUM (VERRILL)[1]

II. THE STRUCTURE AND ORGANIZATION OF THE TADPOLE LARVA

CASWELL GRAVE

Washington University, St. Louis, Missouri

FOUR TEXT FIGURES AND FOUR PLATES

This paper, which deals with the structural organization of the fully developed tadpole larva of Amaroucium constellatum, is a contribution, in part, to the morphology of ascidians, but it is especially intended as a further contribution toward the establishment of a basis for a comparative study of the larval forms of a number of species of ascidian common to the Woods Hole region with a view to the correlation, so far as may be possible, of their specific structural and physiological characters with observable differences in the distribution and habitat of each species.

The structures common to ascidian larvae in general have been so repeatedly described in the many excellent papers published during the fifty years that followed the announcement by Kowalewsky ('66) of his discovery of the chordate affinities of ascidians, that it seems unnecessary to attempt to cite specific references to papers except in connection with results or conclusions that have not found general acceptance. The points added to the morphology of the ascidian larva as a result of this study are enumerated in the concluding paragraphs of the paper.

[1] Since the publication of the first paper of this series (Grave, '20 b) conclusive evidence has been secured that Amaroucium constellatum is not a form of A. pellucidum, but must be considered a true species, hence the change in the general title for this, the second paper. The systematic data referred to will constitute the subject matter of a special paper.

METHODS

Tadpole larvae of Amaroucium may be secured in abundance at Woods Hole during the months of July, August, and September by placing large colonies of the ascidiozooids in glass jars before a window in the laboratory. The best results have been obtained when the colonies were collected the day before tadpoles were desired and kept in running sea-water during the night. Tadpoles escape from the colonies in swarms at and just after sunrise, but they continue to be liberated in small numbers at any hour of the day. When liberated the tadpoles swim immediately to the surface of the water and collect at one side of the jar where they may be easily captured with a pipette.

Immature tadpoles and various earlier developmental stages may be secured by squeezing a colony in the hand over a culture dish of sea-water. With the mass of ascidiozooids, tadpoles, embryos, and eggs thus forced from the colony, a considerable quantity of gelatinous debris is included, which settles very slowly, and may therefore be removed by decantation, two or more changes of water being required. Mature tadpoles may be had in small numbers in this way also. For a few seconds after having been squeezed from the colony they lie motionless upon the bottom of the dish, but soon begin to move slightly, then to swim about, apparently stimulated by light or by contact with pure sea-water.

For the study of the general and histological structure of the tadpole, Bouin's and Flemming's mixtures have been found to give the best fixation. Sections have been made by the paraffin method and stained with Delafield's or iron haematoxylin. For total mounts an excellent transparent stain for tadpoles fixed with Bouin's solution is made by adding to 70 per cent alcohol, slightly acidulated with HCl, an amount of borax carmin sufficient to give a delicate pink color to the solution. Specimens should be allowed to remain in the stain twelve hours or more.

DEFINITIONS

For the purposes of description, the part of the tadpole which contains the adhesive papillae and precedes during locomotion will be referred to as the anterior portion; the tail, as marking the posterior part of the body, and the sensory-vesicle, conspicuous on account of its pigmented sense organs, will be designated as dorsal in position. The relation the body of the tadpole bears to that of the sessile ascidiozooid has been discussed by Maurice et Schulgin ('84). A general readjustment of parts takes place during metamorphosis in which the anterior part of the tadpole becomes the basal portion of the ascidiozooid.

FORM AND SIZE

The general form of this and other ascidian larvae at once suggests that of the amphibian tadpole, hence the name 'tadpole' has been applied to both, but the similarity between ascidian and amphibian larvae, their fundamental chordate characters excepted, is superficial and disappears with any but the most casual comparison of either their form, structure, or activities.

The tadpole larva of Amaroucium, at the period of its complete development, has a total length of approximately 2.25 mm. The body portion varies in length from 0.74 to 0.78 mm. in depth from 0.36 to 0.44 mm. and in width from 0.31 to 0.37 mm.

TUNIC

The body and tail proper of the tadpole are enveloped by a comparatively thick, non-cellular tunic of a glassy, translucent appearance. In the living tadpole the tunic is flecked at its outer surface with numerous whitish points, the nuclei of the flattened test-cells which form a delicate pavement epithelium over its external surface.

The cytoplasm of test-cells in general is homogeneous in appearance, but it is not uncommon to find cells which contain numerous yellowish bodies or are filled with reddish-orange pigment granules. The quantity of pigment present in test-

cells, and in cells of other parts of the body also, varies greatly in tadpoles of different broods, those liberated by highly colored colonies being more highly pigmented than those produced by colonies of lighter color. The pigmentation of each tadpole is approximately the same as that of its parent colony.

The part of the tunic which envelops the tail is greatly compressed dorsoventrally and expanded in the horizontal plane and entirely constitutes the comparatively wide tail fin of the tadpole. Seeliger ('85), in his study of Clavellina, noted that the tunic substance is secreted during the embryonic period when the tail is folded forward and closely compressed between the body of the embryo and its chorionic membrane and that the part which is secreted about the tail is thus caused mechanically to be spread out on either side and to take on a compressed fin-like form. He also noted that the entire tail, the fin included, is twisted on its axis to the left during the embryonic period, but, in the Clavellina tadpole, it apparently untwists when the chorion is ruptured at the time of hatching, for he describes the tail fin of the free-swimming larva as having a vertical position. Damas ('04) noted the horizontal position of the tail fin of the larva of Distaplia magnilarva and called attention to its similarity in this respect to Appendicularia.

The part of the tunic surrounding the body of the tadpole is laterally compressed, but the right and left sides are slightly asymmetrical. Viewed from the dorsal side, a shallow concave depression is seen on the left near the anterior end, and the anterior tip end of the tunic which contains the middle adhesive papilla is found to lie slightly to the right of the medial sagittal plane of the body (fig. A). These asymmetrical features are the result of the pressure of the tail during the period of embryonic development when it is bent forward beneath the chorionic membrane and coiled about the anterior part of the tunic (fig. B). The imprint of the tail in the tunic takes an oblique course from below upward across the left side, and therefore gives to the tunic the form of a screw with a single groove.

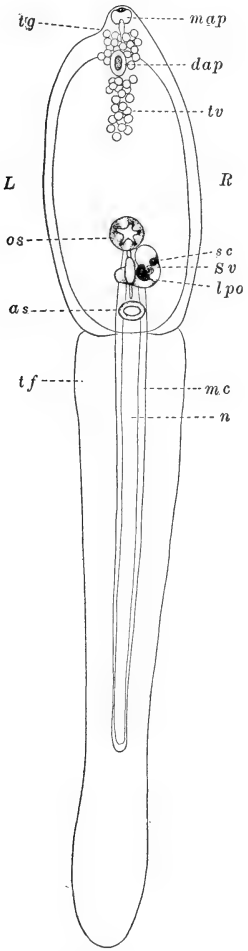


Fig. A Camera outline drawing of the fully developed tadpole larva as seen from the dorsal surface, showing the lateral asymmetry of the body, the horizontal position of the tail fin, and the location of the sense organs in the sensory vesicle. Abbreviations given on page 93.

MANTLE

The mantle (ectoderm) varies in thickness in different regions of the body, but it consists at no point of more than a single layer of cells. The cells of the mantle in general are more or less cubical in form, but are high and columnar in the parts forming the rudiments of the oral and atrial siphons, thin and pavement-like in the region above the sensory vesicle and in the mantle sheath of the tail (figs. 1, C, 3 and 8).

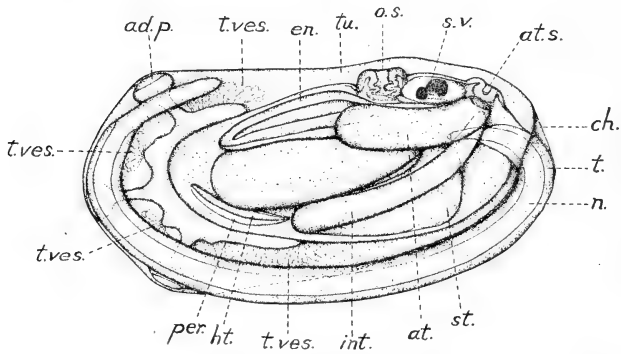


Fig. B A drawing of the embryo within its chorionic membrane, showing the twisted and coiled tail and the four points along the median keel of the body at which the test-clubs grow out from the mantle to form the test vesicles.

ADHESIVE PAPILLAE

The adhesive papillae, of which there are three arranged in a vertical series at the anterior end of the body, are tubular outgrowths of the body wall (fig. 1). Each terminates at the surface of the tunic in an enlarged, goblet-shaped body which opens outwardly and contains a large lens-shaped mass of elongated, richly granular cells probably of mesenchyme origin. The central canal of each papilla is partially filled with mesenchyme cells (fig. 7). Toward the end of the free-swimming period of the tadpole, a contraction of the wall of each papilla takes place, causing the contents of the terminal, cup-shaped enlargement to be extruded upon the surface of the tunic. The viscid

nature of the extruded material is shown by the fact that the tadpole adheres to any foreign body against which it chances to swim, and the most violent movements often fail to release it from such an attachment.

TEST VESICLES

In the fully developed tadpole a large number of blastula-like vesicles occupy a considerable part of the space in the anterior median region of the tunic. They have no organic connection with the body, but lie midway between the mantle and the external surface of the tunic. They are separated by the stalks of the adhesive papillae into four unequal groups (fig. 1). The part of the wall of each test vesicle turned toward the surface of the tunic is composed of cells much larger than those on the side facing the body (fig. 9). The test vesicles maintain this position and orientation during the entire free-swimming period of the tadpole. The function and history of these bodies formed the subject of a paper (Grave, '20b) prepared for the program of the seventeenth annual meeting of the American Society of Zoologists, an abstract of which has been published in the Proceedings of the meeting.

Each test vesicle takes its origin from the mantle wall in the form of a hollow, club-shaped outgrowth or evagination during the late embryonic period. Four clusters of these club-shaped bodies, attached to four median elevations of the body wall, may be seen in immature tadpoles. The dorsal and ventral groups project from keel-like ridges, while the anterior groups are attached to conical papillae situated midway between the bases of the stalks of the adhesive papillae, and each has the appearance of a bouquet or rosette (fig. B). Each club-shaped outgrowth ultimately becomes coverted into a test vesicle, first by the appearance of a constriction near its point of attachment, then at the point of the constriction it separates from the body as a pear-shaped structure which gradually assumes a spherical form and migrates to a position in a zone midway between the body wall and the external surface of the tunic. The test vesicles of Amaroucium probably correspond to the 'bladder

cells' which have been described in other ascidian larvae. In Amaroucium, however, they are not modified cells, but are many-celled bodies derived from the ectoderm. The number of test-vesicles is not constant. As accurately as could be determined, the numbers present in each of eight tadpole larvae are as follows: 62, 52, 55, 52, 53, 60, 58, and 62.

NERVOUS SYSTEM

In the nervous system of the tadpole the following parts may be distinguised: a sensory vesicle, visceral ganglion, and nerve cord which are functional during the brief larval period only; an hypophysial duct, subneural gland, and definitive ganglion which persist to function during the life of the sessile ascidiozooid. The position of these nervous structures in the tadpole and the relations they bear one to another are shown in figures C, D, and 1.

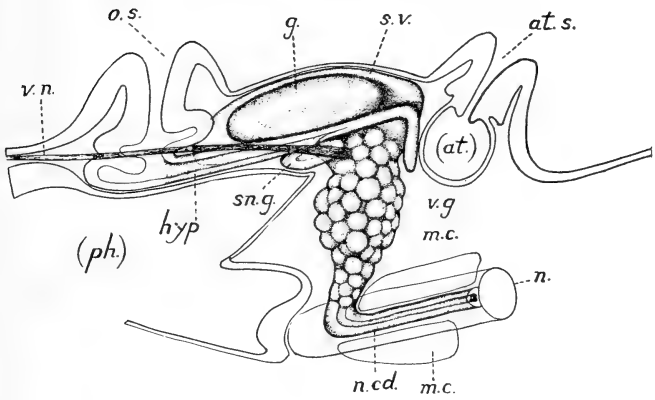
SENSORY VESICLE

The sensory vesicle is situated between the oral and atrial siphons to the right of the median sagittal plane of the body (figs. 1 and 3). It is oval in form and contains a spacious cavity or ventricle filled with a clear liquid. Two sense organs are developed in its wall and project into its central cavity, the eye occupying a considerable portion of the left side and posterior end, the static organ located on its right and ventral sides. Except for the parts which form the sense organs and their ganglia, the wall of the sensory vesicle is thin (figs. D and 5).

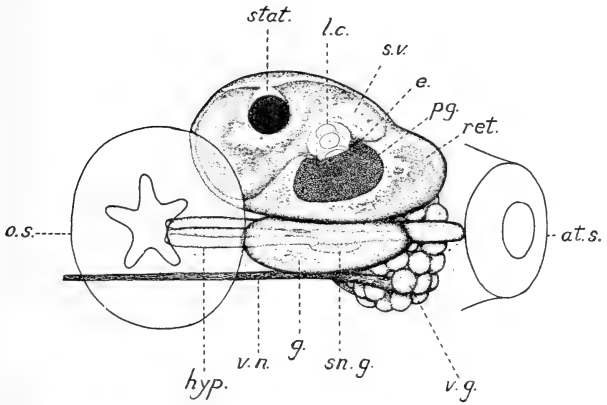
THE EYE

The following parts may be distinguished in the eye; a mass of brownish-black pigment granules arranged in the form of a cup, the mouth of which is directed obliquely upward and forward; three lenses arranged in a linear series in the axis of the pigment cup, and a third part which may be called the retina or retinal ganglion (figs. D, 4, 5, and 6).

A layer of pigment-forming cells in addition to true nerve cells has been described by Salensky ('93) in the developing retina of the embryo of Distaplia, but I have been unable to distinguish



C



D

Figs. C and D Reconstructions of the nervous system from serial sections. Figure C as viewed from the dorsal surface of the larva; figure D as viewed from the left side. Outlines of the siphons are included.

the two types of cells in the eye of either mature or immature tadpoles of Amaroucium, and my observations are therefore in agreement with those of Kowalewsky ('71), who found that the pigment granules lie within the inner ends of the visual cells of the retina. Cell walls are nowhere definite in the nerve tissues of the Amaroucium tadpole, however, and it is possible that the pigment granules are formed in cells distinct from those in which the visual rods are developed. Studies of the eyes of the larvae of other ascidians now in progress may clear up this point.

No migration of pigment granules within the retinal cells was observed in the living tadpole, and sections of the eyes of tadpoles fixed in Flemming's solution after an exposure of thirty minutes in the dark showed no observable difference in the distribution of the pigment granules from that of tadpoles exposed to strong light before similar fixation.

The lenses of the ascidian eye have been described as modified or transformed cells, but in Amaroucium I find they are not modified cells, but are deposition products formed within vacuoles of marginal cells of the retinal ganglion, the nuclei of which, in the embryo, are similar in size and structure to the nuclei of the adjacent nerve cells of the retina (fig. 4). The portion of each lens-forming cell which projects into the cavity of the sensory vesicle is greatly enlarged and contains a large vacuole, in the center of which a transparent spherical droplet of amber-colored substance is deposited. In the earliest stage noted the size of the droplet of lens substance was small. Other stages showed that the lenses gradually increase in volume until they entirely fill the vacuoles. Some cases were noted in which more than one droplet of lens substance were present in the same vacuole, a fact which indicates that the substance is semiliquid in nature, for the droplets presumably flow together to form a single lens. The illustrations which show the lens-forming cells and the lenses (figs. D, 4, 5, 6, and 11) are misleading if they convey the idea that the lenses are lamellated or have a concentric structure. They are homogeneous in appearance both in the living tadpole and in sections. At the center of each lens, how-

ever, one or more granules are usually found. The lenses are easily sectioned and are not crystalline.

The nuclei of the lens-forming cells lose their staining qualities when the lenses have been fully formed and the shrunken cell bodies persist merely as anchors by which the lenses are attached to the superior wall of the sensory vesicle (compare figs. 4, 6, and 11).

The retina consists of a layer of large nerve cells grouped about the pigment cup. In the axis of some and possibly of all of the retinal cells a rod-shaped portion is differentiated which penetrates the pigment zone and ends at the inner surface of the pigment cup. These portions of the retinal cells may be termed the visual rods. They are so placed in the zone of pigment that their long axes coincide with the direction taken by rays of light focused by the lenses into the pigment cup. A visual rod is shown in longitudinal section in figure 11 and four in transverse section are shown in figure 10.

An optic nerve, such as that described by Salensky ('93) in the larva of Distaplia, connecting the retinal portion of the sensory vesicle with the visceral ganglion, I have failed to find, but, as these two parts of the central nervous system of the Amaroucium tadpole are practically in contact, the visceral ganglion probably receives the retinal fibers directly.

The eye of the ascidian tadpole is a true direct brain eye and, as has been pointed out first by Goette ('75) and later by Salensky ('93), Willey ('94), and others, it is similar in its structure and organization to that of the pineal or parietal eye of cyclostomes and lizards. McBride ('14), on the other hand, possibly with the observations of Lahille in mind, commits himself to the view that the eye of the ascidian larva is homologous with one of the paired lateral eyes of vertebrates. Lahille ('90) described what he interpreted to be the remains of an atrophied eye belonging to the right side of the sensory vesicle of the larva of Distaplia, but Salensky ('93) and others, working with the same larva, have failed to find any trace of the rudimentary structure described by Lahille.

Although the structure and organization of the eye are perhaps sufficient to support the interpretation that the eye is the organ by which the tadpole orients with reference to rays of light, it may be worth while to state the physiological evidence, secured since the publication of the paper on the activities and reactions of the tadpole larva (Grave, '20), which shows conclusively that the eye is a functional light-perceiving organ.

During the latter part of their free-swimming period, tadpoles cease to swim continuously, and intervals of rest, when they lie quiescent upon one side, become longer and longer. While examining a tadpole during one of its resting periods with the microscope, the mirror was so turned as to cut off the transmitted light. Immediately the light was cut off the tail began to vibrate. Repeated experiments of the same kind with light reflected from the mirror, alternately turning it off and on, showed that when the tadpole was so lying that light from the mirror entered the pigment cup (on its right side), the tail almost invariably began to vibrate at the instant the light was turned off and in no case when the light was turned on. The actual stimulus to muscular contraction is not transmitted from the eye to the muscle bands of the tail during the illumination of the visual rods of the retina, but immediately after the pigment cup is darkened, following its illumination. This takes place in the course of normal locomotion at the moment in each revolution of the body on its axis when the pigment cup is carried to a position in which rays of light no longer enter its cavity.

It has been noted from the beginning of the investigation that the shadow of the hand when passed over resting tadpoles almost invariably causes immediate renewal of locomotor activity. The observations just described, which show that the eye is a functional light-perceiving organ, incidentally explain this shadow reaction.

THE STATIC ORGAN

The static organ consists of a single sensory cell, at the distal end of which is borne a relatively large, subspherical, black statolith, and a small number of large nerve cells which form

a thickened ganglionic portion in the right lateral and ventral walls of the sensory vesicle.

The statolith-bearing cell projects for its entire diameter into the cavity of the sensory vesicle, and is therefore a pendent structure. In the living tadpole the statolith appears to be contained in a cup-shaped depression at the distal end of the cell, but sections show that it lies wholly within a vacuole-like cavity of the statolith cell and is surrounded by a delicate layer of cytoplasm (figs. D, 5, and 6). The statolith is composed of a substance that is not disintegrated by strong acids and is not bleached by chlorine. It is very hard and, when struck by the edge of the section razor, is usually torn from its base and dragged through the tissues.

Tadpoles with two statolith cells are occasionally found, but they are rare.

The part of the sensory vesicle formed by the ganglionic portion of the static organ is so located that it comes into contact, at its most ventroposterior end, with the side of the visceral ganglion, and nerve fibers probably pass from the former to the latter at this point, but no 'acustic' nerve, such as has been described by Salensky ('93) to connect the 'gehör organ' with the visceral ganglion in the larva of Distaplia, is present in the tadpole of Amaroucium.

VISCERAL GANGLION

The vertically situated part of the larval nervous system which connects the sensory vesicle with the nerve cord has been called the visceral ganglion. A cortex made up of a single layer of large nerve cells and a longitudinally striated medullary portion may be distinguished in it, but no trace of a neural canal can be found either in longitudinal or transverse sections (figs. C, D, 3, and 10).

From a point on the left side of the ganglion, located just below the level of the hypophysial duct, a comparatively large bundle of nerve fibers emerges as a nerve trunk and can be traced obliquely upward and forward to the region above the endostyle a short distance anterior to the oral siphon where it apparently

ends, possibly having a distribution to muscle fibers which are in this region rather richly developed. The origin and course of this nerve are shown in figures C, D, and 3. Its function during the free-swimming period of the larva is not evident and, on account of the rigidity and immobility of the body, is difficult to conceive. Non-striated muscle fibers are present in considerable number in the mesenchyme layer just beneath the body wall in the region into which the nerve can be traced. These muscle fibers take a general course from the region of the endostyle obliquely forward to the ventral side of the body. Slow writhing contractions of the entire body are very evident at the close of the free-swimming period when metamorphic changes have set in, and it is possible the neuromuscular apparatus under consideration first comes into function at this time.

Salensky's conception of the visceral ganglion as the reflex center or brain of the larva seems to be substantiated by physiological as well as by structural data. My observations on the reactions of the Amaroucium tadpole (Grave, '20 b) indicate that the tadpole orients with reference both to light, by means of reflexes originating in the eye, and to gravity, by means of reflexes originating in the static organ. It was found that the normal response to gravity during the latter part of the free-swimming period was greatly modified in the presence of unusual stimulation by light. The visceral ganglion must be the coordinating center for these diverse reflexes.

THE NERVE CORD

Due to a permanent twist of the tail 90° to the left, the nerve cord occupies a position on the left side of the notocord in the space between the projecting edges of the dorsal and ventral muscle bands (figs. C, 3, 8, and 10). Near the anterior end of the notocord the nerve cord bends abruptly upward and slightly to the right to join the ventral end of the visceral ganglion. A definite neural canal is present throughout its length. Small nuclei are present here and there in the thin wall of the cord, but none were found that have the characteristics of nerve cells (figs. 3, 8, and 10). The cord tapers toward the end of the tail,

but it is coextensive with the muscle bands. It no doubt constitutes the pathway for nerve fibers from the visceral ganglion to the muscle cells, but the endings of fibers in muscle cells could not be made out.

DEFINITIVE GANGLION, HYPOPHYSIAL DUCT, AND SUBNEURAL GLAND

The parts of the nervous system described in the foregoing paragraphs are those which function during the free-swimming period of the tadpole and degenerate when the larval period is over. The parts that persist and become the functional nervous system of the sessile ascidiozooid are the hypophysial duct, definitive ganglion and subneural gland. As shown in figure D, they form a vertical series of structures situated immediately to the left of the sensory vesicle in the median sagittal plane of the body.

These structures in their fully differentiated condition in the adult ascidiozooid have been studied by Metcalf ('00). A comparison of his figure 47 with figure D of this paper shows that the entire central nervous sytem of the adult Amaroucium ascidiozooid is fully formed in the larva, and thus shows clearly the relation the larval nervous structures bear to those which persist in the adult.

The hypophysial duct is hollow and its canal is lined with cilia for about two-thirds of its length (figs. C, D, and 1). At its anterior end it is continuous with the wall of the oral siphon and the cavities of these structures are in open communication. As the ectodermal oral siphon at this stage is in no way connected with the endodermal pharynx, there can be no doubt, in the case of Amaroucium, of the primary connection of the hypophysial duct with the ectodermal, and not with the endodermal part of the alimentary tract—a fact of considerable significance for the old controversial question of the homology of the hypophysial duct of ascidians with the hypophysis of vertebrates. The posterior end of the hypophysial duct terminates blindly in the region of the atriopore between the lateral horns of the atrium. The part of the duct which lies posterior to the subneural gland corresponds to the rapheal duct of the ascidiozooid.

The definitive ganglion, so called because of its persistence as the nerve center of the ascidiozooid, lies immediately above the middle portion of the hypophysial duct (fig. 6). These structures are in close contact, but are not at this stage connected. The ganglion is oval in form and is composed of a cortex of cells and a medulla in which no nuclei are found (figs. 3 and 6). The nuclei of the cortex are small and do not have the structure chacteristic of nerve cells, possibly because their functional activity does not begin during the larval period.

The subneural gland has the appearance of an enlargement or outgrowth of the middle portion of the hypophysial duct on the side opposite the definitive ganglion. It is a hollow structure and its cavity is in open communication with the lumen of the hypophysial duct (figs. C and D).

THE MIDDLE GERM LAYER

Mesenchyme cells form a discontinuous layer just beneath the body wall. They are found very infrequently in the posterior part of the body, especially in the region of the sensory vesicle, but in the anterior part they are quite numerous and, in the parts from which the test vesicle and adhesive papillae have developed, they form a continuous layer more than one cell in thickness (fig. 9). Their distribution in the middle portion of the body is shown in figure 3. At no place could they be said to form an epithelium, and nothing comparable to the mesoderm or coelom as they are developed in vertebrates is present.

Mesenchyme cells of at least three varieties can be distinguished; one in which the cytoplasm is apparently homogeneous is the most common, but another in which the cell bodies are loaded with granules is not infrequent. The third variety is associated with non-striated muscle fibers, of which some encircle the body obliquely from the dorsal to the ventral side and are located just beneath the mantle, some are distributed along the walls of the pharynx and atrium, and quite numerous sets of fibers extend in a radial direction from the oral and atrial siphons as centers.

TAIL MUSCLES

Two muscle bands, each consisting of about eighty very large, polygonal muscle cells arranged in four longitudinal rows of twenty cells each, form the dorsal and ventral portions of a relatively thick envelope for the notocord (figs. 2 and 8). A single layer of cross-striated contractile fibrillae are differentiated in the cortical layer of each muscle cell. The fibrillae take a general longitudinal course, but are inclined about 18° to the right of the longitudinal axis of the tail. The fibrillae of adjacent muscle cells join end to end and thus convert the entire series of muscle cells of each muscle band into a single muscle. A further indication that the muscle band, rather than the individual muscle cell, is the morphological as well as the physiological unit, is afforded by the fact that the alternate light and dark segments of the fibrillae are so placed that they form continuous straight transverse rows or lines across the muscle bands, which are not in any way interrupted or interfered with by the muscle cell walls. Each muscle band functions as a unit in a way that indicates that its origin is located at the anterior end of the notocord, its insertion at the posterior end. With each muscular contraction the tail makes a propeller-blade-like stroke, due to the oblique or spiral course of the contractile fibrillae in the muscle bands, and the body of the tadpole is thus made to rotate clockwise during locomotion.

The central portion of each muscle cell contains a nucleus and cytoplasm in which vacuoles, pigment granules, and larger spherical inclusions are usually found (figs. 2 and 8).

ALIMENTARY TRACT

The pharynx, which at this stage is not organically connected with the oral siphon, occupies a large portion of the median dorsal part of the body (fig. 1). In the part of its dorsal wall situated immediately in front of the oral siphon, the endostyle is conspicuously differentiated as a double longitudinal fold. A deep groove is included between the folds, at the bottom of which a tract of long cilia is developed (figs. 1 and 2). The ventral wall of the pharynx is intimately associated, especially at its

anterior end, with a comparatively large cone-shaped mass of yolk material, the yolk granules and masses seeming to be included within enormously developed endodermal cells (figs. 1 and 2). The lateral walls of the posterior portion of the pharynx are in contact with the inner walls of the right and left horns of the atrium and a series of open communications between the pharyngeal and atrial cavities has been formed by the differentiation of three horizontal rows of ciliated gill openings; the first or dorsal row consisting of seven small openings, the middle row of six somewhat larger ones, and the ventral row of five comparatively large gill openings (figs. 1 and 3).

The part of the alimentary canal in which oesophagus, stomach, and intestine are differentiated communicates with the pharynx at a point near the posterior end of the yolk mass and slightly to the right of the longitudinal axis of the body. The stomach and intestine have the form of a horizontal loop in the midventral region of the body beneath the yolk mass. The rectal portion of the intestine pierces the left horn of the atrium and the anus is found near the base of the atrial siphon (fig. 1). It is perhaps needless to state that the alimentary tract is not functional during the larval period.

NOTOCORD AND ENDODERMAL STRAND

The notocord occupies the proximal two-thirds of the axis of the tail and penetrates the body to a point directly below the middle portion of the sensory vesicle, where it ends in contact with a thickened portion of the pharynx just behind the pointed posterior end of the yolk mass (figs. A, C, and 1). It retains no trace of its cellular origin, but at this stage is made up of a thin cortical sheath of dense material in which granules and larger spherical bodies are embedded, and a central medullary core composed of non-staining substance, at the periphery of which a tracery of delicate strands can be made out (figs. 3, 8, and 10).

A longitudinal linear series of cells, known as the 'endodermal strand,' occupies a portion of the space on the right side of the notocord between the overhanging edges of the muscle bands. One or sometimes two 'strand' cells can usually be made out in

transverse sections of the tail, but none have been observed along the part of the notocord that lies within the body (figs. 2, 3, and 8).

PERICARDIAL SAC AND HEART

The pericardial sac, a thin-walled oval structure containing a spacious cavity, is situated in the anterior ventral part of the body beneath the anterior portion of the yolk mass. A shallow but wide invagination of the dorsal wall, which extends the entire length of the pericardial sac, shows the process by which the heart is differentiated (figs. 1 and 2). The formation of the pericardial sac of ascidians has been described as an outgrowth from the embryonic pharynx, but any connection that may have existed at an earlier stage between these structures in the Amaroucium embryo has disappeared in the fully formed tadpole larva.

THE ATRIUM

The atrium is an extensive, U-shaped, thin-walled structure, the lateral horns of which enfold the posterior portion of the pharynx (figs. 1 and 3). As has been stated in the section on the pharynx, the cavities of the atrium and pharynx are connected by means of three rows of gill openings. The middle portion of the atrium is joined to the atrial siphon and the atrial cavity is in open communication with the atrial canal.

SUMMARY

The points that have been added to the morphology and natural history of ascidian larvae as a result of this study of Amaroucium constellatum may be stated as follows:

1. A periodicity in the escape of larvae from the parent colony was observed. Large colonies can be depended upon to liberate tadpole larvae in swarms at or near sunrise, but an occasional larva escapes during other hours of the day.

2. Multicellular, blastula-like test vesicles, about sixty in number, are evaginated from the mantle during a late embryonic stage and lie isolated from the body in the substance of the tunic during the entire free-swimming period of the larva.

3. The lenses of the eye are not modified retinal cells, but are deposition products of a semiliquid or gelatinous nature, formed within vacuoles of cells located in the margin of the retinal ganglion.

4. Visual rods, differentiated in retinal cells of the eye, project through the pigment zone and end at the inner surface of the pigment cup.

5. The eye is proved, by structural and physiological evidence, to function in the orienting responses of the tadpole to light.

6. The statolith is formed in a vacuole of the sensory statolith-cell and is composed of a hard substance that is not disintegrated by strong acids.

7. A visceral nerve, which originates in the visceral ganglion and has a distribution in the region of the endostyle, is described.

8. The nerve cord is not dorsal in position, but, due to a permanent twist of the tail 90° to the left, is located on the left side of the notocord.

9. The two series of muscle cells located on the dorsal and ventral sides of the notocord are shown by morphological and physiological evidence to function as units in a way to produce the rotatory movement of the body during locomotion.

BIBLIOGRAPHY

DAMAS, D. 1904 Contribution à l'étude des Tuniciers. Archiv. de Biol., T. 20.

GRAVE CASWELL 1920 a The origin, function and fate of the test vesicles of Amaroucium constellatum. (Abstract.) Anat. Rec., vol. 17.

1920 b Amaroucium pellucidum form constellatum. I. The activities and reactions of the tadpole larva. Jour. Exp. Zoöl., vol. 30.

GOETTE, A. 1875 Die Entwickelungsgeschichte der Unke als Grundlage einer vergleichenden Morphologie der Wirbeltiere. Leipzig.

KOWALEVSKY, A. 1866 Entwickelung der einfachen Ascidien. Mem. Acad. Sc. Saint-Petersb., ser. 7, vol. 10.

1871 Weitere Studien ueber die Entwickelung der einfachen Ascidien. Arch. mikr. Anat., Bd. 7.

LAHILLE, F. 1890 Recherches sur les Tuniciers des cotes de France. Toulouse.

MCBRIDE, E. W. 1914 Text-book of embryology, Invertebrata, vol. 1. MacMillan & Co.

RITTER, W. E. 1909 Halocynthia Johnsoni. A comprehensive inquiry as to the extent of law and order that prevails in a single animal species. Univ. Cal. Pub., vol. 6.

SALENSKY, W. 1893 Morphologische Studien an Tunicaten: ueber das Nervensystem der Larven und Embryonen von Distaplia magnilarva. Morph. Jahrb., Bd. 20.

SEELIGER, O. 1885 Dik Entwickelungsgeschichte der socialen Ascidien. Jen. Zeitschr. f. Naturw., Bd. 11.

WILLEY, ARTHUR 1894 Amphioxus and the ancestry of the vertebrates. MacMillan & Co.

EXPLANATIONS OF PLATES

ABBREVIATIONS

ad.p., adhesive papilla
at., atrium
at.s., atrial siphon
ch., chorion
e, eye
en., endostyle
end. sd., endodermal strand
g., definitive ganglion
g.o., gill opening
ht., heart
hyp., hypophysial duct
int., intestine
k., keel
l., lens
l.c., lens cell
m., mantle
mc., muscle cell
m.f., muscle fibrilla
mes., mesenchyme
n, notocord
n.c., neural canal
n.cd., nerve cord
o.s. oral siphon
per, pericardium
ph., pharynx
pg, pigment cup
r., rectum
r.c., retinal cell
ret, retina
sn. g., subneural gland
st., stomach
stat., static organ
s.v., sensory vesicle
t., tail
t.c., test cell
t.f., tail fin
tu., tunic
t.ves., test vesicle
v.g., visceral ganglion
v.n., visceral nerve
vis.rd, visual rod
y., yolk mass

With the exception of figure 1, which was drawn by Miss Besse E. Stocking, the drawings have been made by the writer of the paper, camera lucida outlines forming the basis for each. Figures 2 and 4 illustrate structures of the embryo, all others are of the free-swimming tadpole larva of Amaroucium constellatum. Text figure A taken from a paper by the author published in The Journal of Experimental Zoölogy, volume 30, number 2, February 20, 1920, page 243.

ABBREVIATIONS TEXT FIGURE A

as, atrial siphon
dap, dorsal adhesive papilla
L, left side
lpo, light perceiving organ
map, middle adhesive papilla
Mc, muscle-cell sheath
n, notocord
os, oral siphon
R, right side
sc, statolith cell
sv, sensory vesicle
tf, tail fin
tg, groove in test resulting from pressure of the tail during embryonic development
tv, test vesicles

PLATE 1

EXPLANATION OF FIGURE

1 The body of the tadpole larva viewed from the left as a transparent object, showing its structure as reconstructed from serial sections.

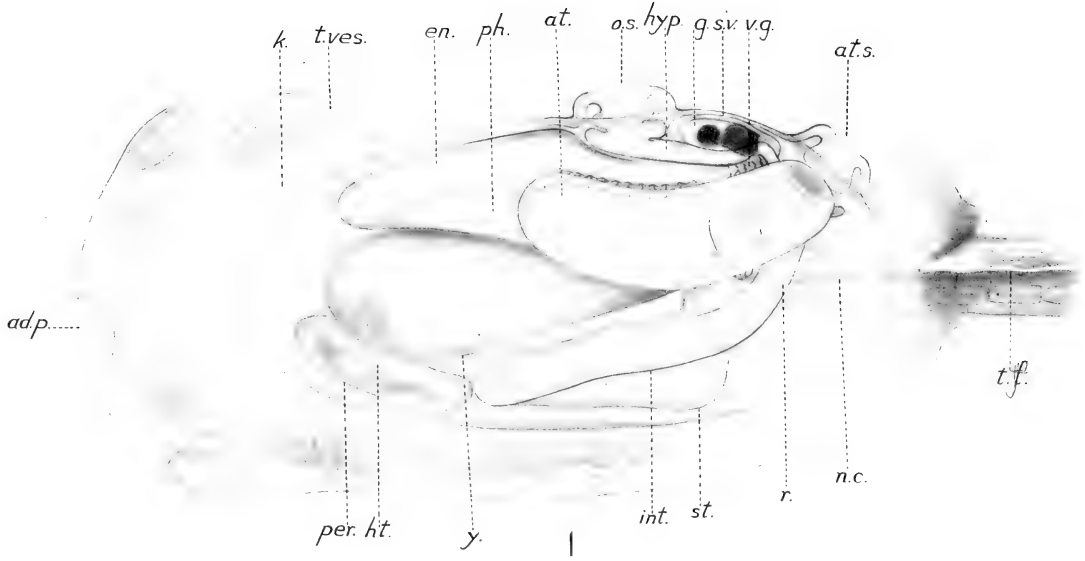
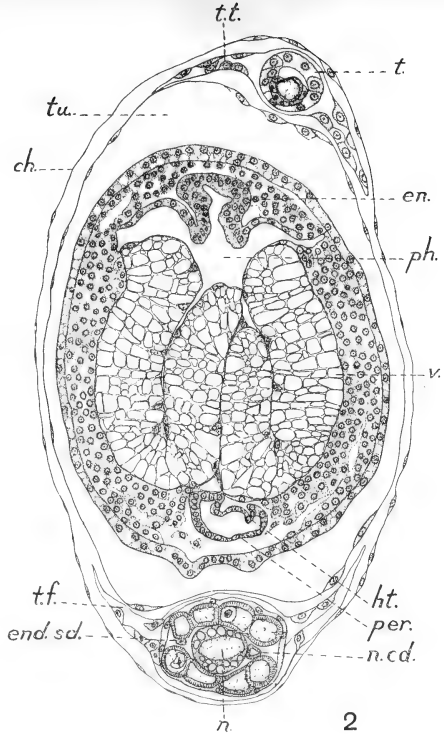

k.
t.ves.
en.
ph.
at.
o.s.
hyp
g.
s.v.
v.g.
at.s.
ad.p.
t.f.
n.c.
r.
int.
st.
per.
hit.
y.

PLATE 2

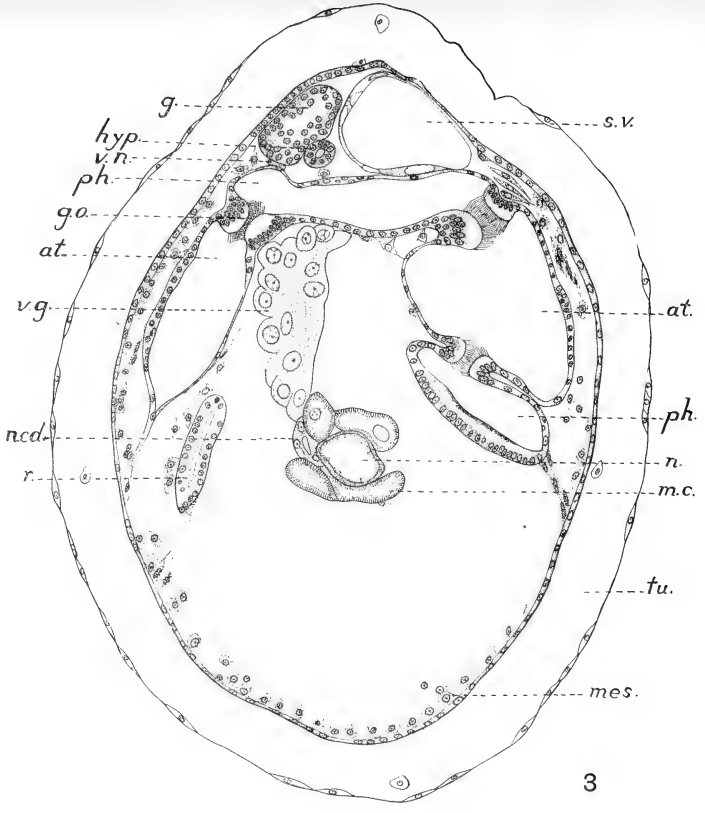
EXPLANATION OF FIGURES

2 A transverse section through the region of the heart of a developing embryo. The twisted position of the tail, the tail-fin formed of tunic material flattened between the chorionic membrane and the body of the embryo and the characteristic structure and position of the yolk mass are shown.

3 A transverse section of the tadpole through the region in which visceral ganglion and nerve cord join. It is reproduced to show especially the position and distribution of mesenchyme cells, the gill openings connecting the pharyngeal and atrial cavities, and the size and position of the visceral nerve.


t.t.
t.
tu.
ch.
en.
ph.
v.
t.f.
ht.
per.
end. sd.
n.cd.
n.

2


g.
s.v.
hyp.
v.n.
ph.
g.o.
at.
v.g.
at.
ph.
n.cd.
n.
r.
m.c.
tu.
mes.

3

PLATE 3

EXPLANATION OF FIGURES

4 A transverse section through the sensory vesicle, hypophysial duct, definitive ganglion, and subneural gland of an embryo. It is reproduced to show especially the developing lenses within vacuoles of lens cells, the similarity of lens cells and nerve cells of the retina, and the relation of the pigment granules of the pigment cup to the bodies of the retinal cells.

5 A drawing of the sensory vesicle and its sense organs as viewed from the dorsal side of a living tadpole.

6 A slightly oblique transverse section through the nervous system in the plane of the statolith cell, the latter displaced slightly by the knife. The entire series of lenses and their position in the axis of the pigment cup are shown.

7 A sagittal section of the middle adhesive papilla, showing its tubular structure and its content of mesenchyme cells.

8 A transverse section of the tail, showing the horizontal fins; the structure of the notocord; the muscle cells, four on the dorsal and four on the ventral side of the notocord; the cut ends of muscle fibrillae in the cortical layer of the muscle cells; the hollow nerve cord on the left side of the notocord in the space between the edges of the muscle bands; the endodermal strand in the corresponding position on the right, and the mantle sheath closely applied to the muscle band beneath the tunic.

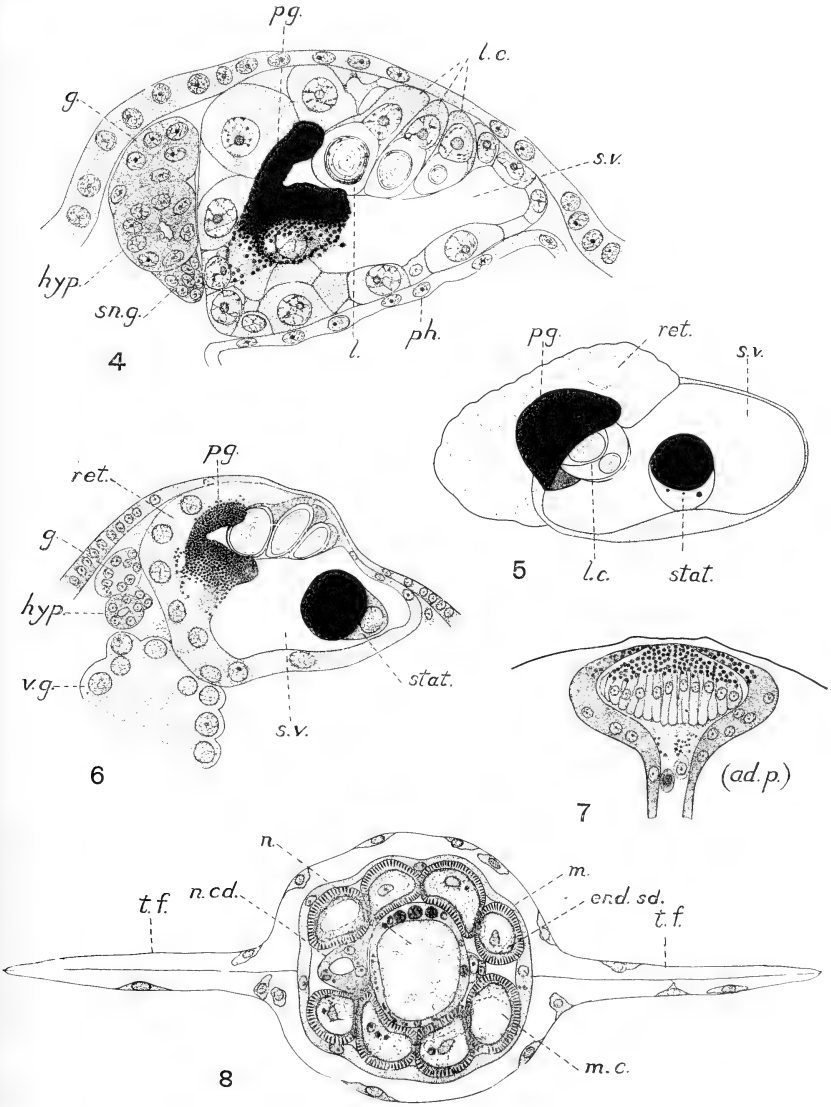

pg.
l.c.
g.
s.v.
hyp.
sn.g.
l.
ph.
4
pg.
ret.
s.v.
5
l.c.
stat.
ret.
pg.
g.
hyp.
v.g.
stat.
s.v.
6
(ad.p.)
7
n.
m.
t.f.
n.cd.
end. sd.
t.f.
8
m.c.

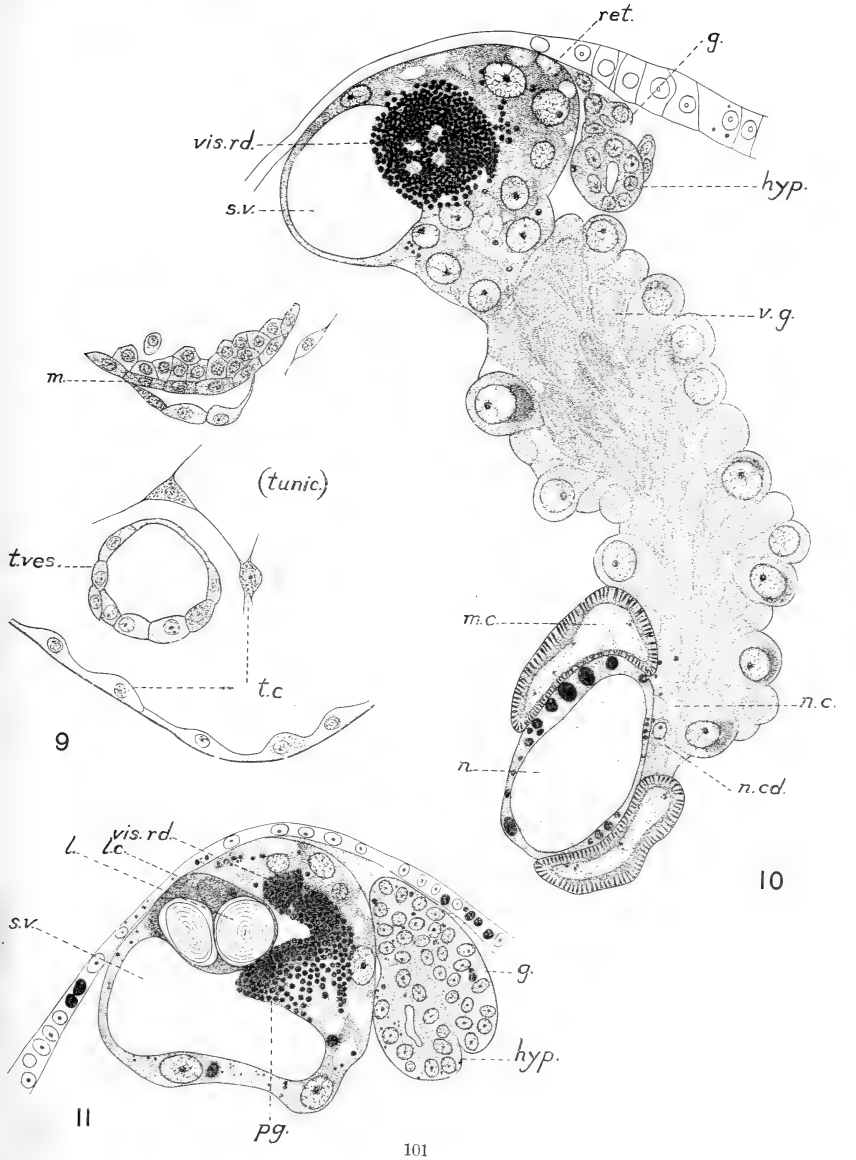
PLATE 4

EXPLANATION OF FIGURES

9 A median section of a test vesicle showing its structure and its position in the substance of the tunic. Test cells are shown in various positions, some located near the surface of the mantle, some above and to the right of the test vesicle, and others in the tunic epithelium. Some contain pigment granules, some are without granules.

10 A portion of a transverse section through the posterior part of the eye. It is reproduced to show especially the four visual rods which are cut transversely in the tangential section of the pigment cup and the extent and structures of the visceral ganglion.

11 A portion of a transverse section in which a visual rod of one of the retina cells is cut lengthwise. Two only of the series of lenses are in the plane of the section.


ret.
g.
vis.rd.
hyp.
s.v.
v.g.
m.
(tunic.)
tves.
t.c
9
m.c.
n.c.
n.
n.cd.
10
l.
lc.
vis.rd.
s.v.
g.
hyp.
11
pg.

Resumen por el autor, Frank Helvestine.

Amitosis en las células ciliadas de los filamentos branquiales de Cyclas.

En los filamentos branquiales de Cyclas las células no ciliadas del epitelio basal producen las células ciliadas del epitelio lateral, latero-frontal y frontal. La mitosis tiene lugar en las células del epitelio basal y en las células no ciliadas del esófago. La amitosis es el método exclusivo de proliferación de las células ciliadas de los filamentos branquiales. Mediante division amitósica la célula madre del epitelio latero-frontal produce células transicionales de las cuales nacen células que reemplazan las células caducas del epitelio frontal. En las células ciliadas de Cyclas no puede demostrarse la existencia de un centrosoma, y entre las mitocondrias y las pestañas vibrátiles no puede discernirse relación genética alguna. Las pruebas indirectas indican que los cuerpos basales de las células son derivados del centrosoma.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, NOVEMBER 7

AMITOSIS IN THE CILIATED CELLS OF THE GILL FILAMENTS OF CYCLAS

FRANK HELVESTINE, JR.

Department of Histology and Embryology, University of Virginia

TWO PLATES (SEVEN FIGURES)

INTRODUCTION

The purpose of this investigation is further to test the validity of the hypothesis first formulated by v. Lenhossék and by Henneguy ('98) that the basal bodies found in ciliated cells are derived from the centrosome, and particularly the corollary of this hypothesis, as expressed by Jordan ('13), that ciliated cells, in consequence of the loss of their centrosome through partition in the formation of basal bodies, must necessarily proliferate by amitosis.

Jordan ('13) supports the hypothesis as regards especially the ciliated epithelium of the ductuli efferentes of the white mouse, and shows for the first time that the cells in this region multiply exclusively by direct division. In no case was he able to demonstrate a centrosome or a mitotic figure.

Saguchi ('17) confirms the observation of exclusively amitotic division in ciliated cells of vertebrates, but he does not agree with the view held by Jordan that these cells divide amitotically because the centrosome has been used up in the formation of the basal granules. He concludes that there is no genetic relation between basal bodies and the centrosome. He states also that the basal granules are formed by differentiation from mitochondria, both in vertebrates and in invertebrates. He further declares that in invertebrates ciliated cells multiply by mitosis exclusively. It may be stated at once that Saguchi's descriptions and illustrations do not adequately support his conclusions.

In my investigation the chief interest centers about the questions: 1, whether ciliated cells in invertebrates divide only by mitosis; 2, the significance of amitosis in ciliated cells; 3, the relation of the basal granules to the centrosome, and, 4, the relation of the basal granules to mitochondria.

The material I have employed in this investigation is the ciliated epithelium of the gill filaments of the fresh-water mussel, Cyclas. I find that in this invertebrate the ciliated cells divide by amitosis exclusively, and no direct relation between mitochondria and ciliogenesis is discernible.

MATERIAL AND METHODS

The material for this study was suggested to me by Prof. William A. Kepner. The mussels were shelled and then fixed in various solutions, of which Meves' was found to be by far the most satisfactory. Paraffin sections were cut at 5μ, and, after mounting, were stained with Heidenhain's iron-hematoxylin. This technic gave results little short of perfect.

DESCRIPTION

For a histologic description of the epithelium of the gill filaments of Cyclas, I shall divide the epithelium into four regions according to its position. I distinguish: *a*, basal epithelium, or the epithelium lying at the base and between adjacent filaments; *b*, lateral epithelium; *c*, laterofrontal epithelium; and, *d*, frontal epithelium.

a. Basal epithelium. The epithelium of this region is nonciliated (fig. 1, *A*). The cells on account of their position are more or less pyramidal. They contain one or two vesicular nuclei. Among these cells mitotic figures are occasionally seen (fig. 3, *M*). The cytoplasm contains many filar and bacillary mitochondria collected for the most part toward the periphery of the cells. From the cells of this region which lie adjacent to the gill filaments, ciliated cells are formed which are pushed up onto the filament, giving rise to the lateral epithelium (fig. 5, *L*).

b. Lateral epithelium. Here the cells are for the most part ciliated. The lateral epithelium of a gill filament at any point may be composed, on one or both sides, of a single row of tall cuboidal cells (fig. 4, *L*) or of several rows of columnar cells (fig. 7, *L*). (The illustrations are of transverse sections of the gill filaments. In longitudinal sections of the filament the several types of cells occur in long rows.)

The cilia of these lateral cells are long and heavy, and are attached to a single row of basal granules lying under the striated border (fig. 1, L, 4, *L*, 5, *L*, 7, *L*). The nuclei are vesicular in character. Amitosis can occasionally be seen in these cells (figs. 1, *L*, 4, *L*, 5, *L*, 7, *L*). This process begins with the indentation of the nuclear membrane (fig. 4, *L*), which indentation deepens until two nuclei are formed (figs. 1, *L*, and 5, *L*). The nuclei separate, after which there follows a cytoplasmic division (fig. 7, *L*). The cytoplasm stains only lightly and contains filar and bacillary mitochondria, most of which are distal to the nucleus. No centrosome can be detected in a single instance. Between these cells and the laterofrontal epithelium several small non-ciliated cells may (fig. 1, *I*) or may not (fig. 4) be interposed.

c. Laterofrontal epithelium. The laterofrontal epithelium is derived from the lateral cells and replaces the worn-out cells of the frontal region. The parent cell or cells of this region comply with the same description as the cells of the lateral epithelium; they are in fact identical with these cells, but in a different position. The nuclei, however, soon enlarge and become hyperchromatic (fig. 1, *P*). The first indication of nuclear division is the indentation of the nuclear membrane (fig. 4, *N*). The nucleus undergoes a multiple division, and from one nucleus, by amitosis, a number of daughter nuclei are formed (figs. 5, *N*, 7, *H*). Coincident with nuclear division there occurs a distinct pairing of the cilia and basal bodies (fig. 5, *N*, 7, *H*). Cytoplasmic division follows, and thus is formed a new type of cell, a columnar cell with a deeply staining, narrow, elongated nucleus (fig. 6, *T*). The cytoplasm contains filamentous and bacillary mitochondria. Each cell possesses four paired flagella-like cilia

and four relatively large basal granules (fig. 6, *T*). The amitotic division involves two vertical cell-planes at right angles to each other. Whether this portion of the epithelium be viewed in sections cut through the gill filament vertically, as in the illustration, or in a plane at right angles to this, that is, in a plane that cuts the filament longitudinally, these cells, thus cut longitudinally, present in either case two basal bodies and two cilia. This condition demonstrates that these daughter cells of the amitotically dividing mother cell are of columnar shape and possess a double pair (that is, four) of basal bodies and cilia. Amitotic division in the laterofrontal epithelium is not always in the same plane (fig. 5, *N*), nor are the daughter nuclei of uniform size (fig. 7, *H*). Neither does the development of the cells of the lateral and laterofrontal epithelium always take place in the order that I have described. In some cases the primitive cell of the lateral epithelium undergoes amitosis before reaching the laterofrontal position (fig. 6, *T*), and the columnar cells which form a transition between the laterofrontal and the frontal epithelium may be both in the lateral and laterofrontal position (fig. 6, *T*).

d. Frontal epithelium. The frontal epithelium is derived from the transitional cells of the laterofrontal epithelium. These transitional cells as they are needed are pushed into the frontal position. Here a transformation occurs. First, there is a partition of the basal granules and a splitting of the cilia, so that the cell, instead of having two pairs of cilia and two pairs of basal granules, now has a tuft of cilia and many basal granules (fig. 5, *R*). The cilia of these cells are originally long, but due to their exposed position on the crest of the filament, where they are constantly in contact with grit and other abrasive materials, the cilia are broken off and give the appearance of a brush-like border (figs. 4, *A*, 5, *A*, 7, *A*). The nuclei of the frontal cells that are adjacent to the laterofrontal region are of the same character as the nuclei of the latter region (fig. 5, *R*). As the cells are moved crestward the nuclei become for a time more vesicular (fig. 5, *A*). When the cells of the frontal epithelium become injured or worn out the nucleus of the cell becomes

pyknotic (figs. 5, *D*, 6, *C*) and finally disintegrates by karyorrhexis. Such necrotic material is resorbed basally, and new cells are pushed over and into place of the degenerated ones.

In the ciliated epithelium of the alimentary tract of Cyclas I find that cellular proliferation takes place by mitosis (fig. 2). The epithelium of this region is of the tall columnar variety. The nuclei of these cells are situated proximally and are pale staining. Each cell has a tuft of moderately long cilia extending from its distal border (fig. 2). The cilia are attached to a double row of basal granules, and from the innermost row of granules a cone of rootlets extends down into the cytoplasm, the apex of the cone falling on one side of the nucleus. The cytoplasm of these cells contains some filar and bacillary mitochondria. Centrosomes could not be detected in the cells with cilia. Mitosis in these cells takes place only before cilia have appeared, or after they have disappeared.

REVIEW OF LITERATURE

In 1877 Peck[5] published a description of the lamellibranch gill, which has formed the basis for all subsequent text book accounts. In this comparative investigation Peck devoted special attention to the histologic details of the gill epithelium in Anodonta. He distinguishes in this form "frontal, latero-frontal, and lateral epithelium." He described all cells as being ciliated. "Those of the latero-frontal rows (a single row on each side of the frontal epithelium) have the longest cilia, far outreaching those of the other cells; the frontal epithelium and the more forward cells of the lateral epithelium come next with finer and much shorter cilia, and lastly, the inlying lateral epithelium has but very short cilia." He calls especial attention to the cells of the laterofrontal epithelium, and describes these cells as being larger than their neighboring cells, and appearing from the surface like goblet cells with a single coarse flagellum issuing from them, while if seen in a transverse section of a filament these cells appear broad and a little flattened, the single flagellum proving to be an adhering group of long cilia. The nucleus of these cells he described as large and clear, and as enveloped by

only a narrow layer of cytoplasm. In regard to this epithelium, Peck gives no further details.

Henneguy[2] and v. Lenhossék,[4] working independently, expressed at about the same time (1898) their opinion regarding the origin of cilia, and especially the basal granules. The Lenhossék-Henneguy hypothesis states that the basal bodies of ciliated cells are identical with the centrosome, that is, derived from it, and it is based on, 1) a series of histologic analogies between the basal bodies and the centrosome and, 2) histologic details that seem significant.

A comparison between the centrosome and the basal corpuscles brings out the following facts: *a*) both bodies have the same form; *b*) they stain alike and with the same intensity; *c*) in unstained preparations the basal bodies refract light to the same degree as does the centrosome and, *d*) the position of the centrosome in certain non-ciliated cells corresponds to the position of the basal corpuscles in adjacent ciliated cells. The evidence furnished by certain workers* seems to lend weight to the hypothesis: *a*) ciliated cells have no centrosome; *b*) certain ciliated cells do not divide by mitosis, and, *c*) ciliated cells resemble the spermatozoon, the flagellum of which is derived from the centrosome.

In 1913 Jordan[3] showed that amitosis is the exclusive method of division in the ciliated cells of the vasa efferentia of the white mouse. That amitosis is the general mode of division in ciliated cells was supported by observations on the epididymis of the rat, horse, bull, mule, rabbit, and dog and the trachea of the cat and the ciliated cells of the gill of Unio. On the basis of his results Jordan suggested that the fundamental cause of amitotic division in the ciliated cells is the destruction of the centrosome in the formation of basal bodies from which the cilia develop.

Saguchi[6] more recently ('17) has made an extensive comparative study of ciliated epithelium from various regions in a number of both invertebrate and vertebrate forms. He claims to be able to detect centrosomes in ciliated cells of both invertebrates

* The literature pro and con has recently been very fully reviewed by Saguchi.[6]

and vertebrates, and he states further that because the centrosome cannot always be demonstrated is no reason for concluding that it is lacking. Mitosis, according to this author, occurs exclusively in the ciliated cells of invertebrates. When these cells divide by mitosis the basal granules and cilia are said to disappear before division and to be lacking until after division has taken place. He agrees that in vertebrates the sole method of division of ciliated cells is by amitosis. In this process the ciliary apparatus remains unchanged. The difference in the mode of proliferation in ciliated cells of vertebrates and invertebrates, he argues, must be due essentially to the degree of differentiation of the cell-plasm. In the development of cilia in cells of embryonic tissue, Saguchi describes a migration of mitochondria from the region distal to the nucleus, where they are grouped, into the cuticle of the cell. Piercing the cuticle, the mitochondria are described as transforming into cilia. In the efferent tubules of the mouse and rat ciliated cells are said to be formed from the cells with brush borders. The mitochondria increase in number and collect distally to the nucleus. They then proceed to the distal cell-border and are transformed into rod-like bodies which sprout short cilia. These cilia pass through the axes of the hairs of the brush border and gradually lengthen. That the ciliary apparatus is formed by the differentiation of the mitochondria and that the centrosome takes no part in the production of cilia are the chief conclusions of Saguchi.

DISCUSSION

In general I agree with Peck, and can distinguish frontal, laterofrontal, and lateral epithelium in the gill filament. Peck, however, did apparently not recognize that such a division must of necessity be an artificial one, as the type of cells in these regions varies with their stage of development from the cells of the basal epithelium, so that one type cannot be said to be peculiar to one special region. The cell that Peck described as resembling "a goblet cell with a single coarse flagellum issuing from it," and which he interprets as the large cell of the latero-

frontal epithelium viewed at right angles, I have identified as a transitional cell arising by amitotic division from the larger parent cell of the laterofrontal epithelium.

Contrary to the conclusion of Saguchi that mitosis is the exclusive mode of division in ciliated epithelium of invertebrates, I find that ciliated cells in the gill filaments of the fresh-water mussel, Cyclas, divide only by amitosis. As to mitosis in ciliated epithelium, I find that the cells undergoing mitotic division possess no cilia. Saguchi in his description also states that before undergoing mitosis the cell loses its cilia. As these cells possess no cilia during division, it cannot properly be said that ciliated cells divide by mitosis. Saguchi confirms Jordan's findings in vertebrates and concludes with him that amitosis is the exclusive method of proliferation of ciliated epithelium in these forms. Since Saguchi admits that the cells in which he saw mitosis in invertebrates possessed no cilia, and since I have found this to be the case also in my material, and further that ciliated cells of invertebrates do divide by amitosis, the conclusion seems justified that ciliated cells where they proliferate as such do so exclusively by amitotic division both in vertebrates and in invertebrates.

From the above it follows that the proximate factor determining whether a cell of ciliated epithelium is going to proliferate by mitosis or by amitosis is the absence or presence of cilia. The question at once arises as to why cells possessing cilia should always divide by amitosis. Is the cause a structural one or a functional one? Jordan suggests that amitosis is due to a lack of a centrosome in these cells, while Saguchi reaches the conclusion that amitosis in these cells is "due essentially to the degree of differentiation of the cell-plasm," which latter may be classed as a functional cause.

Saguchi claims to be able to demonstrate the presence of a centrosome in ciliated cells. His illustrations and descriptions do not unequivocally bear out this assertion. The difficulties attending the identification of such a minute body as the centrosome from among a large mass of mitochondria render such an undertaking practically impossible. In my preparations no

undoubted centrosome is discernible. The absence of a centrosome, or its preemption as basal bodies by the cilia, would seem to be an adequate structural cause to explain the amitotic division in ciliated cells. A relation between the formation of the ciliary apparatus and the centrosome is at once suggested. The Lenhossék-Henneguy hypothesis states that from the centrosome by partition the basal granules are formed, and that from these granules cilia are sprouted. It is very suggestive that the axial filament of the flagellum of the sperm (comparable to a coarse cilium) does grow out from one of the two partition products of the centrosome of the spermatid.

Saguchi's description[6] of the centrosomes in ciliated cells of the vasa efferentia of the mouse and the rat (pp. 254, 255), and his illustrations, both indicate the difficulties and uncertainties involved in an attempt to differentiate centrosomes from mitochondria and other cytoplasmic granules, and particularly from the basal granules. Indeed, his description of the pluricorpuscular centrosome in the cells of the rat, 'curious ring-shaped corpuscles,' which he interprets as 'derived from the centrosome,' stating that "a centrosome divides repeatedly and forms a ring by secondary fusion of separated particles" (p. 255), would seem to accord well with the interpretation of basal bodies as derivatives of a centrosome. Moreover, both in the case of the rat and of the mouse, Saguchi describes a diplosome in the nonciliated brush-border cells of the efferent tubules, the upper member of which pair of centrosomes 'often bears a cilium' (p. 255). These observations would seem to support the conclusion that basal corpuscles of ciliated cells are derived from centrosomes; but Saguchi refuses to ascribe to them any such significance.

I find no relationship, other than spatial, between mitochondria and the ciliary apparatus. Recent investigations on mitochondria have demonstrated that these cytoplasmic elements have no direct genetic relationship to structures such as nerve, muscle, or connective-tissue fibrils, but are fundamental vital elements of the cytoplasm, probably associated with metabolism. Saguchi, however, concludes and asserts that cilia are formed from mitochondria. Such a transformation would necessitate not

only a morphological change, but also a chemical change. His illustrations representing a migration of mitochondria into and through the cuticle or distal cell-border to form cilia are far from convincing. The fact that the mitochondria lie between the nucleus and the distal cell-border in ciliated epithelium holds some significance for this author. I might suggest that this is the natural place to look for mitochondria in ciliated cells, or any other epithelium of the columnar type, as normally these cells show a marked polarity, and with the nucleus situated well to the base of the cell, the only position left for the main mass of mitochondria to occupy is between the nucleus and the distal border of the cell. Moreover, the analogy between the segregation of mitochondria about the idiozome of spermatocytes and the basal bodies of ciliated cells is very suggestive as regards the homology between centrosomes and basal bodies. These facts render Saguchi's claim of a mitochondrial origin of cilia dubious.

SUMMARY

1. Basal, lateral, laterofrontal, and frontal epithelium can be distinguished in the gill filaments of Cyclas. The cells of the lateral, laterofrontal, and frontal epithelium are ciliated and are derived successively from the non-ciliated cells at the base of the filaments.

2. Mitosis may occur in the non-ciliated basal epithelium. The ciliated cells of the lateral, and especially of the laterofrontal epithelium divide exclusively by amitosis. Mitosis occurs in the ciliated epithelium of the intestine, but the cells dividing by this method do not possess cilia and cannot therefore be classified as ciliated cells.

3. The parent ciliated cell of the laterofrontal epithelium divides by amitosis, thus producing a group of narrow, cylindric transitional cells with four basal granules and four cilia each. These transitional cells, by a partition of the basal granules and the splitting of the cilia, form cells with tufts of long cilia which renew worn-out cells of the frontal epithelium. Worn-out cells of the frontal epithelium disintegrate, passing through a stage of karyorrhexis, and are resorbed.

4. The ciliated cells of the gill filaments of Cyclas reveal no centrosome.

5. These ciliated cells contain mitochondria in their cytoplasm between the nuclei and the distal borders of the cells, but no genetic relation between mitochondria and cilia is discernible.

6. Indirect evidence points to the conclusion that the basal bodies of ciliated cells are centrosomal derivatives.

I am indebted to Prof. H. E. Jordan for suggesting this problem to me and for assistance in the prosecution of this research.

LITERATURE CITED

1 Heidenhain, M. 1907 Plasma und Zelle, S. 284. Fisher, Jena.
2 Henneguy, L. F. 1898 Sur le rapports des cils vibratiles avec les centrosomes. Arch. d'Anat. micr., T 1 (cited from Heidenhain).
3 Jordan, H. E. 1913 Amitosis in the epididymis of the mouse. Anat. Anz., Bd. 43, S. 589.
4 Lenhossék, M. v. 1898 Über Flimmerzellen. Verh. d. anat. Ges. zu Kiel (cited from Heidenhain).
5 Peck, R. H. 1877 The minute structure of the gill of lamellibranch Mollusca. Quart. Jour. of Micr. Sci., vol. 17, p. 43.
6 Saguchi, S. 1917 Studies on ciliated cells. Jour. Morph., vol. 29, p. 217.

PLATE 1

EXPLANATION OF FIGURES

All figures except figure 2 were drawn from transverse sections of the gill filaments of Cyclas. The tissue was fixed with Meves' fluid, cut at 5μ, and stained with iron hematoxylin. The magnification is 1300 diameters, except in figure 5 where is it 1500 diameters. In order not to obscure the basal bodies and their cilia, cytoplasmic details, including mitochondria and the cytoreticulum, are added only in figure 5. A $\frac{1}{16}$ Leitz oil-immersion lens was employed in this study.

1 Transverse section showing basal epithelium and portions of two adjacent gill filaments. The cell in the laterofrontal position is of the primitive type with a tuft of long cilia (*P*). Interposed between the laterofrontal and lateral epithelium is a small non-ciliated cell (*I*). The cell of the lateral epithelium which is also ciliated (*L*) contains two nuclei and is evidently in a phase of amitotic division. The cells of the basal epithelium are roughly pyramidal in shape and are non-ciliated (*A*).

2 Area from the ciliated epithelium of the intestine. The cells are tall columnar and have a tuft of cilia, a double row of basal granules, and a cone of rootlets extending into the cytoplasm. One of the cells is undergoing mitosis, but this cell has no cilia.

3 Transverse section showing basal epithelium and a small portion of the filament. A mitotic figure (*M*) is seen in a cell of the non-ciliated basal epithelium.

4 Section of a complete filament. The frontal epithelium (*A*) has short, broken-off cilia. Two pyknotic nuclei of degenerating cells are seen close to the basement membrane (*D*). The cell of the laterofrontal epithelium shows a nuclear indentation, the initial step in amitosis. The cell of the lateral epithelium (*L*) lies next below the laterofrontal epithelium.

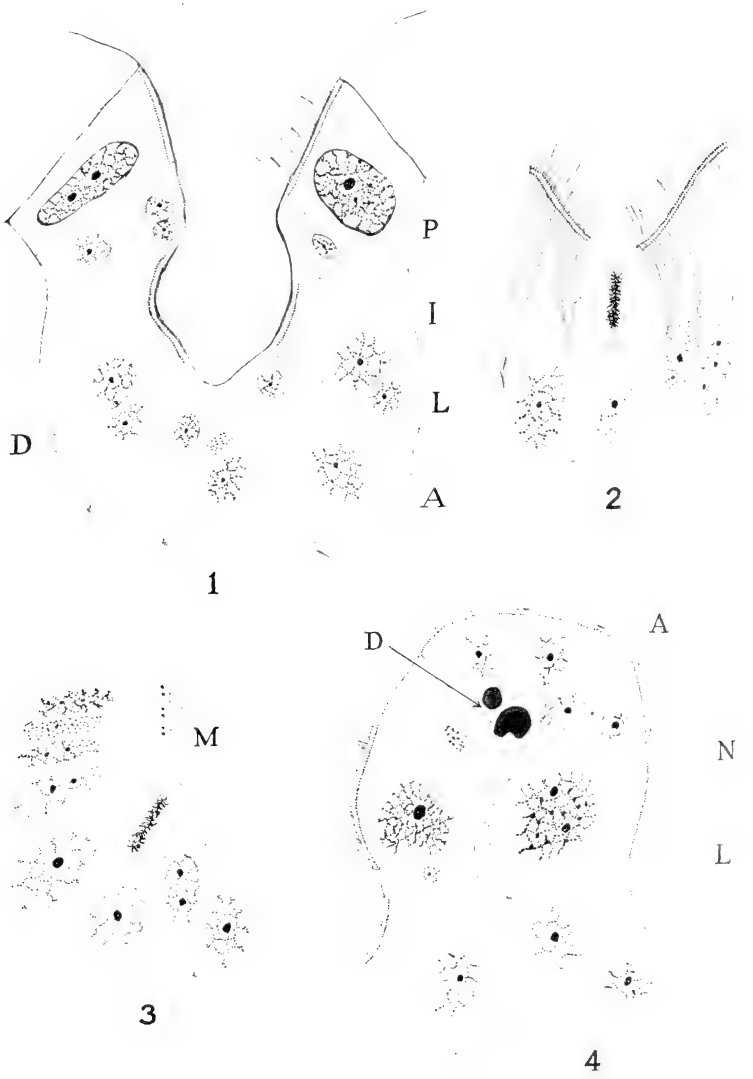

P
I
L
A
D
1
2
M
3
D
A
N
L
4

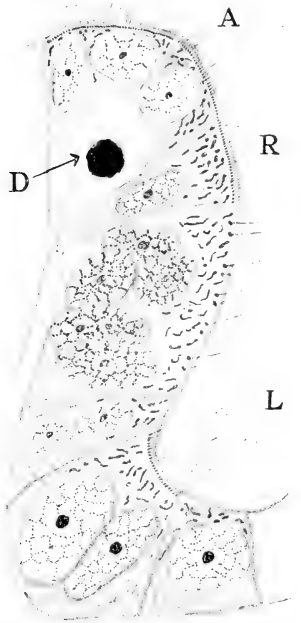
PLATE 2

EXPLANATION OF FIGURES

5 Right half of complete filament. A binulceated cell of the lateral epithelium (*L*), differentiated from the non-ciliated cells of the basal epithelium, is still almost in a basal position. Amitotic nuclear division has occurred in the cell (*N*), in the laterofrontal position, with the pairing of the cilia and the basal granules. A degenerating cell (*D*) of the frontal epithelium, with a pyknotic nucleus surrounded by a vacuole, is situated toward the interior of the filament. The cells of the frontal epithelium (*A*) have short worn-off cilia and vesicular nuclei. The mitochondria, predominantly of bacillary and filar form, are aggregated in the distal border of the cells.

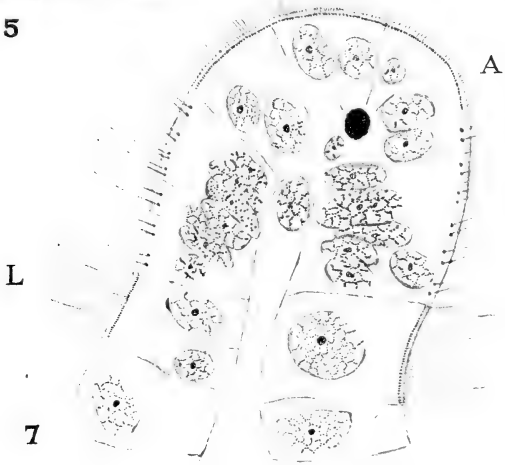
6 Transverse section of half of a filament. The primitive cell of the lateral epithelium has suffered amitotic division, forming transitional cells (*T*). Each daughter cell is of the tall columnar variety and shows in vertical sections a pair of cilia and a pair of basal bodies. A remnant (*M*) of the primitive cell (*M* and *T*) is undergoing belated nuclear division. The pairing of the basal granules and the cilia is conspicuous next the uppermost of the group of daughter cells (*T*). Two of the frontal cells (*C*) are in stages of degeneration.

7 Transverse section of gill filament, showing frontal epithelium (*A*), multiple direct nuclear division with pairing of cilia and basal bodies in the laterofrontal epithelium (*H*), and the lateral epithelium of one side composed of two cells (*L*).



5

6



7

Resumen por el autor, D. H. Wenrich.

La estructura y división de Trichomonas muris (Hartmann).

Este flagelado que habita en el ciego del ratón mide 12 a 16 micras de longitud por 5 a 10 micras de espesor, y posee los siguientes orgánulos: Núcleo, citostoma y blefaroplasto con las estructuras que en él se insertan—los tres flagelos anteriores, el flagelo posterior que corre a lo largo del márgen de la membrana ondulante, el bastón basal cromático en la base de dicha membrana, el axostilo, las filas externa e interna de gránulos cromáticos y el cuerpo parabasal. Esta última estructura es el cuerpo parabasal de Janicki ('11) pero aparece solamente con ciertos métodos técnicos. En la división pueden reconocerse estados comparables a la profase, metafase, anafase y telofase de las células de los metazoarios. Durante la profase se forman seis cromosomas dobles (hendidos longitudinalmente), mientras que el cariosoma desaparece gradualmente como en el caso del nucleolo de los metazoarios.

El nuevo bastón basilar cromático se origina como una hilera de pequeños gránulos que se inserta por uno de sus extremos en el blefaroplasto. La nueva membrana ondulante y el flagelo posterior se desarrollan al mismo tiempo que el bastón cromático basal. Un pequeño blefaroplasto nace por gemación del primitivo, y ambos permanecen reunidos por una paradesmosis durante la división. La membrana nuclear persiste durante la mitosis. El comportamiento de los cromosomas durante la metafase y anafase es semejante a los dellas células de los metazoarios. El axostilo primitivo degenera, formándose uno nuevo a expensas de cada blefaroplasto. El borde interno del bastón cromático basal produce por gemación una nueva fila de gránulos cromáticos. La división de la célula se retrasa hasta que las dos series de orgánulos están completas. El núcleo y el cuerpo celular son las únicas partes que se dividen ecuacionalmente, mientras que todas las demás partes necesarias aparecen como crecimientos de las estructuras primitivas correspondientes.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, NOVEMBER 7

THE STRUCTURE AND DIVISION OF TRICHOMONAS MURIS (HARTMANN)

D. H. WENRICH

Department of Zoology, University of Pennsylvania

ONE TEXT FIGURE AND FOUR PLATES (THIRTY-SIX FIGURES)

CONTENTS

INTRODUCTION

The structure and division processes of various species of Trichomonas have received the attention of several investigators, but there is much disagreement among them regarding details of structure in the vegetative condition and events during division even in the same species. Such differences are very noticeable, for example, in the most extensive accounts in recent years, one

by Kofoid and Swezy ('15) and two by Kuczynski ('14, '18). The structure and division of Trichomonas muris were described in these papers as well as in the earlier one of Wenyon ('07). Since I have been able to secure some material which seems to be especially favorable for the study of cell structure and division in this species, and since my findings are not in entire agreement with any of the authors mentioned above, it would seem to be worth while to place on record my observations. I have begun an investigation of the various intestinal protozoa of rats and mice, but the present account will be limited to the one species.

MATERIALS AND METHODS

A. Materials

Trichomonas muris (Hartmann) is found chiefly in the coecum of mice and to a less extent in the large intestine. Only rarely has it been found in the small intestine and then only at the lower end.

The first material from the coecum of a mouse (Mus musculus) in which I found the division stages numerous was secured in December, 1916. Slides made from this material have proved to be the most valuable in the collection, and many of my figures have been made from them. Since that time 102 additional mice have been examined, of which fifty-one were wild and fifty-one were albinos. Of the wild mice, nine were Peromyscus leucopus and the others were the house mouse, Mus musculus. Only two of the Peromyscus and only five of the forty-two house mice showed infection with Trichomonas muris, while fifteen of the fifty-one white mice were found to harbor this species. Young mice showed less tendency to infection than adults and the degree of infection was extremely variable. It ranged from occasional specimens to cases when the entire contents of the coecum appeared to consist of Trichomonas and a few bacteria. In these latter cases division stages were common in the mass of coecal contents as well as near the mucous membrane.

B. Methods

Aside from preparations of living flagellates in fresh coecal contents mixed with salt solution, cover-glass preparations fixed and stained in various ways and mounted in balsam were employed. Coecal material, usually from the region adjoining the mucous lining, was mixed with a little salt solution and smeared out thin on clean cover-glasses. With few exceptions these smears were fixed without allowing them to dry, although occasionally some were dried and subsequently stained with some modification of the Romanowsky method. These dried smears do not give satisfactory preparations and have not been used as the basis of the observations here recorded.

For the wet smears the following fixatives, usually heated to about 40°C., have been tried: Schaudinn's sublimate and alcohol, with and without the addition of acetic acid; Worcester's formol-sublimate-acetic; Flemming's stronger and weaker solutions; Perenyi's chrom-nitric acid; Carnoy's alcohol-chloroform-acetic; Bouin's picro-formol-acetic; Allen's ('16) modification of Bouin's (B 15); sublimate-acetic, and picro-mercuric. The most satisfactory of these have proved to be Schaudinn's, Bouin's, Allen's, and Flemming's, in about the order named. Some other fixatives were used in special experiments which will be described elsewhere.

For staining, Delafield's, Heidenhain's iron alum-haematoxylin, and safranin (after Flemming's) have been tried, but most of the smears have been stained with Heidenhain's haematoxylin, which has always given the most satisfactory results. Alcoholic solutions of haematin, haematoxylin, and iron-alum, according to the methods described by Dobell ('14) and Kofoid and Swezy ('15), were tried, but did not give results as satisfactory as the twenty-four-hour staining in iron alum-haematoxylin, so their use was not continued. Various counterstains were tried, but none of them appeared to add to the value of the preparations, and were not generally employed.

THE VEGETATIVE INDIVIDUALS

A. Form

Both in the living and the fixed condition the body of this species of Trichomonas is rather fusiform, with a length of from one and a half to two times the greatest width. There is some tendency for the so-called dorsal side to be more convex than the opposite, somewhat flattened, ventral side. In the free-living condition the region of greatest width is usually near the middle, but the flexibility of the pellicle permits a variety of shapes, especially when the animals are creeping or forcing their way through the coecal debris. Then the body may change shape rapidly and some of the variations are to be seen in the fixed material. For example, figure 7 shows an animal with the anterior end much more pointed than the one in figure 8. Figures 8 and 17 show animals with the greatest width at the posterior end instead of in the middle, as is more common.

Adverse conditions, such as lowered temperature, changes in the constitution of the surrounding fluid, or desiccation, often lead to considerable changes in form, the most common modification being the rounded-up condition (figs. 11, 13, and 15). The rounded form also seems to be characteristically assumed during the process of division (figs. 20 to 30). When confined in cramped quarters the form changes are exceedingly various.

In free-swimming animals the undulating membrane is spirally arranged on the surface of the body, and they rotate on the long axis, without any appreciable changes in diameter. On the other hand, fixed and stained individuals often give the impression of being flattened and of lying on one side with the undulating membrane at one edge, as seen, for example, in figures 1 to 4. In those specimens showing the spiral arrangement of the undulating membrane and accompanying structures, it is seen that the direction of the spiral is from the left over to the right, as shown in figures 5 and 17 and in text figure A.

B. Size

Wenyon ('07) called attention to the great variation in size in the Trichomonas of mice giving the length as from 3 to 20 μ. Kofoid and Swezy ('15) emphasized a similar variation in size for T. augusta. In both cases the authors raise the point that differences in size alone do not furnish sufficient criteria for the separation of species. I have found two species in mice which do differ as to size, and it may be that the range of sizes observed by Wenyon had a greater significance than he supposed. Careful study and measurement of the flagellates found in mouse no. 1, for example, revealed a larger species which I take to be T. muris, ranging in length from 8 to 20μ, with an average of

TABLE 1

Table showing results of measurements, from certain host mice and for certain fixatives

MOUSE NUMBER	FIXATIVE	NUMBER OF INDIVIDUALS MEASURED	AVERAGE LENGTH, MICRONS	RANGE, MICRONS
1	Schaudinn's	100	13.1	10–16
19	Schaudinn's	50	12.7	10–16
24	Schaudinn's	100	12.8	10–16
24	Allen's	100	15.7	11–22

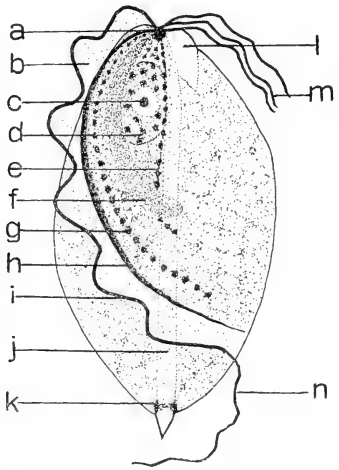
12.9μ, and a smaller species ranging in length from 6 to 9μ, with an average of 7.2μ. The smaller species may be T. parva Alexeieff, and can be differentiated on morphological grounds other than size, and shows only three chromosomes in division. In other mice pure infections of each species were found as well as other mixed infections.

Measurements have been made from several series of slides, and the results indicate slight racial differences for the different hosts as well as differences due to various methods of fixation. Table 1 indicates some of these differences.

The results for different fixatives is strikingly illustrated by mouse no. 24, where the average length for animals fixed with Allen's fluid (15.7μ) is 22.6 per cent more than the average length for those fixed in Schaudinn's fluid (12.8μ). It is prob-

able that Schaudinn's fluid causes shrinkage, and possibly Allen's fluid may cause swelling.

In making these measurements great care has been taken to secure an unbiased selection of individuals for measurement. Only individuals which appeared normal and had the axostyle approximately straight were measured. All such individuals in



Text figure A Vegetative individual of T. muris, partly diagrammatic; *a*, blepharoplast; *b*, undulating membrane: *c*, caryosome; *d*, nucleus; *e*, inner row of chromatic granules; *f*, parabasal body; *g*, outer row of chromatic granules; *h*, chromatic basal rod; *i*, posterior flagellum as chromatic margin of undulating membrane; *j*, axostyle; *k*, chromatic ring at point of emergence of axostyle; *l*, cytostome; *m*, anterior free flagella; *n*, posterior free flagellum.

any one field of the microscope were measured. Successive fields were treated the same way, duplication of fields being prevented by the use of a mechanical stage. All measurements were made by the aid of an eye-piece micrometer which had been calibrated for the set of lenses used. The measurements obtained agree very well in a general way with those of Kuczynski ('14) and Kofoid and Swezy ('15).

C. Organization of the cell

The organelles of this species of Trichomonas are those typical of the genus and are indicated in text figure A. They include besides the nucleus (*d*) and cytostome (*l*) the series of structures attached to the blepharoplast (*a*), consisting of the three anterior free flagella (*m*), the long posterior flagellum running as the chromatic margin (*i*) of the undulating membrane (*b*) and continuing posteriorly as a free flagellum (*n*), the chromatic basal rod (*h*) at the base of the undulating membrane, the axostyle (*j*), the parabasal body (*f*) and the inner (*e*) and outer (*g*) rows of chromatic granules.

The protoplasm is enclosed in a cell membrane or pellicle, which, as previously noted, is flexible enough to permit variations in form. These form variations may be classed as 'euglenoid' in type. Pseudopodia formation has been described by several authors, for example, by Kuczynski ('14) and by Kofoid and Swezy ('15), but I have seen such apparent pseudopodia only under conditions which appeared to be either degenerative or precystic, and therefore I do not regard this phenomenon as normal for the active individual. The protoplasmic projection shown in figure 16 is probably the result of mechanical injury in making the smear, and is not a pseudopodium.

The protoplasm itself is rather fluid in nature, as is indicated by the rapidity with which form changes occur. It appears to be somewhat vacuolated, although not to the extent seen in some other species, such as T. augusta, as figured by Kofoid and Swezy (15), or T. mirabilis, as figured by Kuczynski ('18). The appearance or non-appearance of vacuoles seems to vary somewhat from host to host and from cell to cell. Variations from one fixative to another are discussed further on.

The nucleus (text fig. A, *d*) lies in the anterior third of the body dorsal to and usually a little to the left of the axostyle which occupies the position of the principal axis. It is usually oval or broadly elliptical in shape, being approximately 4 to 5μ long and 2.5 to 3μ wide. At the periphery is a delicate nuclear membrane or caryotheca, which is sometimes difficult to see.

Within the membrane the chromatin occurs as small granules scattered upon a fibrous network, and as a caryosome (*c*) of relatively large size which is usually surrounded by a clear area. The exact number of the small chromatin granules had not been determined, but in the early prophases they are reduced to six, which are paired or split.

The clear area about the caryosome is sometimes large with a diameter as much as one-half to two-thirds that of the nucleus (figs. 1, 2, 7, 8, etc.). In other instances it is much smaller (fig. 13). In figure 1 the caryosome appears to be double, but this condition is rare. Careful focusing usually discloses fine fibrous connections between the caryosome and the network at the periphery of the clear area (figs. 7, 8, 10, 12). There is no apparent constancy in the position of the caryosome, since it is found at either extremity of the nucleus or in any intermediate position.

Occasionally there is seen a nucleus like that shown in figure 9, but such nuclei often are accompanied by signs of degeneration, and the condition is regarded as abnormal.

I have not been able to make out a rhizoplast connecting the nucleus with the blepharoplast as described by Kofoid and Swezy ('15).

The cytostome is an opening at the anterior margin of the body on the side of the major axis opposite the nucleus. This side is usually considered as ventral. The cytostome is not so large as that described and figured for T. augusta. There appears to be a short cavity leading into the interior along the ventral side of the axostyle.

The blepharoplast is a deeply staining granule, or possibly a pair of granules at the anterior end of the major axis of the body. To it a series of other organelles are attached, as already mentioned. The nature of this focus of organization is difficult to determine. By some authors it is regarded as homologous with the similarly named structure in some of the simpler flagellates, such as the haemoflagellates, and by others it is assumed that in the Trichonomads it is composite, being composed of a number of granules equal to the number of flagella attached. Martin

and Robertson ('11) thus describe it for Trichomonas (Tetratrichomonas?) gallinarum. Kofoid and Swezy ('15) believe it is composed of two parts, one of which is a centrosome and the other the basal granule for the flagella. In the material that I have studied this structure frequently appears to be double, that is, composed of two approximately equal parts, and the posterior flagellum is attached to the anterior moiety, while the chromatic basal rod is connected with the posterior one. Such a condition was also described by Wenyon ('07). Since the three anterior flagella take the stain so slightly, it is difficult to determine what their relation is to the blepharoplast components.

Because the three anterior flagella do not stain very deeply, they are difficult to make out. This difficulty is often increased by the presence of spirochaetes of similar caliber and staining power and by the flagella taking a position in contact with, or under, the body. In the drawings they have been omitted when not plainly seen. In all cases in which they could be clearly discerned, they appeared to be of equal length, wavy, and about one-half the length of the body, although sometimes shorter. Figures 2 to 5 and 7 to 15 show the flagella in their typical condition.

Hartmann ('10), Wenyon ('07), and Kuczynski ('14, '18) figure these flagella just as I have found them, but the figures for this species given by Kofoid and Swezy ('15) have the anterior flagella as long as or longer than the body of the animal. On account of this and other differences, one may be led to suppose that the latter authors were dealing with a different species.

The posteriorly directed flagellum running as the chromatic margin of the undulating membrane is very much longer than the others, extending the length of the body, making six to eight undulations in its course and projecting posteriorly as a free flagellum as long as the anterior flagella. This posterior part is similar to the anterior flagella in caliber and staining power, but the intracytoplasmic portion appears to be much thicker and takes the stain intensely. There is some variation in stainability depending on the fixative employed, as will be noted elsewhere.

As has been observed by other authors, the undulating membrane seems occasionally to be broken, allowing the entire flagellum to become free. Individuals with the posterior flagellum free are not rare in fixed and stained preparations (fig. 15).

The chromatic basal rod takes origin in the blepharoplast, or possibly the posterior portion of it, and extends along the surface of the body at the base of the undulating membrane. It, together with the undulating membrane, takes a spiral course on the living animals, as in figures 5 and 17, as previously noted, passing posteriorly from the left over to the right. It ends free in the cytoplasm. It appears to be a body of some rigidity because changes in its position are usually accompanied by corresponding changes in the form of the body. As described by Wenyon ('07), it may project from the body as a stiff thread. It is broadest near the middle, tapering to a slender distal terminus, and to a less slender proximal or anterior end attached to the blepharoplast. Near the anterior end it often exhibits a bend, which may even be S-shaped, which suggests a high degree of flexibility of that region (fig. 14, e.g.).

Most observers have represented this structure as a homogeneous rod. In this species I have been considerably puzzled about its organization, for frequently it appears to have embedded within it a row of granules on the inner side, similar to the row which lies close to it, but deeper in the cytoplasm (figs. 11 and 14). At other times the additional row seems to be just in contact with the rod (figs. 2, 10, 13, 21), and again the row may be adjacent to but not in contact with the rod (fig. 8). These observations indicate that new rows of granules take their origin from the basal rod and migrate inward, possibly replacing, during division, the one that is always found close to and parallel with the rod.

This outer row of chromatic granules close to the chromatic basal rod is very characteristic of this species and extends from 60 per cent to 90 per cent of the length of the rod out from the blepharoplast. It is figured by Hartmann ('10), Wenyon ('07), Kuczynski ('14, '18), and Kofoid and Swezy ('15). Another row of similar granules is found deeper in the cytoplasm, and

close to the axostyle on its dorsal side. It is easily seen in the region posterior to the nucleus, but its anterior extension is frequently obscured (figs. 1, 2, 4, 7, 9, etc.). In some cases it is traceable forward outside the nucleus up to the blepharoplast. Posterior to the nucleus this row is nearly parallel to the longer, more peripheral one. The inner row of granules is mentioned and figured by Wenyon ('07) and by Kuczynski ('14), but seems to be absent from the form described by Kofoid and Swezy ('15) under the name of T. muris.

In the region between the nucleus and the blepharoplast there are often additional granules similar to those in the two rows (figs. 7, 10, 14). The presence of these extra granules often makes it difficult to determine the anterior limit of the nucleus, on account of their resemblence to the granules of chromatin within the nucleus and the faintness of the nuclear membrane.

The axostyle is a hyaline cylindrical rod attached to the blepharoplast and it traverses the major axis to project slightly at the posterior end, where it tapers rapidly to a sharp point. At the point of emergence there is the ring of deeply staining substance (text fig. A, *k*) mentioned by Kofoid and Swezy. In the region of the nucleus the axostyle is frequently somewhat curved around that body which appears to lie slightly to the left of it. The axostyle seems narrower in the region near the blepharoplast than elsewhere. I have never seen any cases of a capitulum in this species such as Kuczynski ('18) mentions.

The flexibility of the axostyle is indicated by the frequent occurrence in fixed material of a decided bend at the most flexible region just posterior to the nucleus (figs. 2, 3, 11, 13, 15), but I have never seen this structure used as an organ of locomotion, as maintained by Kofoid and Swezy ('15) for T. augusta.

There are no chromatic granules in the axostyle except in new ones growing out from the blepharoplast in the telophase of division. However, the deeper row of granules often appears to be in contact with the axostyle in the region immediately anterior to the nucleus (figs. 1, 2, 3, 7, 8, etc.).

The parabasal body is a cylindrical curved rod, of a diameter comparable to that of the axostyle, connected by a narrow attachment to the blepharoplast and lying dorsal to and to the right of the nucleus. Its texture is apparently different from that of any other structure in the cell and its staining reaction with haematoxylin is different from the other structures. While it appears to be homogeneous, its texture is of a looser, more spongy nature than that of the structures so far mentioned. Its appearance compares well with the figures of it given by Janicki ('11). It is quite variable in length, as indicated by figures 3, 4, 5, 6, and 16, but when it is longer it often has a constriction (figs. 4, 5), or a thinner place (fig. 16), marking off two regions. One wonders if the distal portion may not become detached and serve some function in metabolism.

I have never seen any indication of a central core or thread as described by Cutler ('19) for the parabasal of Ditrichomonas termitis. On the contrary, in an animal which was either rounding up for encystment or else had started to degenerate (fig. 6), the parabasal appeared as a granular peripheral case enclosing a non-staining area.

Since Kofoid and Swezy ('15) employed mainly Schaudinn's fluid which seems to dissolve out the parabasal, this elusive organelle was apparently overlooked by them, and they applied the term 'parabasal' to the chromatic basal rod. The homology of the above-described parabasal in Trichomonas muris with the similar structures figured by Janicki ('11) for Devescovina, Parajoenia, Stephanonympha, and Trichomonas and by Cutler ('19) for Ditrichomonas termitis seems to me to be justifiable, but a homology between the chromatic basal rod and these parabasals of Janicki, as claimed by Swezy ('16), would, in my opinion, be open to some question.

D. Differences due to different fixatives

It will be profitable, I think, to consider at some length some differences of appearance in the organization of Trichomonas muris which are correlated with the use of different fixatives. The conditions found in the series of slides from mouse no. 24

illustrates this point. In this case the entire set of instruments, reagents, glassware, microscope, etc., were placed in a warm room at 37°C. a number of hours before the mouse was killed. The mouse was taken into the same warm room, killed, opened, and the coecal contents examined. The coecum was found to be swarming with Trichomonas, so fixations were made with Allen's, Bouin's, Carnoy's, Schaudinn's, sublimate-acetic and weak Flemming's fluids. After fixing for half an hour at 37°C., the subsequent washing and further treatment were carried out at room temperature, and all the slides were stained at the same time and in the same way with the same stock solutions of iron alum and haematoxylin. The chemical differences in the different fixatives would therefore appear to be the variable factors in this experiment, so that differences in appearance can, I think, be attributed to different effects of the fixatives on the organisms. In any smear of this kind, of course, there are always thicker and thinner areas, and the intensity of the stain varies with the thickness of the film on the cover-glass. It is therefore possible to compare for a wide range of intensities of the stain.

The general cytoplasm may first be considered. Figure 1 indicates the results from fixation with Carnoy's fluid. Little vacuolization is indicated, and such vacuoles as there are do not show any stainable contents. Figure 2 is from a smear fixed in sublimate-acetic, and here not only are the vacuoles well defined, but the contents have taken the stain. Some few individuals on this smear did not show the vacuole contents stained, but the great majority did. The smears of this series fixed in Schaudinn's fluid showed an occasional individual with vacuole contents stained. In the other series which were fixed with Schaudinn's fluid vacuole contents did not usually take the stain. Figure 3 is from a smear fixed in weak Flemming's fluid, and the structure of the protoplasm is much like that in figure 1.

The various organelles may next be considered. Schaudinn's fluid and sublimate-acetic gave somewhat similar results except for the protoplasmic vacuoles already mentioned. The nucleus, blepharoplast, posterior flagellum, chromatic basal rod, and specific granules are all sharply differentiated, although in the

sublimate-acetic slides the chromatic basal rod was not so intensely stained as in those fixed in Schaudinn's. Similar results were obtained by the use of Allen's and Bouin's fluids, except all structures appeared swollen in comparison with those prepared with other fixatives. Also the free flagella were better stained after the last two fixatives named than after the first two. In the case of Carnoy's fluid (fig. 1) the results varied considerably with the stain. In the animals showing an average intensity of the stain, the nucleus was very black, often failing to show any structure, while the chromatic basal rod and the chromatic margin of the membrane failed to stain. In contrast, the two rows of chromatic granules were stained very deeply. In the specimen drawn (fig. 1) the chromatic margin was not so strongly stained as is indicated and the nucleus was lighter than in the majority of individuals. The blepharoplast was also faintly stained on these slides, while the free flagella and the axostyle were farely well defined in most cases. After weak Flemming's fluid all the structures were rather indistinctly differentiated by the stain, and yet these slides were the only ones in which the parabasal body appeared.

I did not find the parabasal body until after reading the paper by Cutler ('19), who describes its occurrence in Ditrichomonas termitis. According to Cutler, this structure was not constant in material prepared with the usual fixatives, but by employing Flemming's without acetic acid and other fixatives which contained neither acetic acid nor corrosive sublimate, he was able to demonstrate it consistently. Following his suggestion, I employed on the same lot of material from mouse no. 29 Allen's, Bouin's, and Flemming's fluids each without acetic; also 1 per cent chromic acid containing 1 per cent urea and several strengths of formalin, together with unmodified Schaudinn's and Allen's fluids as controls. The latter two fluids gave the best general fixation, but the Flemming's without acetic and the 1 per cent chromic acid both brought out the parabasal in some individuals when subsequently stained with iron-alum haematoxylin. Since Janicki ('11) found the parabasal in T. bactrachorum which had been fixed with an 'osmic acid mixture,' I was led to scrutin-

ize all of my slides which had been fixed with Flemming's fluid, with the result that I detected this structure in T. batrachorum and T. augusta from the leopard frog and in some slides of T. muris fixed with weak Flemming's. Later I found the same structure in T. caviae in material fixed with weak Flemming's and Flemming's without actic. The parabasal was most clearly differentiated in the slides of T. muris fixed in weak Flemming. Since in the weak Flemming the amount of osmic is reduced and since, further, the parabasal appeared in slides fixed with 1 per cent chromic acid, it would seem that the chromic acid is as much if not more responsible for bringing out this structure than is the osmic acid. Also, my experience does not parallel that of Cutler ('18) in the case of formol, since none of my formol-fixed preparations showed the structure.

In the slides fixed with weak Flemming from mouse no. 24 a great majority of the flagellates showed the parabasal plainly, while in a few it was difficult or impossible to make it out. In the slides from mouse no. 29 fixed with 1 per cent chromic and with Flemming's without acetic only a small percentage of the flagellates exhibited the parabasal. There thus appear to be individual variations with the same technique as well as differences due to differences in technique. Kuczynski ('14) found the parabasal in only four out of more than fifty guinea-pigs and in none of the mice, although over a hundred were examined.

The above results point to the necessity of employing a variety of methods of technique, since reliance upon a single method might readily lead to erroneous conclusions.

E. Encystment

Encystment in Trichomonas has been much disputed, there being few observations of a conclusive nature showing the existence of cysts. Wenyon ('07) called attention to the existence in the faeces of the mouse of large numbers of rounded-up individuals which he stated could live for a week or more outside the host if kept moist. Some others, which were much contracted and rounded up, he thought were encysted, and he figures such a specimen in his figure 35, plate 11. I have seen many of the

rounded-up kind, especially in material from hosts which had been dead several hours. I have also seen in some hosts considerable numbers of the contracted forms in the coecal contents. In figure 36 I have represented one of these, and it is very similar to the one figured by Wenyon. In figure 35 there is shown one which is apparently in the process of changing to the rounded and contracted condition. I am inclined to the belief that these animals are preparing to encyst, since there is no sign of degeneration except the apparent disappearance of the free flagella.

DIVISION

All authors who have studied carefully the division of any of the species of Trichomonas agree that the process is complicated and appears to take a relatively long time for its accomplishment. Kuczynski ('14) gives eight hours as the time for T. augusta. It is also generally agreed that the flagellates remain active during the entire process, the flagella and undulating membrane continuing to vibrate even in the rounded-up condition which is characteristically assumed during part of the time. The extensive activities of the post mitotic phase have been well described and illustrated for T. augusta by Kofoid and Swezy ('15).

Since it is possible to recognize in the division of the nucleus stages comparable to those of mitosis in metazoan cells, it will be convenient to refer to these stages under the conventional terms, prophase, metaphase, anaphase, and telophase.

A. Prophase

1. The nucleus. The first changes in the nucleus which indicate the approach of mitosis result in the formation of the prophase chromosomes out of the scattered chromatin granules of the 'resting' nucleus. There are always six of these chromosomes, and each one consists of a pair of closely associated moieties. The parts are often somewhat elongated and the two components lie side by side. These prophase elements remain connected with each other and with the caryosome, until the end of the prophase stage, by the fine strands of non-chromatin

reticulum of the nucleus (figs. 10 to 17 and 21). Occasionally the six elements become arranged in the form of a chain, recalling the chains of split chromomeres sometimes seen in metazoan prophases (fig. 15). In cases where the fixation has not been good, the two parts of each element appear to be fused together, so that the nucleus seems to have six single granules in it in addition to the caryosome. This condition seems to be more prevalent in the later than in the earlier prophases (fig. 19). Since in the earliest stage in which the prophase chromosomes can be distinguished they are already double, it has been impossible to determine whether or not the doubling is the result of antecedent splitting.

In the earlier stages the six chromosomes are always outside the clear area surrounding the caryosome, but later the boundary of the clear area disappears, and the caryosome then seems to be more directly connected with adjoining chromosomes by the non-chromatic reticulum (fig. 15). In cases where the chromosomes appear to be single, due to fusion, and where the pericaryosomal space can no longer be defined there seem to be seven chromosomes instead of six, since the caryosome is not always easily distinguishable from the chromosomes. In all such cases, however, careful study has resolved the group of seven into six chromosomes and one caryosome. During the progress of the prophase changes the caryosome gradually loses its staining power just as do nucleoli of metazoan cells, and at the metaphase no trace of it is visible. Figure 22 shows a very late prophase or early metaphase with the spindle partly formed and a faintly defined vestige of the caryosome.

Number of prophase chromosomes. Wenyon ('07) reports the number of prophase chromosomes as six and says that they early divide into two, giving six pairs of granules. In his figure 2, plate 11, for example, he shows six pairs of granules besides a caryosome. My results are thus in agreement with his. Kuczynski ('14) describes eight prophase and four metaphase chromosomes and again insists on these numbers in his later paper ('18). In this later paper, however, he admits (p. 128) that "Over 70 per cent of the observed prophase nuclei of the Tri-

chomonads named (T. muris, T. augusta, T. Caviae, and T. batrachorum) contain seven sharply outlined chromosomes although in many cases, of which a number have been pictured (e.g., plate I, figs, 16, 17; plate II, fig. 20; plate IV, fig. 57; plate VII, fig. 96), the probability is great that the position of the chromosomes interferes with the certain recognition of an eighth. Chromosome-groups of only six, of uncertain separation, occur much more seldom." In all the figures mentioned in the quotation (except in fig. 96), and in some others not mentioned, the groups can be resolved into six split prophase chromosomes and one caryosome. In the few cases where Kuczynski thinks he finds eight, I am inclined to the belief that he may have counted as separate chromosomes the two parts of one which had become rather widely separated; then, with the caryosome, the number eight is obtained.

Kofoid and Swezy ('15) give five as the chromosome number both for the prophases and the metaphase for T. muris and T. augusta. If the form which they called T. muris is the same species as the one I have been studying, the difference in chromosome number needs to be accounted for. I will merely refer to the great difficulty in elucidating these small details in such minute organisms, even when the technique has been good, and to the further possibility that the form studied by them was of a different species.

As for other species, since Kuczynski finds and figures conditions in T. caviae so similar to those in T. muris, I am inclined to believe that there are six chromosomes in T. caviae. Dobell ('09) found six chromatin bodies in T. batrachorum, but hesitated to call them chromosomes. Martin and Robertson ('11), on the other hand, described for T. eberthi eight prophase and four metaphase chromatin units, although they prefer not to call them chromosomes. It can hardly be argued that all species of Trichomonas should have the same number of chromosomes, but since Dobell and Wenyon have both found six and since the numbers in the species studied by Kuczynski are probably six instead of eight, the situation in T. eberthi might bear reinvestigation.

I have not found stages with the so-called 'nuclear cloud' as described by Kofoid and Swezy ('15) as shown in their figure 49, nor have I seen the spirene stage shown in their figure 50. My figure 9 shows a condition somewhat similar to their figures 46 and 47, but I think such nuclei are abnormal, particularly since they are so much larger than usual and often accompany other evidences of degeneration.

2. Chromatic basal rod. Coincident with the intranuclear changes of the early prophase, the new chromatic basal rod makes its appearance. Usually it appears some time before the blepharoplast has divided and is very difficult to recognize in its earliest stages. Figure 11 shows the earliest stage in which I have been able to find this structure, and here it will be seen to consist of a row of very fine granules closely connected together and joined to the blepharoplast. Figure 10 shows a stage which seems to be a little later, judging by the nuclear changes, and here also the new chromatic basal rod is a row of granules, but much longer than the one in figure 11. I was unable to trace it past the nucleus and up to the blepharoplast.

The new rod is always in a characteristic position, dorsal to, and to the right of, the nucleus (figs. 10 to 17). Although relatively slender at first, it gradually increases in size until by the time the blepharoplast divides it is easily recognizable. After the division of the blepharoplast the new rod does not always maintain its position near the surface of the body. In figure 19, for example, the new blepharoplast is at the upper surface, while the new rod extends from it around the nucleus, deep into the protoplasm to the lower surface.

3. The new undulating membrane and chromatic margin. As the new chromatic basal rod grows, irregular thickenings appear along its length, as indicated in figures 12 and 15. A little later one can see the new chromatic margin of the new undulating membrane closely applied to the new rod (figs. 19 to 21). In its first recognizable condition this chromatic margin is of much smaller caliber than the old one, its undulations are low and in length it cannot be traced beyond the distal end of the new rod (figs. 20 to 26). In figure 19 it was possible to trace the new

chromatic margin along only apart of the course of the new rod, although presumably it extended the whole distance. In the part which could be made out, however, it remained close to the rod, and hence transversed the deeper protoplasm along with the latter organelle. This deeper position would hardly be expected if the new chromatic margin, or posterior flagellum, had been split off from the peripherally placed old one.

I have not been able to see evidence of a splitting of the undulating membrane and the chromatic margin, as described by Kofoid and Swezy ('15), although I have searched long and diligently for such evidence. My evidence indicates that the new chromatic margin grows out along the new chromatic basal rod as a new structure just as the other flagella grow out as new structures. In figure 18 I have drawn an individual which appeared to have the old chromatic margin double for the anterior half of its length. The two portions appear to be of equal caliber. The nucleus could not be made out distinctly and there are other indications of degeneration, so that I regard this individual as abnormal, especially since I have carefully examined such large numbers in all stages of division without ever finding any other specimen that indicated a splitting of the membrane.

Wenyon ('07), Martin and Robertson ('11), and Kuczynski ('14, '18) also find the new posterior flagellum growing out as a new structure, although Dobell ('09) describes the splitting of the undulating membrane in T. batrachorum. I am inclined to agree with Kuczynski that Dobell, and Kofoid and Swezy have been misled by the secondary filament in the undulating membrane of T. augusta and T. batrachorum, and I am quite convinced that splitting of the undulating membrane does not normally occur in T. muris.

4. The blepharoplast. After the new chromatic basal rod has been formed, the new blepharoplast appears, connected to the old one by the paradesmose (Kofoid and Swezy, '15). Figure 17 shows a relatively early prophase with the new rod attached to a small granule, which in turn is connected with the old blepharoplast. In my opinion, this small granule is the new

blepharoplast. In nearly all cases the new blepharoplast is smaller than the one attached to the old chromatic margin, and it would not be unexpected if it should begin as a small bud from the main or mother blepharoplast. The daughter blepharoplast continues to separate from the old, until the two are on opposite sides of the nucleus. The paradesmose connecting them remains on the outside of the nuclear membrane which appears to persist during division (fig. 23). Figure 22 shows the two blepharoplasts in place and the spindle forming in the nucleus, while the chromosomes are not quite completely aligned in the equatorial plate.

5. Other structures. On account of the poor stainability of the anterior flagella and on account of their frequent position on or close to the cell to which they belong, and on account of the presence ofttimes of large numbers of slender bacilli and wavy spirochaetes, the behavior of these structures in division has been difficult to follow. I am convinced, however, that the accounts of other authors are correct to the effect that one or two of these flagella accompany the new blepharoplast, while the other two or one remain with the old or parent blepharoplast (figs. 25 and 26). New flagella to make the full number appear to be formed as new outgrowths from the blepharoplasts (fig. 32).

Late in the prophase the axostyle becomes separated from the blepharoplast and begins to degenerate (figs. 20 to 22). New axostyles grow out from the blepharoplasts in the telophase, as will be described later.

I have not been able to detect any peculiarities in the behavior of the parabasal body during the prophases. I have drawn figure 16 to show that there cannot possibly be any confusion between the parabasal and the outgrowing new chromatic basal rod. The parabasal is unusually long in this specimen and there is a thin region over the nucleus which suggests that the distal end may possibly become detached. This idea is also suggested by figures 4 and 5, where there is a constriction; but in these latter cases there is no evidence of approaching division.

I have already suggested that new long rows of chromatic granules grow out from the chromatic basal rod. On the other

hand, there is some evidence of division of these granules, as seen, for instance, in figure 8. Here the long row seems to be double in the distal part and the two rows appear to lie close together. The distance between them is foreshortened, however, in this position. The duplication in connection with the short row behind the nucleus is difficult to interpret, and I am not sure that division of the granules is indicated.

B. Metaphase

Figures 22 to 26 show a series which includes a very late prophase or early metaphase (fig. 22), metaphases, and early anaphases, which indicate very well the behavior of the chromosomes in these stages. In figure 22 the chromosomes are still similar to those of the earlier prophases, the two parts of each being closely approximated with their long axes parallel. Although the fibers of the forming spindle have already become attached to the chromosomes, the latter have not as yet lined up into a definite plate. It appears from these figures that whatever directive influence the spindle fibers may have in the separation of the chromosomes, it is exercised for some of them before the plate has become established. All the figures with an equatorial plate show the two parts of some of the chromosomes already drawn out so that they are in contact only at their ends, while others are just in the process of being separated. Since I have seen a great many animals in the stage indicated by figures 23, 24, and 25 and none showing stages between them and figure 22, I judge that some of the chromosomes are separated during the formation of the metaphase plate.

As seen in the figures mentioned, the number of chromosomes in the metaphase is definitely six, the number found in the prophases. Martin and Robertson ('11) and Kuczynski ('14, '18), as previously noted, believe that eight(?) prophase chromosomes are reduced to four multiple elements in the metaphase. I think I have demonstrated the probability that the prophase number in Kuczynski's figures is six, and the tendency for the metaphase chromosomes to clump probably accounts for the apparent number, four. Kofoid and Swezy ('15) do not show

any metaphase figures for T. muris, and even in their extensive figures for T. augusta they have nothing corresponding with my figure 22. They therefore missed the evidence showing that the process of separation at the metaphase and anaphase corresponds to the details as seen in the corresponding stages in metazoan mitoses, except for the precocious separation toward the two poles before the equatorial plate is completely formed. Their figures 20 and 21 for T. augusta in which the metaphase chromosomes are seen as single elements elongated in the direction of the spindle axis possibly show conditions in which the constriction between the separating chromosomes has been eliminated by contraction of the chromatin in the process of fixation.

C. Anaphase

Figures 26 and 27 illustrate anaphases. I have not seen so many anaphases as I have metaphases, and presume that this phase is of shorter duration. During this stage the chromosomes appear to become elongated (fig. 26) and constricted (fig. 27). Figure 27 shows the smallest chromosome as having divided precociously and the daughter elements are much nearer the poles than those of the other chromosomes.

D. Telophase

After the chromosomes have been completely separated and the two daughter groups have arrived at positions some distance apart, the nucleus which has been elongating during the anaphase (fig. 27) becomes constricted in the middle (figs. 28 and 29), thus forming the two daughter nuclei. The nuclear membrane persists throughout this process. In the early telophases the chromosomes begin to change their appearance, becoming less dense and more granular. The constriction which first appeared in the anaphase becomes more pronounced and each of the former chromosomes appears to be made up of two rounded or slightly elongated parts in contact at the ends (fig. 31). These eventually give rise to the scattered granules seen in the resting nucleus and the new caryosome becomes established surrounded by its

characteristic clear area. I have not been able to make out the precise method of origin for the caryosome.

The entire number of six chromosomes can usually be seen when a polar view of the telophase group can be had, such as is shown for the lower nucleus in figure 30. The complete number is also seen in the side views of figure 31. Kuczynski ('14) likewise shows six in a similar stage in his figure 66 of T. muris. His figures 64 to 67 and 69 also show well the constriction of the anaphase and early telophase chromosomes that I have mentioned.

Since the telophase chromosomes appear to resolve themselves each into two chromomeres, and since the earliest prophase chromosomes which can be recognized as such are already double, one naturally wonders if the two parts of a prophase chromosome may not be represented by the two telophase chromomeres. Since the two telophase chromomeres are arranged end to end, while the two parts of a prophase chromosome are arranged side by side, and since the number of chromatin granules in the resting nucleus is rather large and indefinite, the direct relationship suggested is improbable.

While the two daughter nuclei are becoming reorganized into typical resting nuclei, complete sets of other organelles are being established for the two new individuals. The origins of most of these organelles have been discussed in connection with the prophase. The chromatic basal rod and the flagella merely complete a development initiated at the earlier phase. The new axostyles, however, apparently do not begin to grow out until the telophase. There is a suggestion of a new axostyle growing out from the old blepharoplast in the early stage shown in figure 29, but in figures 32, 33, and 34 the new axostyles are distinctly seen. In figure 34 it will be noted that the new axostyle growing out from the older, larger blepharoplast is longer than the other one, as might be expected. It will also be seen from these figures that there is a row of chromatic granules along the new axostyles. These appear to be imbedded in the axostyles and are probably intimately concerned in the formation of these organelles. These granules must go to the surface later or disappear, for they do not occur within the adult

axostyle. It is possible that the chromatic granules seen in the adult along the axostyle from the blepharoplast to behind the nucleus (figs. 3, 7, and 8) are the same as the ones which appear to be concerned in the formation of the new axostyles.

It is probable that the degeneration of the axostyle in the late prophases accounts for the rounding up of these animals at about that stage in the division process.

Kuczynski ('14, '18) saw and figured the degeneration of the old axostyle and the growing out of the new ones from the blepharoplasts, and Martin and Robertson ('11) report the same thing for T. eberthi. Wenyon states that the axostyle ('pointed organ') divides by longitudinal division, but offers no evidence in support of this statement. Kofoid and Swezy ('15) show one figure (fig. 60) which they interpret as showing division of the axostyle in T. muris. But the figure is also open to the interpretation as a partial superposition of two independently formed elements, and since it is the only one they could find after prolonged search, the evidence is not very conclusive. Since the evidence of the degeneration of the old axostyle and the origin of new ones as outgrowths from the blepharoplasts is so conclusive in my material and in the results reported by Kuczynski, the origin of this structure by splitting may be regarded as extremely doubtful, at least for T. muris. Dobell ('09) and others were undoubtedly in error in believing that the new axostyles developed from the paradesmose. This structure retains its connection with the two blepharoplasts for some time after the division of the nucleus (figs. 31, 34), but eventually disappears.

Kofoid and Swezy state for T. muris that the long row of granules disappears during metaphase and reappears in the telophase. I have been able to find them at practically all stages of division but, as previously noted (p. 128), there is evidence that the old row may be replaced by a new one which is budded off from the ventral (inner) side of the chromatic basal rod. Just how the new chromatic basal comes to have an associated row of granules has not been determined.

I have not been able to find stages showing any division of the parabasal body. In all the anaphases and later stages (figs. 26 and 28) there appears to be a parabasal for each blepharoplast. Whether the old one disappears and two new ones grow out, or whether the old one remains and one new one grows out, or whether some other mode of origin may prevail has not been determined. One point should be noted, however, namely, that in these anaphases and telophases the parabasal attached to the daughter blepharoplast is always smaller than the one connected with the old blepharoplast. Considering a possible analogy with the chromatic basal rod, this fact might be interpreted as indicating that the old parabasal persists and a new one grows out from the new blepharoplast. The one attached to the old blepharoplast is not so long as the longest ones seen in the non-dividing and earlier prophase stages (figs. 3, 4, 5, 16), but is comparable in length to the portion proximal to the constriction as seen in figures 4 and 5 or proximal to the fainter area in figure 16. The suggestion already made that the portion distal to the constriction may become detached will be recalled.

The origin of all the new structures has been discussed, except the cytostome. This structure is not much in evidence during the metaphases and anaphase, but two cytostomes appear in the telophase. It is possible that the old one, like the axostyle, disappears, and two new ones are formed. Before division of the cell body, all the organelles in the two sets apparently become developed to a condition corresponding to that of the original set.

No cases have been found showing the constriction of the cell body in my fixed and stained slides, but I have frequently observed this process in the living animals. It takes place rapidly and the two separating individuals always appear to be of equal size and completely developed. The long interval between the division of the nucleus and the division of the cell body doubtless serves to allow the new organelles to attain complete development before the daughter cells separate.

I think it is worth while to point out that according to the evidence which I have presented there appear to be only two

parts of this complicated flagellate that divide equationally. They are, 1) the nucleus, including the chromosomes, and, 2) the cell body. The blepharoplast, chromatic basal rod, posterior flagellum, and possibly also the parabasal body and one or two of the anterior flagella of the parent appear to be retained by one of the new daughter individuals, while the other daughter is supplied by new outgrowths, including a new small blepharoplast budded off from the parent one. The old axostyle, and possibly also the old cytostome, disappear and a new one is formed for each new cell. New chromatic granules appear to have a different origin, as previously described. This behavior is paralleled by that of the Infusoria, exemplified by Paramecium, which remains active during the process of division. Some of the cilia and one of the contractile vacuoles are taken by each daughter cell, and new ones are formed to make the complete set of organelles. Part of this development in Paramecium takes place after the separation of the daughter cells, whereas in Trichomonas development of the new organelles appears to be completed before the daughters separate. Since in both cases the daughter cells come to resemble each other completely, their hereditary potentialities must be equally descended from the parent. An equational division of the nuclear material would therefore be sufficient to insure equality between the daughter cells, granting that the nuclear material constitutes the physical basis of heredity.

SUMMARY OF THE MORE IMPORTANT RESULTS

1. Trichomonas muris (Hartmann) from the coecum of the mouse measures 10 to 16μ long by 5 to 10μ wide, but varies in size slightly from host to host and to a larger extent as a result of the use of different fixatives.

2. Different fixatives also give rise to different staining reactions of the protoplasmic vacuoles, nuclei, and other organelles.

3. The anterior free flagella are short, not more than half the length of the body, and stain faintly with iron-alum haematoxylin stain. The posterior flagellum stains intensely as the chromatic margin of the undulating membrane, but its posterior

free extension is similar to the anterior flagella in length and staining capacity.

4. The chromatic basal rod is thicker in the middle and tapers toward both ends. It appears to give origin to the outer row of chromatic granules by a kind of budding process.

5. There is a deeper row of chromatic granules near the axostyle extending from behind the nucleus up to the blepharoplast.

6. There is a parabasal body similar to the one described by Janicki and Kuczynski. It has a position dorsal and to the right of the nucleus. It varies in appearance and occurrence from host to host, from flagellate to flagellate, and from one fixative to another. It has appeared after the use of weak Flemming's, Flemming's without acetic, and 1 per cent chromic acid solutions.

7. In the prophase of division the chromatin becomes organized into six double (split ?) prophase chromosomes and the caryosome gradually disappears. A new chromatic basal rod grows out from the blepharoplast and appears first as a row of fine granules. It is connected with the small new blepharoplast which a little later becomes budded off from the main, or parent, one. The two blepharoplasts are connected by a paradesmose.

8. Before the metaphase has been reached the axostyle becomes detached from the blepharoplast and begins to disintegrate.

9. In the metaphase six definite chromosomes are found, but the two parts of each tend to separate in the late prophase while the equatorial plate is forming.

10. In the anaphase the chromosomes become granular and each divided into two equal parts by a transverse constriction. The body of the nucleus divides by simple constriction, the nuclear membrane persisting through the process.

11. In the telophase six chromosomes, each doubled by the transverse constriction, can be seen. These become organized into the 'resting' nucleus. A new axostyle grows out from each blepharoplast. The origins of the new parabasal bodies and cytostomes were not definitely made out.

12. The two sets of organelles retain the common protoplasmic body until development is complete, and then the cell body divides rapidly.

13. The only parts of the cell to divide equationally are, *a*) the nucleus, including the chromosomes, and, *b*) the cell body.

LITERATURE CITED

Allen, Ezra 1916 Studies on cell division in the albino rat (Mus norvegicus, var. alba). II. Experiments on technique, etc. Anat. Rec., vol. 10.

Cutler, D. Ward 1919 Observations on the protozoa parasitic in the hind gut of Archotermopsis wroughtoni Desm. Part I. Ditrichomonas (Trichomonas) termitis Imms. Quart. Jour. Mic. Sci., vol. 63.

Dobell, Clifford C. 1909 Researches on the intestinal protozoa of frogs and toads. Quart. Jour. Mic. Sci., vol. 53.

1914 Cytological studies on three species of amoeba, etc. Arch. f. Protist., Bd. 34.

Hartmann und Kisskalt 1910 Practikum der bakteriologie und protozoologie. II Teil. Protozoologie, von M. Hartmann. 2te aufl. Jena.

Janicki, C. 1911 Zur Kenntniss der Parabasalapparat bei parasitischen Flagellaten. Biol. Cent., Bd. 31.

Kofoid, C. A., and Swezy, Olive 1915 Mitosis and multiple fission in trichomonad flagellates. Proc. Amer. Acad. of A. and S., vol. 51.

Kuczynski, M. 1914 Untersuchungen an Trichomonaden. Arch. f. Protist., Bd. 33.

1918 Ueber die Teilungs vorgänge verschiedener Trichomonaden und ihre Organisation im allgemeinen. Arch. f. Protist., Bd. 39

Martin, C. H., and Robertson, Muriel 1911 Further observations on the coecal parasites of fowls with some references to the rectal fauna of other vertebrates. Part I. Quart Jour. Mic. Sci., vol. 57.

Swezy, Olive 1916 The kinetonucleus of flagellates and the binuclear theory of Hartmann. Univ. of Cal. Publ. in Zool., vol. 16.

Wenyon, C. M. 1907 Observations on the protozoa in the intestine of mice. Arch. f. Protist., Suppl., Bd. 1.

EXPLANATION OF PLATES

The drawings have all been outlined with the aid of a camera lucida, using a Spencer 1.8 mm, oil-immersion objective and a Zeiss no. 12 compensating ocular. The draw-tube was set to make a magnification of 4000 at the level of the table where the tracing was done. In reproduction the magnification has been reduced to 3000. Details of structure were completed with ink while the object remained under observation, then each drawing has been checked two or three times by subsequent comparison with the object. All figures from material stained with iron-alum haematoxylin. Fixation will be indicated for each figure. For these fixing fluids the following abbreviations will be used: Allen's for Allen's 'B–15,' Carn. for Carnoy's fluid, Schaud. for Schaudinn's fluid, sub.-acet. for sublimate-acetic, and wk. Flem. for the weaker fluid of Flemming.

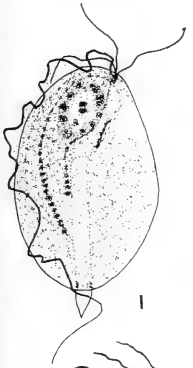
PLATE 1

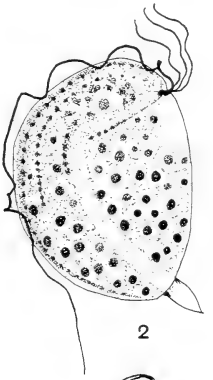
EXPLANATION OF FIGURES

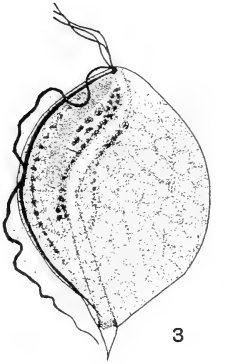
1 to 3 Vegetative individuals showing some differences due to use of different fixatives; fig. 1, Carn., chromatic basal rod not stained; fig. 2, sub.-acet., vacuole contents stained; fig. 3, wk. Flem., parabasal body stained.

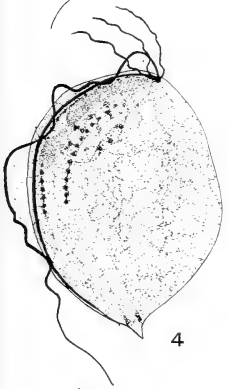
4 to 6 Wk. Flem., parabasal body stained, constricted in figs. 4 and 5, undergoing change in fig. 6.

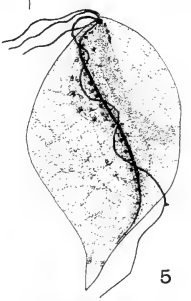
7 to 9 Schaud., vegetative individuals; fig. 7, view from left side; fig. 8, possible division of rows of chromatic granules; fig. 9, hypertrophied (abnormal ?) nucleus.

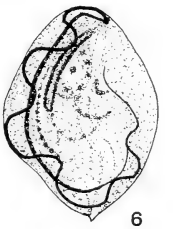

1

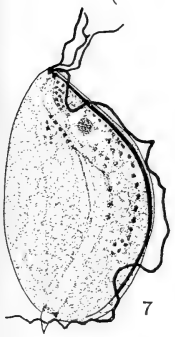

2

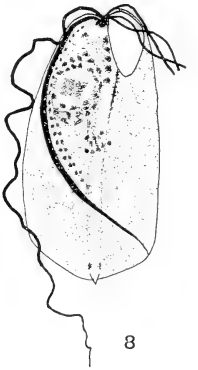

3


4


5


6


7


8

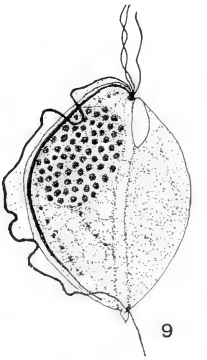
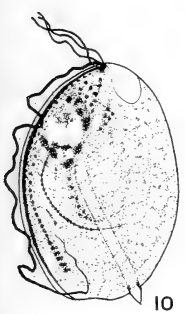

9

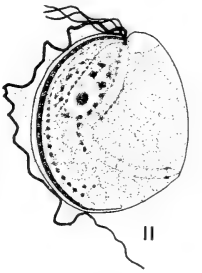
PLATE 2

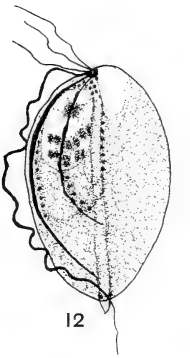
EXPLANATION OF FIGURES

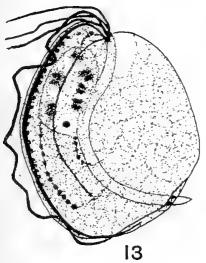
Figs. 10 to 15, 17, 18, Schaud.; fig. 16, wk. Flem.

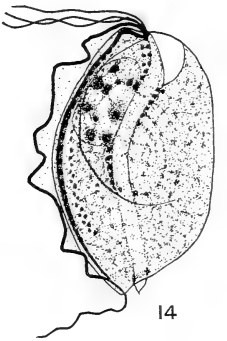
10 to 17 Prophases; fig. 18, apparent division of chromatic margin of undulating membrane. Figs. 10 and 11, early granular stage in the formation of the new chromatic basal rod. Figs. 12 to 15 and 17, six double split chromosomes besides the caryosome which gradually loses its staining capacity. Fig. 16, new chromatic basal rod together with the parabasal body. Fig. 17, budding of a small new blepharoplast to which the new chromatic basal rod is attached.

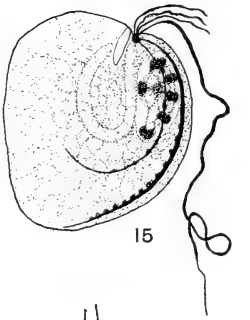

10

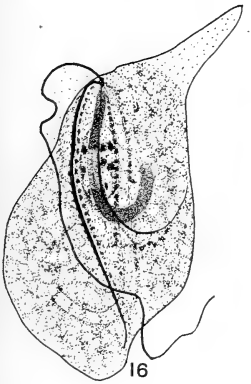

11

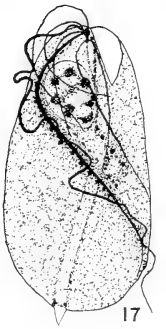

12


13


14


15


16


17

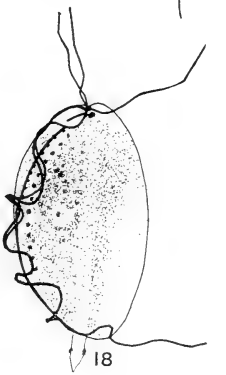

18

PLATE 3

EXPLANATION OF FIGURES

Figs. 19 to 21, 25, Schaud; figs. 22 to 24, 27, Allen's; fig. 26, wk. Flem. Figs. 19 to 21, late prophases; figs. 22 to 25, metaphases; figs. 26 and 27, anaphases.

19 Paradesmose between blepharoplasts; new chromatic basal rod and new chromatic margin penetrating deep into the cytoplasm; chromosome moieties fused, giving the appearance of six single elements.

20 Blepharoplasts 180 apart; axostyle detached and beginning to degenerate.

22 Begininng of the spindle and beginning of separation of daughter chromosomes; remnant of caryosome seem.

26 New parabasal body attached to the daughter blepharoplast.

27 Constriction in the anaphase chromosomes; a small chromosomes has divided precociously.

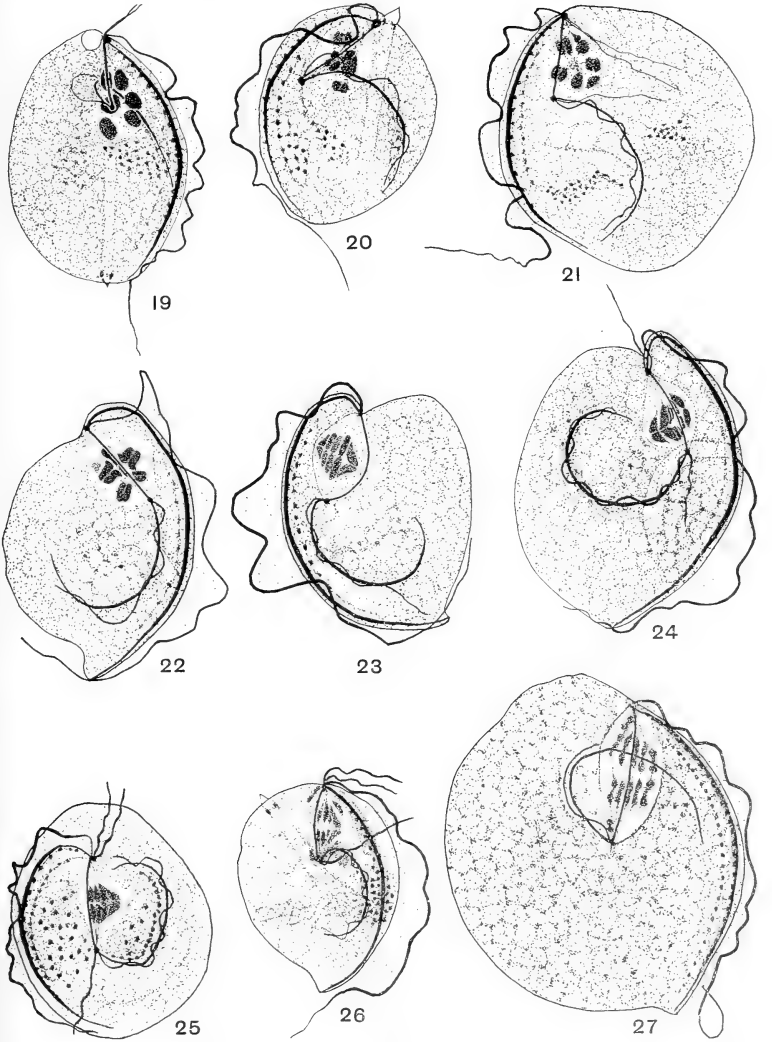

19
20
21
22
23
24
25
26
27

PLATE 4

EXPLANATION OF FIGURES

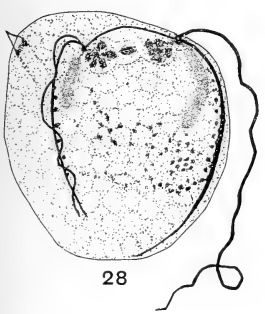
Fig. 28, wk. Flem.; figs. 29 to 32 and 36, Schaud.; figs. 33 to 35, Allen's.

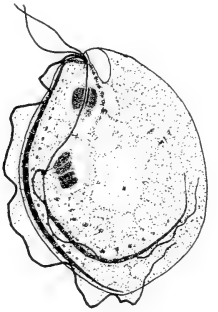
28 Early telophase: delayed separation of chromosomes; two parabasal bodies.

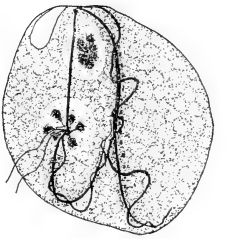
29 Constriction of nuclear membrane; degenerating axostyle.

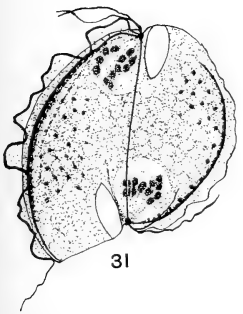
30 to 34 Telophases. Fig. 30, side view of one, and polar view of the other daughter nucleus. Fig. 31, constriction in daughter chromosomes; paradesmose intact. Figs. 32 to 34, formation of 'resting' nuclei and outgrowth of new axostyles.

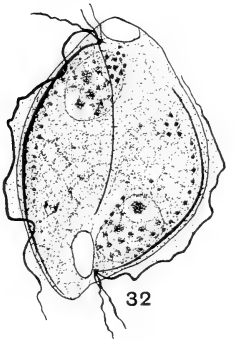
35 and 36 Precystic changes.

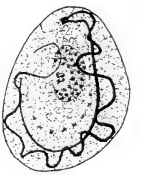

28

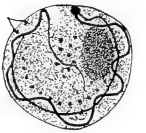

29

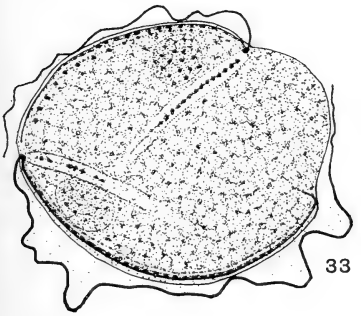

30

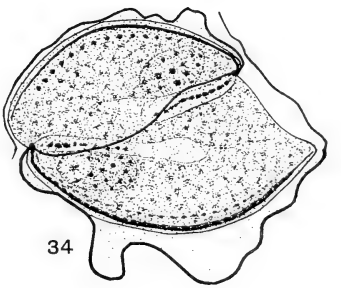

31


32


35


36


33


34

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

THE CIRCULATORY SYSTEM AND SEGMENTATION IN ARACHNIDA

ALEXANDER PETRUNKEVITCH
Yale University

TWO TEXT FIGURES AND TWO PLATES (SEVEN FIGURES)

The circulatory system in Arachnida has been made repeatedly the subject of studies and is fairly well known. Nevertheless, several points have escaped observation, partly on account of technical difficulties, partly because the attention of the investigators was directed toward other aspects of their study. Thus it came to pass that the relation of the circulatory system to the problem of segmentation in arthropods received less attention than it deserves. Indeed, in this respect the circulatory system may be more valuable than the nervous system and may, with a certain portion of the alimentary canal, of which I shall speak in a later contribution, help to establish definite homologies within the phylum of arthropods, and thus not only clear the relationships between the various classes belonging to that phylum, but also throw light on their phylogeny.

Many years ago, in the spring of 1905, while on a trip to Jamaica, West Indies, I collected scorpions and spiders, preserving them in the only fluid then obtainable in Jamaica, a mixture of alcohol with ether. The specimens were simply thrown into a jar and left in the fluid, as I did not intend to use them for any anatomical or microscopical study. Quite recently I wanted a few sections through scorpion embryos for class demonstration, so I imbedded and sectioned some quite young scorpions which had been carried by their mother on her back and of which I had many specimens representing stages before and after the first moult. I also took embryos out of the uterus. My surprise was great when I found that the fixation of the tissues was

remarkably good and that prolonged sojourn in the preserving fluid made the material considerably less brittle than it usually is on account of the voluminous yolk. A cursory examination of the sections has revealed so many interesting deviations from the usually accepted descriptions of the anatomy and embryology of scorpions, that a number of carefully oriented and sectioned series of various stages were made. All sections were purposely made 20μ thick. Exact orientation for sagittal, frontal, and transverse series was comparatively simple on account of the size, shape, and coloration of the material. A few of the series are absolutely symmetrical. The most satisfactory staining proved to be haematoxylin followed by orange G.

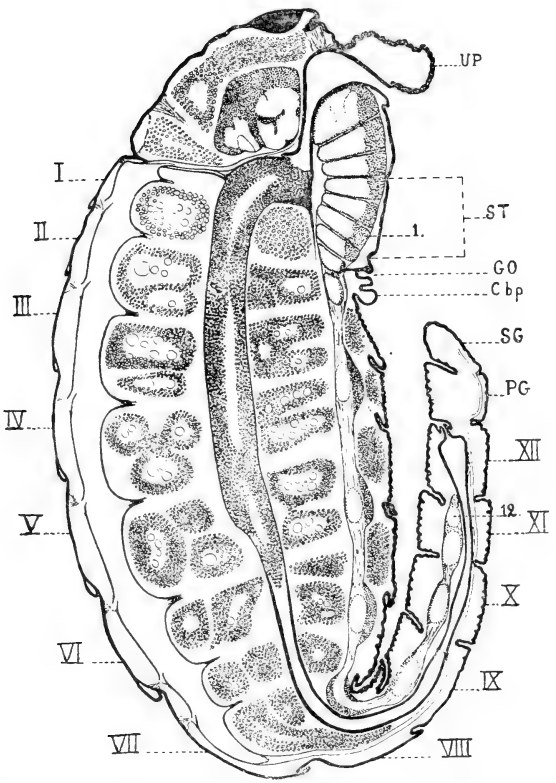
When the study of the circulatory system revealed the remarkable similarity in this respect between scorpions and spiders, I prepared corresponding series through very young spiders fixed for the purpose in my sublimate mixture. Two species of scorpion (Centrurus insulanus and C. carolinianus) and three species of spiders (Agelena naevia, Lycosa carolinensis, and Pholcus phalangioides), belonging to three different families, form the basis of the present study. While it would be very interesting to extend it over other groups of Arachnida, the diversity of the material leaves no doubt that similarity here is not accidental, but is the expression of true homology, and that generalization is therefore warranted and helpful.

To avoid possible misinterpretation of results, obtained only from the study of sections however perfect, a few scorpions were injected through the heart. These scorpions, Centrurus carolinianus of Texas, were obtained alive through the courtesy of Professor Painter, of the University of Texas, who kindly took the trouble to collect and mail to me a dozen specimens. Injection was made in a manner similar to that which I used for Lycosa several years ago, but the technique in the case of the scorpion is somewhat more complicated, owing to the fact that the heart is only imperfectly visible through the chitin. For this reason it becomes necessary to open the chloroformed specimen in a saline solution to expose the heart. The tergites of the second to sixth abdominal segments are carefully removed,

beginning with the posterior edge of the sixth tergite. The ligaments of the heart are cut close to the hypodermis with a sharp scalpel, as otherwise the heart would sustain injury. Freshly precipitated carmine as injection fluid proved to be quite satisfactory. Not only the large vessels become injected to their end near the base of the claws in the legs, but many ramifications of pedal arteries appear dark red. The injected specimen is next fixed in 95 per cent alcohol, dehydrated in absolute alcohol, and cleared in cedar oil, in which it becomes sufficiently transparent for further preparation. All organs obstructing the view are now carefully removed with the aid of two needles under a binocular dissecting microscope and the entire circulatory system exposed to view.

Usually the circulatory system in scorpions is described as consisting of a dorsally situated heart which gives rise anteriorly to the cephalic aorta and posteriorly to the posterior aorta. The heart itself is said to consist of eight chambers with a pair of ostia each, or eight pairs of ostia altogether, typically one pair for each segment of the body. The cephalic aorta is described as giving rise to a pair of arteries near its base, and a little further to another pair. The latter assume a downward course, pass on each side of the oesophagus, forming a ring from which six pairs of arteries are said to be given off to the appendages, while a single median supraneural artery runs from the ring backward above the nervous system. The usual description of the finer ramifications, as well as of the arteries given off by the heart, is irrelevant to our purposes and may be entirely omitted.

The microscopical structure of the heart seems in all Arthropoda to be more or less the same. Its wall is composed of three layers. The outer layer, the adventitia, consists of connective tissue. The heavy media or muscularis is formed either by a spiral muscle or by symmetrically arranged semicircular muscle fibers which meet in the middorsal and midventral line, as has been described by Bergh for insects. The inner layer or intima is, whenever present, nothing but a very thin transparent membrane which, according to Verson, may be nothing but the sarcolemma of the muscle fibers of the media.



Text figure A. Centrurus insulanus, late embryo. Sagittal section in the plane of symmetry, showing all organs developed at that age. The black spot above is one of the median eyes. In front of the eye is the dorsal dilator of the pharynx. Abdominal tergites marked with Roman numerals *I* to *XII* without reference to their embryological history. *1*, the first abdominal neuromere; 12, the last neuromere; *Cbp*, basal plate of comb; *GO*, genital opening; *PG*, poison gland; *SG*, sting; *ST*, sternum; the dotted lines showing its anterior and posterior margins; *UP*, upper lip (rostrum). Further explanation in the text.

This description of the gross anatomy of the circulatory system is, as we shall see, not quite correct, but served to show the similarity between the scorpion and Limulus. On the other hand, it created differences between scorpions and spiders especially, since the circulatory system of the latter had only recently been described correctly. Moreover, the exact position of the heart in both groups remained unnoticed, and yet therein lies its value from the morphological point of view.

The best material for the study of the circulatory system in the scorpion is furnished by that stage of embryos represented in our text figure A, in which all organs are already fully developed, but the nervous system has not yet reached its final state of contraction. The postabdomen is still short, the length of each segment being smaller than the diameter. The last neuromere has already begun to fuse with the preceding one and is almost entirely withdrawn into the fourth postabdominal segment. The first and second abdominal neuromeres have moved forward, passed the diaphragm, are completely within the cephalothorax, and are fused with the thoracic ganglia into one mass in which the separate neuromeres remain however clearly defined as they do, even in adult scorpions. The third abdominal neuromere is just on the verge of passing the diaphragm, while the fourth, which in the adult scorpion forms the last portion of the thoracic ganglionic mass, is still in the abdomen just behind the genital opening and on a level with the basal plate of the comb. In this connection we may state that Buxton had recently shown that the comb receives its nerves from the eighth postoral ganglion of the suboesophageal mass, i.e., from the third abdominal neuromere—an observation which I am able fully to confirm.

In median sagittal sections the diaphragm appears as a thin line. Its ventral portion runs from the ventral body wall just in front of the genital opening to the endosternite which lies above the nervous system. Its dorsal portion arises from a vertical transverse crest of the endosternite and proceeds upward to the dorsal body wall, where it is attached between the carapace and the first abdominal tergite.

The midgut may easily be recognized because of the presence of a cardiac valve, because of its thin walls and, further, because it is still filled with embryonic yolk. It has the appearance of a wide tube with larger lateral branches or diverticula usually known under the name of 'liver.' The anterior portion of the midgut proper runs at almost a right angle to the longitudinal axis of the body and belongs to the cephalothorax because it lies in front of the diaphragm. Its posterior end extends only to the end of the fifth abdominal segment. The diverticula of the midgut extend forward almost to the anterior end of the cephalothorax and backward through the entire preabdomen, the last dorsal diverticulum reaching even into the first post-abdominal segment. The gross anatomy and segmental arrangement of the diverticula are not easy to understand. Sagittal sections show a clear separation of the diverticula into ten metamerically arranged groups, two of which are in the cephalothorax and eight in the preabdomen. Their metameric nature is especially emphasized by the dorsoventral muscles and the hypocardiac ligaments. The ligaments shown in text figure A as ventral projections of the heart exist only in the preabdomen. Of the muscles, one pair is in the cephalothorax where they are attached by one end to the carapace between the two cephalothoracic diverticula and by the other to the endosternite above the nervous system. There are eight pairs of dorsoventral muscles in the preabdomen passing between the diverticula, right and left of the midgut, from the back to the ventral surface of the body. The first pair is easily overlooked as it lies closely applied to the posterior surface of the diaphragm. Dorsally, these muscles are attached to the anterior end of the first abdominal tergite on the outside of the epicardiac ligaments. Ventrally, they are attached, like the cephalothoracic pair, to the endosternite above the nervous system. All other dorsoventral muscles are attached at both ends to the chitin of the body wall. The dorsal attachments are to the outside of the epicardiac ligaments. The ventral attachments are slightly farther apart than the dorsal, one pair for each abdominal sternite, the second pair lying at the sides of the genital opening, and the third at the sides of the basal plate of the comb.

Although the diverticula are divided by the dorsoventral muscles into ten groups, there are only six pairs of ducts connecting them with the midgut proper. The first pair of these transverse ducts is in the cephalothorax and connects the two cephalothoracic diverticula with the anterior portion of the midgut. The first, second, third, and fourth abdominal diverticula have a pair of transverse ducts each in the corresponding segments. The last pair of connecting ducts opens into the midgut in the fifth abdominal segment. These ducts are long, extending backward over three segments and establishing a connection between the midgut and all four posterior metameric groups of diverticula.

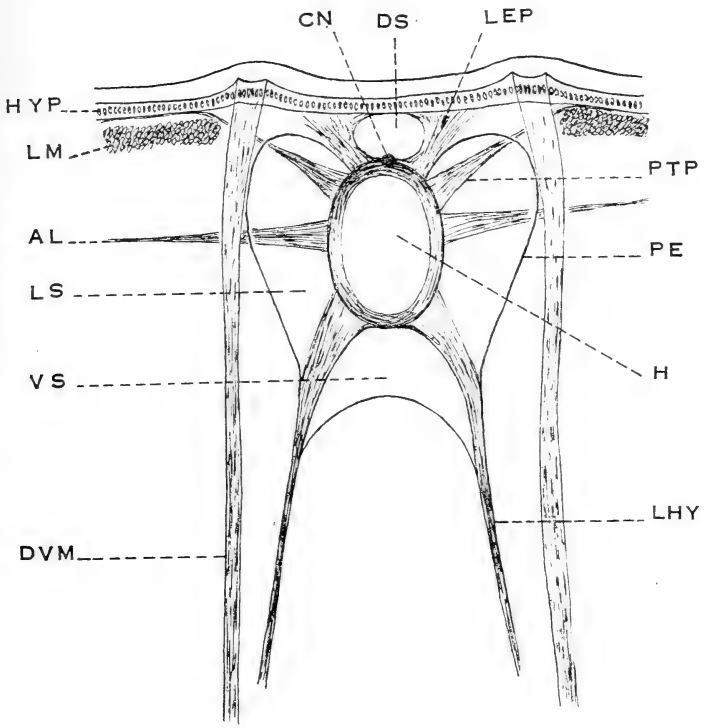
Transverse sections through the second to fourth abdominal segments show that each pair of diverticula is composed of two dorsal, two ventral, and one lateral lobes. All lobes are filled with embryonic yolk and all those on the same side are naturally connected with each other near their base. The dorsal right and left lobes are separated by the pair of dorsoventral muscles. Below the midgut proper, which occupies approximately the center of a transverse section, the dorsoventral muscles pass on the outside of the inner pair of ventral lobes. In the cephalothorax only dorsal lobes are present. The last pair of transverse ducts gives off side branches to the fifth and then to the sixth abdominal diverticula, while the ends of the ducts lead into the seventh pair into which the eighth pair also opens.

The hindgut begins in the terminal region of the fifth abdominal segment. It is considerably smaller than the midgut, but has both relatively and absolutely much thicker walls. It is devoid of embryonic yolk and in the last postabdominal segment forms a considerable widening beyond which it is again suddenly constricted and opens with a small anus in the midventral line at the end of the segment.

The heart lies entirely in the preabdomen, extending from the diaphragm which separates the latter from the cephalothorax, almost to the end of the seventh abdominal tergite. Its anterior and posterior limits are clearly defined by the valves of which I shall speak later. The heart has seven pairs of ostia

(not eight as usually erroneously stated). The presence of the ostia gives the heart the appearance of an eight-chambered organ. In reality there are neither valves nor any constriction or impediment in the spaces between the successive ostia. There is, therefore, nothing that would have the morphological value of chambers. In transverse sections the ostia occupy a position approximately half-way between the dorsal pole and the equator of the heart. The first pair lies exactly at the posterior edge of the first abdominal tergite. The position of all seven pairs may be best understood from text figure A. It is the same in all specimens and does not change with maturity. The structure of an ostium with its valve is represented in figure 3 on a large scale. This is a frontal section, and the media or muscularis is therefore sectioned at right angles to its muscle fibers. Each fiber has the appearance of a rectangle. The adventitia of connective tissue is well defined and shows long, darkly stained nuclei. Multinuclear fibers of connective tissue run from the edges of the ostium laterally, converging and forming a ligament, the so-called pteripyle.

The position of the ligaments by which the heart is suspended is well known in spiders, owing to several researches, especially those of Causard. There is scarcely any difference in this respect between spiders and scorpions. Text figure B, drawn from a complete series of transverse sections through a late embryo, represents the heart of the scorpion with all its ligaments of a single group projected into the same plane. All told, there are eight metamerically arranged groups of ligaments, each group, except the first, composed of four pairs. The shortest of these are the epicardiac ligaments which pass on each side of the dorsal sinus and are attached to the basal membrane of the hypodermis, thus clearly demonstrating their connective nature, since all muscular fibres, as for example those of the dorsoventral muscles represented in the figure, pass between the hypodermal cells and are inserted in the base of the cuticle. The second pair are the pteripyles. Their distal end merges with the somatic connective-tissue layer which separates the dorsal longitudinal muscles of the abdomen from the hypodermis. The third pair,



Text figure B. Centrurus insulanus, late embryo. Transverse section through the heart in the region of the third abdominal segment, showing the ligaments. The drawing was made from two sections, as not all ligaments are in the same plane. The epi- and hypocardiac ligaments are in one plane, while the pteripyles and alar are ligaments in another. *AL*, alar ligaments; *CN*, cardiac nerve; *DS*, dorsal pericardial sinus; *DVM*, dorsoventral muscle; *H*, heart; *HYP*, hypodermis; *LEP*, epicardiac ligaments; *LHY*, hypocardiac ligaments; *LM*, dorsal longitudinal muscles; *LS*, lateral pericardial sinus; *PE*, pericardium; *PTP*, pteripyle; *VS*, ventral pericardial sinus.

often called alary muscles, are the alary ligaments. Their ligamentary nature has been elucidated by Causard. They are directed at almost right angles to the longitudinal axis of the heart. Distally they are not attached to the body wall, as usually stated, but merge with a layer of connective tissue, evidently representing the splanchnic coelom covering and separating the diverticula of the midgut from other organs situated above the latter. The fourth pair are the hypocardiac ligaments. They are by far the strongest and longest, and are easily mistaken for muscles, especially where they intercross with the dorsoventral muscles. From here on they continue diverging and unmistakably and finally merge with the splanchnic layer of connective tissue which covers the diverticula of the midgut from below. The first group of ligaments consists of two pairs only. The epicardiac ligaments are attached to the anterior edge of the first tergite. The hypocardiac ligaments are more or less normally developed, but the pteripyles and alary ligaments are wanting.

Since there are no muscles for the dilatation of the heart, diastole is accomplished through the elasticity of the heart ligaments. This explains why the muscularis of the heart is so powerfully developed. During systole the heart has to overcome the resistance of the ligaments, while the contraction of the latter during diastole is not impeded by the relaxed muscles. There is nothing unusual in such arrangement, as a similar condition exists in almost all joints of the appendages in Arachnida, where flexing is accomplished by muscular contraction and extension by the elasticity of the interarticular chitinous membrane. I have counted 120 pulsations of the heart in one minute.

The pericardium appears as a thin membrane, and the space between it and the heart is, in sections, invariably filled with coagulated blood plasm, and consequently is clearly discernible. Owing to the presence of epicardial and hypocardial ligaments, this space is subdivided into four regions which may be termed sinuses, though they communicate with each other in those regions of the heart where there are no ligaments. The lateral sinuses are the largest, next in size is the ventral sinus, while the dorsal sinus, almost round in shape, is the smallest of the four.

In the dorsal midline of the heart, partly imbedded in a groove in the wall of the heart, the cardiac nerve extends from one end of the heart to the other (text figure B, *CN*). The nerve is clearly visible in all transverse sections and unquestionably corresponds to the cardiac nerve described in Chilopoda (Duboscq), Protracheata, and other Arthropoda. As my material is not specially prepared for the study of nerves, I am unable to find a connection of the nerve with the brain, but such connection has been described by Police in Euscorpius.

The structure of the anterior aortic valve is best understood from median sagittal sections and sections which traverse the valve more or less at right angles. In the first (fig. 1, *AV*) the valve appears as a line attached to the dorsal wall of the heart exactly under the epicardiac ligaments, inclined downward, and about two and a half times as long as the diameter of the heart at the place of the attachment of the valve. In reality the valve is a muscular membrane arising from the dorsal half of the wall of of the heart and attached to the sides of the vessel throughout its length. The anterior edge of the valve is longer than the diameter of the vessel. The valve has, therefore, a peculiar shape, being concave or troughlike at its free edge and convex or arched at its base. About half-way between its base and end the valve is drawn tight in the equator of the transverse section of the vessel. Such a section is represented in figure 4, which also shows that the valve is not a fold, but consists of a single layer of transverse muscular fibers with elongated nuclei. There is always a greater accumulation of blood-cells above the valve than below it, showing that the action of the valve is perfect.

The structure of the posterior aortic valve is more difficult to ascertain, and is somewhat different from the anterior one. Text figure A represents the position of the posterior valve as being not far from the posterior edge of the seventh abdominal tergite. This position is constant in specimens of all ages. The valve seems to have the shape of a cone, the open free apex of which is directed posteriorly, while the broad base is attached to the wall of the heart along its entire circumference. This valve, too, has a single layer of circular muscle fibers composing it,

but the length of the posterior valve is many times smaller than that of the anterior valve. Indeed, the posterior aorta which begins at this place is a thin vessel gradually becoming smaller as it traverses all the segments of the postabdomen. It may be traced through the poison gland into the sting, where it ends apparently without any ramifications.

I have stated that the valves are muscular in structure. It may be objected that I have adduced no evidence in support of this assertion and that one may just as well claim that the valves are internal projections of the same connective tissue which as its adventitia surrounds the heart. Indeed, I have no sections through either the anterior or posterior aortic valves to prove or disprove either of the contentions. But I have already mentioned the fact that spiders possess the same types of valves. A comparison of figure 6 with figure 1 will show that the position and appearance of the anterior valve in both scorpions and spiders is the same. Similarly, a comparison of figures 4 and 7 will disclose the identity in structure of the anterior aortic valve in these two orders of Arachnida. Now I happen to have a great many sections through young spiders, and these show the intima lining both surfaces of the valve and continuing directly as intima of the heart itself. In many cases there is a slight loosening of the intima from the muscularis, with the consequence that it appears as an uninterrupted line. The intima of the heart being the sarcolemma of the muscle fibers, it is not conceivable that it could line any but muscular tissue.

Let us now turn our attention to the anterior aorta and the arterial blood vessels of the cephalothorax (fig. 1). The anterior aortic valve, having the exact direction of and lying immediately in front of the diaphragm represents the exact demarkation line between the heart and the aorta. Otherwise, the transition from the heart to the aorta would be scarcely perceptible. Shortly beyond the valve the aorta gives rise to a pair of small arteries supplying with blood the pair of dorsoventral muscles which separate the first cephalothoracic diverticula of the midgut from the second.

The aorta itself continues as a considerable vessel under the brain until it reaches the third neuromere of the suboesophageal ganglionic mass. Here the aortic arch around the oesophagus is formed. The arch is very short and connects the aorta with the right and left thoracic sinus opposite the base of the second pedal artery as shown in figures 1 and 2. Two pairs of vessels issue from the aortic arch. The first pair are the large cephalic arteries shown in figure 1. The second pair cannot be shown to advantage in the planes chosen for our drawings and is therefore not represented there. But these vessels are nevertheless constant in their position and easily found. Their roots are in the right and left anterior inner edge of the aortic arch, near its junction with the sinus and almost directly above the third vessel connecting the sinus with the supraneural artery. The two vessels run convergingly upward, feeding the wall of the oesophagus.

Each thoracic sinus gives rise to small and thin vessels connecting the sinus with the supraneural artery, and to four large vessels for the appendages. Of these vessels the first is the largest and splits almost immediately into two branches. The outer branch (fig. 1 and 2, *1*, *AP*) is the first pedal artery, while the inner, longer, and stouter branch is the pedipalpal artery (*APP*). The latter gives off a thin branch directed inwardly, following in its course the curvature of the ganglionic mass, and connecting with the supraneural artery just behind the pharynx. A branch of this supplies the tissues in front of the pharynx.

Posteriorly, the right and left sinus merge with each other and form a connection with the supraneural artery. This artery is single and runs in the median line above the ventral nervous system and closely applied to it. Anteriorly it runs to the very end of the ganglionic mass, turning downward in its course and now continuing backward in the midventral line below the ganglionic mass as subneural artery. Posterior to the thoracic sinus the supraneural artery continues as a straight vessel in the median line above the nervous system and closely applied to it through the entire abdomen. I have not followed its course in the postabdomen.

Nine single interneural vertical arteries connect the supraneural with the subneural artery. These vertical arteries pass exactly between adjoining neuromeres, the first artery separating the pedipalpal from the first pedal neuromere, the ninth lying immediately behind the fourth abdominal neuromere of the suboesophageal ganglionic mass. Median longitudinal connecting vessels seem to exist between all vertical arteries, but only between the fifth and sixth, and between the sixth and seventh vertical arteries the connecting vessels are invariably well discernible, as shown in figure 1.

The subneural artery does not extend beyond the suboesophageal ganglionic mass, but ends behind the fourth abdominal neuromere, where it receives the ninth vertical artery, which may be, therefore, in a way regarded as a direct continuation of the subneural artery. At the place of their junction a single blood vessel is given off ventrally. This is the comb-artery shown in figures 1 and 5. It gives off a pair of branches, one for each comb, and another pair of smaller branches for the genital opercula.

Each cephalic artery gives off several branches, the most important of which is the ophthalmic artery shown in figure 1. Beyond the ophthalmic artery the main vessel may be termed cheliceral artery. Inside the chelicera the cheliceral artery forms two ramified branches, one for the flexor and the other for the extensor of the movable finger.

COMPARISON WITH SPIDERS

Having thus described the most important features of the circulatory system in scorpions, we now may proceed to its comparison with the corresponding system in spiders. A glance at plate 3 of my paper published in the Zoologische Jahrbücher for 1920, vol. 31, will reveal both the similarities and diversities of structure. What I described there as 'Kopfarterie' corresponds with the cephalic artery of the scorpion almost to the minutest details, and shows the same ramifications. On the other hand, in the spider the cephalic arteries represent the upper branches of the thoracic arteries, the lower branches of which

lead to the thoracic sinuses, whereas in the scorpion the cephalic arteries arise from the aortic arch. This means simply a further extension of the arch in the spider, so that the aortic arch of the scorpion corresponds with the thoracic and connecting arteries of the spider. The homology is unmistakable, and it may be wiser to speak in the spider also of an aortic arch instead of a thoracic and a connecting artery.

The thoracic arch, then, of the spider opens into the thoracic sinus at the base of the second pedal artery as in the scorpion. As in the scorpion, the pedipalpal and the first pedal arteries are branches of the first arterial stem given off by the thoracic sinus. The aorta recurrens of the spider, shown in my drawings, is the supraneural artery. But for the comparison of the supraneural and subneural arteries of the scorpion and spider we have to consult the description of these arteries given by Causard, and his figures on plate IV. Instead of quoting passages in the original, I translate them with such omissions as have no relation to our subject.

"We will now consider the arteries which issue from the aortic arches. For a long time two roots were described issuing from the posterior end of each goose-foot [my thoracic sinus,—*A. P.*], forming by their junction a sort of supraganglionic anastomosis which gives rise to a longitudinal artery directed backward and running along the dorsal surface of the ganglionic mass. Schneider gave this artery the name *supraneural.* He has also shown that in front of this anastomosis there are five others. There are therefore altogether six anastomoses which this author describes as thin and delicate. This is true of the five anterior ones, but cannot be accepted as characteristic of the last one, which has a considerable diameter. Moreover, the supraneural artery is rather stout; how could it be fed by two such fine roots? These, as he shows, are often incomplete, the supraneural artery arising from a single root which may be either the right or the left one. When the root is complete this anastomosis has the shape of a V.

"The anterior anastomosis has the shape of a V open posteriorly [i.e., of an angle with a vertex directed forward—*A. P.*].

It is situated immediately under the oesophagus and gives rise anteriorly to a thin artery which is closely applied to the inferior surface of the oesophagus. This is the *suboesophageal* artery. The four following anastomoses are rectilinear and each gives rise to a vessel which issues from the middle of their ventral surface and traverses the ganglionic mass from end to end to its ventral surface. Schneider gave to these arteries the name of *median cerebellar arteries*, as he did in the case of the scorpion. I prefer to call them *ganglionic median arteries* [my vertical or interneural arteries—*A. P.*]. The supraneural artery gives also rise to a certain number of more or less short arteries of this kind, the first being omitted at the beginning of the supraneural artery and corresponding therefore with the sixth supraganglionic anastomosis. I was able to find seven or eight such arteries, thus bringing their total number to 12 or 13 . . . . What Schneider does not mention is the fact that all these arteries connect on the ventral surface of the ganglionic mass with a median longitudinal lacune" . . . . (pp. 61–62).

Although I have no conclusive evidence at this moment, either to confirm or to disprove some of the statements contained in the above quotation, it seems to me that Causard has been misled by imperfect injections. We easily recognize in the suboesophageal artery of Causard that portion of the supraneural artery, which is shown in the scorpion in our figure 2 as *SOA*. But I think that both Causard and Schneider have overlooked the connection of the 'anastomoses' with the supraneural artery. Causard, indeed, has seen their connection with the subneural artery by means of the vertical arteries (ganglionic median arteries). On the other hand, the number of these vertical arteries given by Causard as 12 or 13 seems to be decidedly too great. A careful examination of sagittal series of sections through young spiders shows invariably the presence of eleven distinct neuromeres in the thoracic ganglionic mass. The first belongs to the pedipalpi, the second to fifth to the legs; the tenth corresponds to the same neuromere in the scorpion, which in the latter is already in the abdomen. The eleventh neuromere is imperfectly divided into two. The anterior portion

is the eleventh neuromere proper, while the posterior portion represents the remnant of the abdominal neuromeres, whether contracted and fused or lost altogether makes no difference as regarding our proposition. We thus have at the most eleven interganglionic surfaces, if we count the partition of the eleventh neuromere as complete. Therefore, there cannot be more than eleven vertical arteries, since arteries passing through instead of between ganglia are not known.

The heart of the spider has four pairs of ostia in the Theraphosidae and only three pairs in the true spiders, as against seven pairs in the scorpions. From the position of the aortic valve, it is safe, however, to accept that the reduction in the number of ostia took place in a progressive direction from the rear end of the heart forward. What has happened to the rear portion of the heart, which has lost the ostia? I think it must have shrunk in size, become considerably thinner and changed into what became the proximal end of the posterior aorta. We have seen that the posterior aortic valve has a structure distinctly different from that of the anterior valve. It would be scarcely necessary to assume a progression of the posterior aortic valve, a shifting of its position with the loss of ostia. Is it not more likely that the posterior aortic valve is a modified remnant of the last pair of ostia valves which have become functionless as such, when the ostia themselves closed? With other words, that the posterior aortic valve of a Theraphosid is the remnant of the fifth pair of ostia valves, while in true spiders it is the remnant of the fourth pair?

From the above comparison of the circulatory system of the scorpion with that of the spider we may now draw the following important conclusions: the scorpion represents the more generalized and therefore more primitive circulatory system among Arachnida, the spider the more modified and therefore the more advanced. The most permanent structure in the circulatory system of Arachnida is the anterior aortic valve which is attached at the anterior edge of the first abdominal tergite and therefore marks the limit between cephalothorax and abdomen. The reduction in the number of ostia stands in direct relation-

ship with the loss of segmentation in the abdomen and proceeds in the same direction, that is, from the posterior end forward. The changes in the neural portion of the circulatory system do not extend over the thoracic haemomeres because of the permanency of the thoracic appendages, but follow the changes in the position of abdominal neuromeres. As the contraction of the longitudinal connectives between neuromeres brings abdominal neuromeres into the thorax, abdominal vertical arteries are also shifted in position, while the complete disappearance of the last abdominal neuromeres brought about a corresponding complete disappearance of the last vertical arteries.

COMPARISON WITH LIMULUS

The circulatory system of Limulus has been excellently described by Milne-Edwards, and such errors as he has admitted in his description have been later corrected by Patten and Redenbaugh. I have made injections of adult large specimens to verify the results, and can only confirm their correctness. It is different, however, with the interpretation of the structures, and here I disagree both with the older and later investigators.

Alphonse Milne-Edwards worked eight years before Lankester, and although the idea that Limulus is an Arachnid had been already advanced by Latreille and later by Owen, yet the knowledge was not sufficient to admit of incontrovertible homologies. Consequently, notwithstanding the great similarity in the structure of the nervous and circulatory systems, Milne-Edwards felt justified in pointing out the differences and in refusing to place Limulus either among Crustacea or among arachnids. For reasons which it is not worth while reviewing at present, Milne-Edwards considered the first pair of appendages in Limulus homologous, not with the chelicera, but of the pedipalpi in scorpions.

Lankester's interpretation of Limulus was colored by his theory of tagmata into which (according to him) the body of an arthropod is divided. He finds that the body of Arachnida is composed of three tagmata of six somites each and that the genital openings are placed on the first somite of the second

tagma or mesosoma. Following this idea, he finds the same tagmata in Limulus, the mesosoma being represented by the genital opercula and the five gill-plates, while the metasoma is reduced to a very small area around the anus, including the last pair of lateral spines.

Patten and Redenbaugh do not attempt to change the interpretation of Lankester, but correct it in regard to the chilaria. The presence of a distinct neuromere for this pair of appendages having been established by Kingsley, our authors naturally ascribe to them the value of a distinct metamere and consider the chilarial somite as belonging to the cephalothorax. For them, as for all previous investigators, the articulation between the carapace and the abdomen is the segmentation line separating the cephalothorax from the abdomen.

It would be useless to describe here in detail the entire circulatory system of Limulus, but certain features of it must be considered. The heart occupies the same position as in other arthropods and extends from about the middle of the line passing through the side eyes back to about the middle of the abdomen. The heart has no opening posteriorly and the superior abdominal artery is connected with the heart only indirectly through the collateral arteries. Therefore, though occupying the same position as the posterior aorta of Arachnida, the superior abdominal artery of Limulus cannot be regarded as homologous with the latter. The number of ostia is greater than in the scorpion, inasmuch as Limulus has eight pairs. Patten and Redenbaugh describe and figure a pair of rudimentary ostia in front of the aortic valve. These may be the last remnants reminiscent of a still older time when the ancestor had a heart extending farther forward. The aortic valve has almost the same structure as in Arachnida. In front of the valve "a pair of tendinous bands, comparable to a pair of alary muscles, run forward and upward a short distance beyond the limits of the pericardium, and attach themselves to the carapace close to the insertions of the tergoproplastral muscles" (p. 127). I may add that this connection is so strong that in removing the carapace the heart is easily injured, unless particular care is given to sever the connection of

these tendinous bands, which is certainly not the case with the heart ligaments of the subsequent metameres.

The aorta is exceedingly short and forms almost at once two vessels which are rightly regarded as the aortic arch. These vessels are large and long, run at first forward, then curve downward, pass the oesophagus on each side and open into the 'vascular' ring a little to the inside of and above the base of the first pedal artery. The entire ventral circulatory system of Limulus is perineural; i.e., it sheaths completely the nervous system. Not only the postoral neuromeres of the suboesophageal ganglionic mass, but the supraoesophageal forebrain as well is enclosed in this perineural circulatory system. The haemal sheath extends through the entire length of the ventral nervous cord in the abdomen. Accordingly, neither supraneural, nor subneural, nor interneural or vertical arteries are present. The cheliceral arteries issue from the ventral surface (actual, not morphological) of the vascular ring. In all this Limulus is very different from the scorpion and other Arachnida. Yet the similarity is nevertheless quite striking. If the forebrain portion of the vascular ring were removed, the rest of it would present an identical appearance with the two thoracic sinuses of the Arachnida. The similarity is increased by the existence of five nervous bridges connecting the right and left ganglia of the five pedal neuromeres. These nervous commissures are naturally ensheathed by the corresponding perineural vessels which, therefore, represent the five arteries in the scorpion connecting the thoracic sinuses with the supraneural artery. But in what way could we explain the origin of the scorpion type of neural circulatory system from the Limulus type or vice versa? Has the perineural system broken up into two sinuses and neural blood vessels, or have the latter altogether a separate origin?

The relatively great size of the oesophagus and the position of the forebrain in front of and not above the suboesophageal ganglionic mass in Limulus may have something to do with the differences between this animal and Arachnida. But this position itself is by no means original. Notice the position of the mouth in the middle of the ventral surface of the cephalothorax

and the position of organs in front of the mouth, which morphologically have to be considered as postoral. Notice the plastrobuccal muscles going "from the anterior neural side of the plastron to the oesophagus" and the strands of muscles attaching the proventriculus to the carapace in the region of the median eyes. The former undoubtedly represent the pharyngeal dilators of Archnida, the latter the dilators of the sucking-stomach in spiders and the corresponding pair of dorsoventral muscles in the scorpion. Although considerably in front of the posterior edge of the carapace in Limulus, these muscles are not far in front of the aortic valve. Notice that in severing the carapace from the abdomen with a knife, the opercular plate remains with the carapace. Notice, further, that the suboesophageal ganglionic mass in Limulus consists of seven neuromeres, the sixth belonging to the chilaria and the seventh to the opercula; that, owing to the perihaemal type of blood system, the vessels for the chilaria and opercula issue from the vascular ring; notice all this and you get the idea of what happened to Limulus in the course of its phylogenetic development. On the ventral surface two somites, corresponding to the first and second abdominal somites in Arachnida and characterized by the chilaria and opercula, became fused with the thoracic somites, while at the same time the corresponding neuromeres moved forward and fused with the suboesophageal ganglionic mass. On the dorsal surface a general displacement forward took place. In this displacement two things remained unchanged: the position of the mouth and the attachment points of the foregut and of the heart in the region of the aortic valve. What was above and behind the mouth, with the forward bending of the back came to lie in front and above the mouth. Part of the heart followed the displacement because of the permanent attachment at the aortic valve. Of the tergites, those of the chilarial and opercular somites had to follow the forward motion of the original carapace and were drawn into the hollow of the horseshoe-shaped carapace as it was formed through the forward displacement. These tergites fused with the carapace along their front and sides, but are still visible even in the adult and especially in the so-called trilobite stage of the

young. The cephalothorax of Limulus is therefore the result of fusion of the original cephalothorax with the chilarial and opercular somites, and the articulation between the carapace and abdomen is in reality an articulation between the opercular and first gill somite, or what corresponds to the division line between the second and third abdominal somites in Arachnida, as exemplified by the genital and comb somites in the scorpion. The division line between the last thoracic and first abdominal (chilarial) tergites lies immediately in front of the attachment of the heart, i.e., somewhat in front of the line passing through the two lateral eyes.

The history of this forward displacement and fusion of originally abdominal somites cannot be gleaned from a study of the external segmentation of Limulus embryos. On the ventral surface segmentation is clear, but on the dorsal the first visible segment is already the first gill segment. Something similar may be seen in the scorpion. Here, in the adult, the third abdominal tergite corresponds to the first lung sternite and therefore in reality representing the fourth abdominal somite. The second tergite, corresponding to the comb, represents the third abdominal somite. But the first abdominal tergite is the result of a fusion of the first and second tergites of the corresponding embryonic somites. The external segmentation is clear in young embryos on the ventral surface, and in quite young embryos is at least indicated by the even segmentation of the nervous system as seen in longitudinal sections through these stages. But when segmentation appears in the shape of transverse depressions on the dorsal surface, the first visible abdominal tergite corresponds already with the same tergite of the adult and is therefore already the result of fusion. It may be argued that if in Limulus abdominal tergites fused with the carapace, the same may have happened in the case of the missing first tergite of the scorpion. But this interpretation meets with too many objections. Of these perhaps the clearest is presented in the case of solpugids in which the thorax is still externally segmented.

In my monograph of Palaeozoic Arachnida ('13) I have pointed out that the Xiphosura must have developed independently

of the scorpions. The idea that Limulus is an arachnid as it is usually expressed, or more correctly that the Arachnida have a common ancestor with Xiphosura, must now be completely abandoned. Geologically, Limulus is older than the scorpion and already the oldest limuloid shows the same type of segmentation as the recent. Neither has the idea of Versluys on the origin of gills from lung books any bearing upon the question of origin of Limulus or Arachnida. With a stress on imagination one may derive Limulus from a eurypteroid ancestor, but to derive the latter from originally air-breathing Arachnida on the basis of no other evidence than conjectures which rest on a comparison of gill-plates with lung books and in the absence of any remains of air-breathing Arachnida antedating eurypteroids, seems to be a rather hazardous undertaking.

It may be interesting to mention in this connection that early stages in the embryonic development of scorpions show clearly eighteen postoral neuromeres, the first of which soon passes in front of the mouth and represents the cheliceral somite. The study of preoral neuromeres in the same stages is too complicated to admit of impartial judgment. In later stages, after the passage of the mouth behind the cheliceral neuromere, one may clearly count three pairs of nerves issuing from what appears to be three corresponding neuromeres. The first pair are the optic nerves of the median eyes, the second the nerves of the lateral eyes, and the third the nerves of the upper lip. These nerves are much finer than both optic nerves and can be traced with certainty only in sections parallel to the plane of symmetry (sagittal). The adult scorpion has therefore four preoral and seventeen postoral neuromeres. Five of the latter belong to the thorax and twelve to the abdomen. It happens that the abdomen of the scorpion shows twelve tergites. Yet one should not conclude from this coincidence of figures that each of the neuromeres mentioned belongs to a corresponding tergite. Nothing of the kind. I have already mentioned that the comb receives its nerves from the third abdominal neuromere, as may be easily demonstrated on sagittal and frontal sections. In early stages the neuromeres do not possess longitudinal connectives and

are recognizable without difficulty only because of the constriction between adjoining neuromeres. The last neuromere is clearly situated in the last abdominal segment, and only later moves forward and fuses partially with the penultimate neuromere. There is, therefore, in the adult scorpion an abdominal segment in excess of neuromeres. From an examination of numerous series I have no doubt that it is the first postabdominal or caudal segment and which therefore may have the value not of a true somite, but of an anterior subdivision or segment of the same somite to which the second postcaudal segment also belongs. Here, then, something happened the reverse of the fusion of sclerites in the first two abdominal somites, namely, the subdivision of the sclerite ring of a single somite into two distinct sclerite rings or segments, without a corresponding subdivision of other structures in the same somite.

It may be objected that such formation of pseudo-segments has not as yet been described, either for Arachnida or other Arthropoda, and that it were simpler to accept that the neuromeres really correspond to the visible segments, but in moving forward lost connection with them and began to furnish nerves to the next following. In other words, that the first abdominal neuromere originally furnished the nerves for the genital opercula, lost connection with the latter, and ceded this morphological and physiological function to the second neuromere; that the same happened to the second neuromere in relation to the comb, which now received its nerves from the third neuromere. But this explanation, besides being more complicated, suffers from another weakness. The roots of nerves follow the displacements of their neuromeres, but the nerves themselves obtain their connection with the original appendages, even if some branch of the nerve passes to another somite. This may be seen in Limulus and in many other arthropods. But in the case of the first abdominal neuromere of the scorpion there is not even a considerable or appreciable displacement forward, so that there would be no morphological reason of any kind for a loss of connection with the genital opercula if these belonged to the first neuromere.

We may therefore form the following conclusions regarding segmentation in scorpions and in Arachnida in general. The body of an Arachnid is composed of twenty one somites, to wit: 1 the first ocular (median eyes in the scorpion, anterior median eyes in the spider); 2 second ocular (lateral eyes in the scorpion, eyes with inverted retina in spiders, i.e., anterior lateral, and the four posterior ones); 3 rostral (upper lip); 4 cheliceral; 5 pedipalpal; 6 to 9 thoracic pedal; 10 to 21 abdominal. The first three are originally preoral in position. The fourth or cheliceral becomes preoral during development. The attachment of the heart to the anterior edge of the dorsal wall of the first abdominal somite and the formation at this place of the aortic valve indicate the division line between the thorax and the abdomen. The tenth somite is always rudimentary, having lost its identity in all but its neuromere. The genital opening is on the eleventh somite (second abdominal). A further fusion and ultimate loss of the identity of somites in Arachnida involves the posterior end of their body, beginning with the twenty-first somite and proceeding forward. In some cases, as in the eighteenth somite in the scorpion, secondary or spurious segmentation may take place, which has no relation to the original metamerism. If there be more than three originally preoral somites, these would have to be sought for in front of the first ocular somite.

Turning once more our attention to Limulus, we may first of all consider the homology of the thoracic and abdominal somites established in a way excluding all doubt. The six pairs of appendages belong to the same somites as in Arachnida, the chilaria represent the tenth, the opercula the eleventh somite, the five branchial neuromeres correspond to the twelfth to sixteenth somites, and of the three postbranchial ganglia the last is the result of fusion of the nineteenth to twenty-first neuromeres, if the ancestor of Limulus possessed that many postbranchial somites.

The homology of the preoral somites is more troublesome. Patten and Redenbaugh describe three preoral neuromeres, the olfactory, median ocular, and lateral ocular. Shipley following Carpenter recognizes only two somites, the median ocular and

the rostral. I think we may consider it as fairly conclusive that the median ocular and lateral ocular somites are homologous in Limulus and the scorpion. Whether the rostral somite of the scorpion corresponds with the somite designated as rostral in Limulus by Carpenter, is not so sure, but if it does not, then Limulus must possess just the same some evanescent somite between the lateral ocular and cheliceral. As for the olfactory somite, its homologue in Arachnida would have to be sought in one of those two pairs of obscure parietal ganglia described by Schimkewitsch for tetraneumonous spiders.

In the presence of a perineural circulatory system, in the existence of eight pairs of ostia in the heart, and of a pair of chilarial nerves, Limulus shows evidence of its origin from an arthropod ancestor lower and more primitive than the Arachnida. But in every other respect Limulus shows advanced development different from that in Arachnida and most likely standing in direct relationship to its particular mode of life. It seems as if the older interpretation of Limulus as a descendant of Trilobites must be revived. The shape of the trilobite carapace, the position of the mouth, the probable similarity in the position of the foregut as suggested in figure 24 of Raymond's beautiful monograph, the larval stages showing segments which were interpreted as cephalothoracic, but some of which probably are abdominal tergites drawn into and fused with the thoracic ones, point to a similarity more than casual. At any rate, the problem should be reinvestigated from the new point of veiw.

COMPARISON WITH OTHER ARTHROPODA

We have seen that the aortic valve has a uniform structure and a permanent position in Arachnida, permitting of strict homologies within that class. We have also seen to what conclusions we arrive through the assumption that the rule holds good in the case of Limulus also. One would expect that a structure so permanent in one or perhaps two classes would prove to be the same in the case of all other Arthropoda, if the diverse forms united under this immense phylum are of monophyletic origin. Unfortunately, this is not the case.

In his work on the organs of circulation in Schizopoda, published in 1883, Delage writes (I translate the original): "To determine exactly the length of the heart one should first of all well define its limits. It happens that these limits are not easily traceable because the diameter of the heart is not greatly different from that of the aortae at their points of origin. (Delage has in mind both the anterior and posterior aorta.) They are marked by the presence of cardioaortic valvules which have not yet been described by anyone. Moreover, these valvules are identical with those which are found in the same place in Amphipoda." "Within these limits the heart extends from the level of the last maxillary segment to the superior portion of the last thoracic segment." The anterior aorta, the median stem of which ends in the upper lip, gives off four branches in its course: the common trunk of the ophthalmic arteries, the cerebral artery, and the two antennal arteries. It may be of interest to notice that the sternal artery in Schizopoda arises from the heart.

The structure of the aortic valves themselves is different from those of Arachnida. They are paired lateral structures, as in all other Crustacea. The position indicated by Delage, taking into account evidence derived from the study of all other organs, is two somites nearer the head than in Arachnida. In such Decapoda as the crayfish and the lobster the heart is distinctly limited and considerably modified. Instead of arising from the aorta, the antennal arteries arise directly from the heart and have their own valves. Yet the aorta has also valves at its base and these are of the same type as in Schizopoda and Amphipoda. The position of these valves coincides exactly with the semilunar sulcus of the carapace, the two ends of which open into the so-called cervical groove. There are therefore differences in regard to structure of the circulatory system in closely related orders of the same sub-class—differences which cannot be understood without special study directed to their elucidation.

We know still less of the Protracheata, Pycnogonida, and the four classes formerly comprised under the general name of Myriapoda. Although I have some investigations under way, I am not prepared as yet to make any definite statement.

The circulatory system of insects is somewhat better known in this respect, yet here also the data are quite inadequate to form a clear judgement. Popovici-Baznosanu has described the heart in the Chironomus larva and states that the aortic valves are situated close to the anterior end of the fifth segment. In other larvae the heart had been described by other authors as situated near the rear end of the body. In some larvae the heart is not even situated directly under the dorsal body wall, but lies considerably deeper in the body cavity. The structure of the cardio-aortic valves, too, seems to be not only different from that of the aortic valve in Arachnida, but not always of the same type in all insects. Moreover, according to Zawarzin there are modified ostia in the aorta itself. It is evident that the first step must be in finding the true limits of the heart itself in insects. Meanwhile all conjectures would be entirely out of place.

SUMMARY AND CONCLUSIONS

1. In comparing the segmentation in arthropods the uncertain method of counting somites beginning with the anterior end should be abandoned. Instead, some structure should be chosen which has permanent value for a number of forms within a class and used as a starting-point of comparison.

2. Such structure in the case of Arachnida is furnished by the cardio-aortic valve which marks the division line between the last thoracic and first abdominal segments.

3. The method applied to Limulus leads to the conclusion that the carapace of Limulus is more complicated than in Arachnida, having two abdominal tergites drawn into the horseshoe-shaped thoracic tergite with which they have fused anteriorly and laterally.

4. A further conclusion is that the midcorporal articulation in Limulus is not between thorax and abdomen, but between the second and third abdominal somites.

5. The structure of the circulatory system in Arachnida follows a general plan given in the text.

6. The number of postoral somites in adult Archnida is seventeen. Five of these are thoracic and twelve abdominal.

7. The genital opening is on the second abdominal somite.

8. The first caudal segment in scorpions is not a true somite, but merely the anterior division of the fourteenth postoral somite.

9. If the number of preoral somites in Arachnida is not more than four, as represented by the median ocular, lateral ocular, rostral, and cheliceral somites, then the total number of somites in Arachnida is twenty-one.

EXPLANATION OF PLATES

ABBREVIATIONS

AC, Cheliceral artery
AO, Aorta
AP, Pedal artery
APP, Pedipalpal artery
1. AT, First abdominal tergite
AV, Anterior aortic valve
BR, Brain
CA, Comb-artery (ninth vertical artery)
Cbp, Basal plate of comb
CO, Aortic arch connection with thoracic sinus
D, Diaphragm
DP, Dilator muscle of the pharynx
DS, Dorsoventral muscle separating the first cephalothoracic diverticle of the midgut from the second
GB. 2, Gnathobase of the second leg
GP, Genital plate (operculum)
H, Heart
LC, Longitudinal connective between the fourth and fifth abdominal ganglia
M, Mouth
MA, Anterior edge of carapace
ME, Median eyes
MG, Midgut
MP, Posterior edge of carapace
PH, Pharynx
SAA, Supraneural (epineural) artery
SBA, Subneural (hyponeural) artery
ST, Sternum
UP, Upper lip (rostrum)
VA, Vertical or interneural artery

PLATE 1

EXPLANATION OF FIGURE

1 The circulatory system of a young scorpion, Centrurus insulanus. A very perfect median sagittal section, 20μ thick, was first drawn with the Edinger drawing apparatus. The section contained everything shown in the figure except the cephalic arteries, the thoracic sinus and the arteries leading to the appendages. All these were introduced into the drawing on the basis of results obtained from the study of a series of frontal sections through scorpions of the same age and species and of adult injected specimens of Centrurus carolinianus from Texas. To avoid confusion, the corresponding arteries of the right side are omitted. The cephalothoracic diverticula of the midgut are also omitted. In the section from which the figure was made they extend as far as the median eyes.

JOURNAL OF MORPHOLOGY, VOL. 35, NO. 2

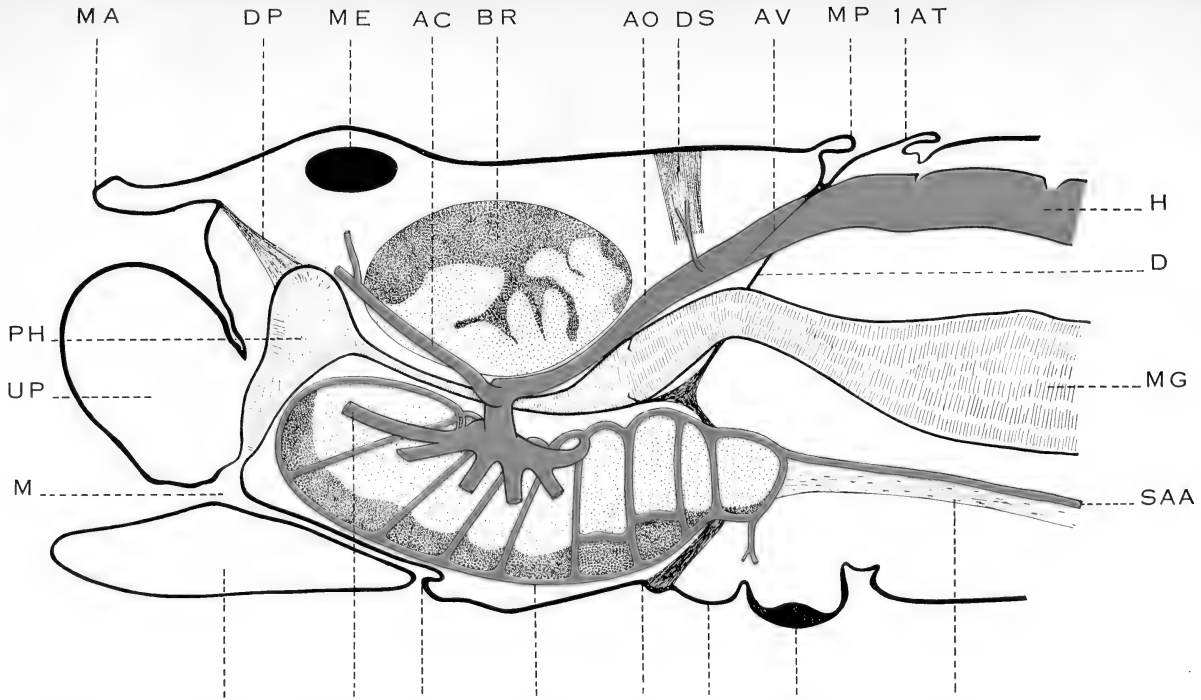


FIG 1

PLATE 2

EXPLANATION OF FIGURES

2 The circulatory system of a young scorpion, Centrurus insulanus. Dorsal view of the thoracic sinus with its arteries and of the supraneural artery. This figure represents a combination drawing of three consecutive sections of a frontal series. The drawing was made with the Edinger drawing apparatus under the same magnification as figure 1. Heart, aorta, cephalic arteries and subneural artery are not shown because they belong to quite different levels. The place where the aortic arch connects with the thoracic sinus of the corresponding side is indicated by the black oval opposite the base of the second pedal artery. Similarly, the vertical arteries connecting the supraneural with the subneural artery are represented by small black circles. The dotted parts represent the nervous system.

3 One of the right ostia in the heart of a young scorpion, Centrurus insulanus, drawn from a frontal section under high power. Between the fibers of the peripyle ligament blood corpuscles are shown.

4 Transverse section through the aorta of a young scorpion, Centrurus insulanus, showing at a high power the aortic valve.

5 Comb-artery of a young scorpion, Centrurus insulanus, drawn from a thick transverse section. The drawing shows the fourth abdominal ganglion and the genital duct with its muscles.

6 Sagittal section through the heart of a young spider, Lycosa carolinensis, in the region of the aortic valve. The drawing shows the relation of the valve to the carapace for comparison with figure 1.

7 Transverse section through the aorta of a young spider, Lycosa carolinensis, showing the aortic valve, for comparison with figure 4.

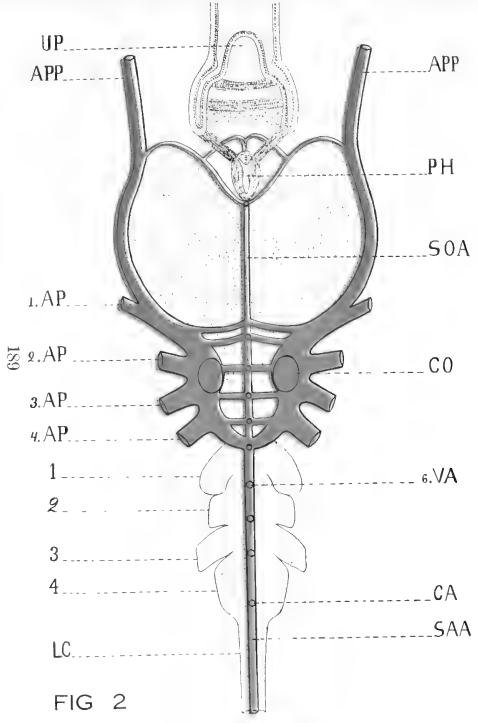


FIG 2

FIG 3

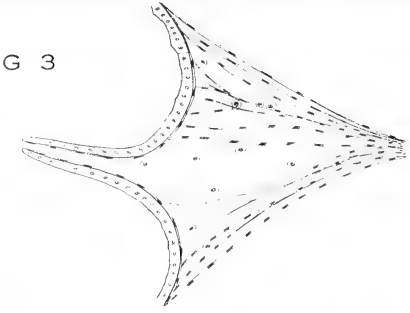
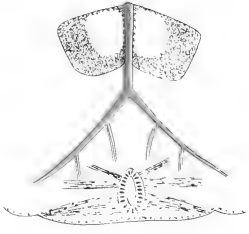


FIG 4

FIG 5



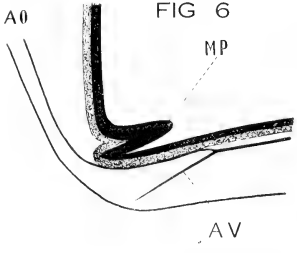


FIG 6

FIG 7

Resumen por el autor, William H. Leigh-Sharpe.

Morfología comparada de los caracteres sexuales secundarios de los peces elasmobranquios—los órganos copuladores, sus sifones y glándulas. Memoria III.

Los elasmobranquios más antiguos carecen de órganos copuladores; también puede afirmarse con certeza que carecen de sifones y glándulas de dichos órganos. Los fósiles más próximos a estos presentan un tipo directo de órgano copulador; probablemente no existen en ellos sifones o solamente aparecen bajo una forma rudimentaria. Más tarde el tipo de órganos copuladores en forma de rollo apareció, prediciendo á los Scyllidae; probablemente estos órganos iban acompañados de un sifón. Los Lamnidae son geológicamente más recientes que los Scyllidae, y poseen una glándula en el órgano copulador, por lo menos en las formas recientes. Más tarde se produjeron las rayas, las cuales se asemejan a las de los tiempos recientes.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 27

THE COMPARATIVE MORPHOLOGY OF THE SECONDARY SEXUAL CHARACTERS OF ELASMOBRANCH FISHES

THE CLASPERS, CLASPER SIPHONS, AND CLASPER GLANDS

MEMOIR III

W. HAROLD LEIGH-SHARPE

London, England

FIVE FIGURES

The previous memoirs appeared in the Journal of Morphology as follows: Memoir I, volume 34, page 245, 1920; Memoir II, volume 35, page 359, 1921. The first contained a general introduction to the subject and an account of Scyllium catulus, S. canicula, Acanthias vulgaris, and Raia circularis. The second dealt with Galeus vulgaris, Mustelus vulgaris, Lamna cornubica, and Rhina squatina.

The present memoir describes the following species:

The fossil aspect of this subject is unsatisfactory. Soft parts, the clasper siphons and clasper glands, as is only natural, are not preserved. The claspers, mainly the skeleton, alone are indicated. After an examination of the fossil collection in the Natural History branch of the British Museum, South Kensington, London, England, by the courtesy and under the personal superintendence of Dr. A. S. Woodward, I have selected five examples which have some bearing on the matter.

The figures have been executed from them especially for me by Miss Edith C. Humphreys, and the catalogue number is appended. A summary of the conclusions drawn from these observations follows.

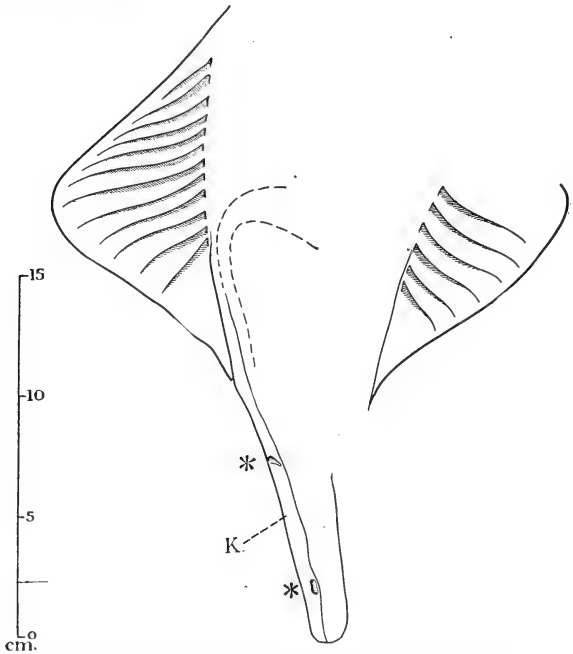


Fig. 1 Cladoselache kepleri. *K.*, kidney; *, see text.

CLADOSELACHE KEPLERI

This primitive Palaeozoic fish occupies the unique position of the oldest known elasmobranch. It possesses no claspers; in consequence, the bases of the pelvic fins appear wider apart than is usual. Figure 1 represents the specimen P. 9269, the Newberry sp. from the Upper Devonian (Cleveland Shale), Berea, Ohio, U. S. A. (William Clark collection).

The streak marked *K* in the figure has been misinterpreted as a clasper, an error which, owing to the influence of E. Stromer von Reichenbach, has found its way into the text-books. However, this is the only specimen in which this streak occurs, and, further, as recently as about 1914 at the points marked * in the figure, Doctor Woodward has caused excavations to be made and microscope slides prepared of the abstracted fragments. These sections reveal the same structure as that of the kidney of recent forms, the organ having become calcified.

There being no claspers, it is a safe assumption that there are also neither clasper siphons nor clasper glands.

PLEURACANTHUS PARALLELUS

Subsequently, in late Palaeozoic times, there appeared elasmobranchs with claspers—the Pleuracanthei. Figure 2 represents the specimen from the Carboniferous 'gaskohle' at Třemošna near Pilsen. I have not seen this fossil. Dermal denticles appear to be preserved, and, if the restoration of Xenacanthus decheni is to be relied on, the spoon-shaped conformation at the tip of the claspers had already been evolved.

The point of interest is that the clasper, from its skeleton, appears to be of the 'straight' or 'direct' type, by which is meant, not that it is without a gentle curve longitudinally, but that it is not rolled up in a scroll-like manner; on the contrary, the groove is very wide open. Such a condition I interpret as being primitive here, and secondary in the skates. The type of clasper suggests that possibly clasper siphons were not yet evolved, or were present only in a rudimentary form.

Acanthodes wardi is devoid of claspers, and therefore of no use in this investigation.

SQUALORAJA POLYSPONDYLA

This early Mesozoic chimaeroid fish, to be compared subsequently with Chimaera, is beautifully preserved in the specimen P 2276 found in the lower lias at Lyme Regis, Dorsetshire, England, a jurassic formation (fig. 3).

"Arising immediately within the point of union of the pubic and iliac regions is the basal cartilage which . . . . is prolonged backwards into a powerful clasper . . . . the cartilage becomes more calcified and . . . . broader. The

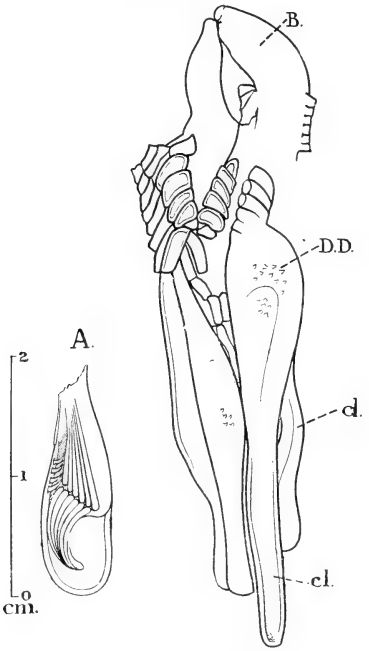


Fig. 2 Pleuracanthus parallelus (after Fritsch.[1]) *B.*, basipterygium; *D.D.*, dermal denticles; *cl.*, claspers. *A.*, restoration of Xenacanthus decheni.[2]

inner edge is straight, but the outer edge exhibits a gentle sigmoid curve which results in the widening of the rounded terminal extremity; and at the end of each clasper (especially the left)

[1] Fritsch, Ant., Fauna der Gaskohle und der Kalksteine der Permformation Böhmens—Prag. Bd. 3, Heft I, Taf. 93.

[2] Op. cit., p. 31.

a small tuft of dermal hooklets is preserved. The fin rays . . . . completely shown on the right are altogether twelve in number, and the length of the supporting cartilage is scarcely more than one-half of the appended clasper."[3]

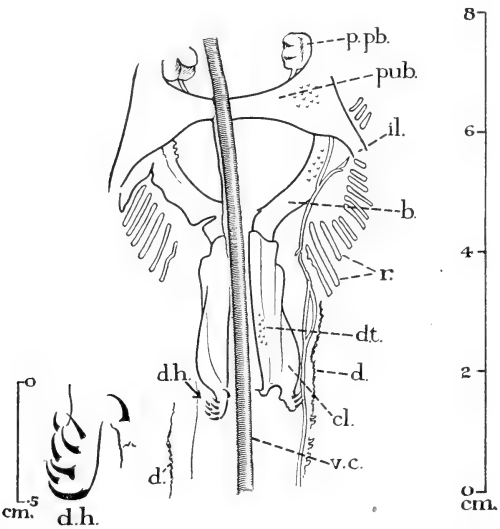


Fig. 3 Squaloraja polyspondyla, dorsal aspect. *p.pb.*, prepubic process; *pub.*, pubic bar; *il.*, iliac process; *b.*, basipterygium; *r.*, cartilaginous fin rays; *d.*, edge of skin; *cl.*, claspers; *d.h.*, dermal hooklets; *d. t.* dermal tubercles; *V.c.*, vertebral column.

The prepubic processes no doubt formed the base of attachment of the anterior claspers, which Parker has given reasons for believing a third pair of limbs, metameric with the pelvic claspers, making the Holocephali the sole exemplars of hexapodous vertebrates. This suggestion has not met with support.[4]

[3] Quoted from Woodward, A. S., Squaloraja polyspondyla, Proc. Zool. Socy., 1886, p. 527.

[4] Parker, T. J., Nature, 1886, vol. 34, p. 635.

The analogy and possible homology between the basipterygium and a femur probably led to the introduction of the term of pterygopodia applied to the claspers.

Dermal denticles and tubercles are preserved in places, and the tips of the claspers are provided with recurved dermal hooklets. These appear to be seven close together with one more remote,

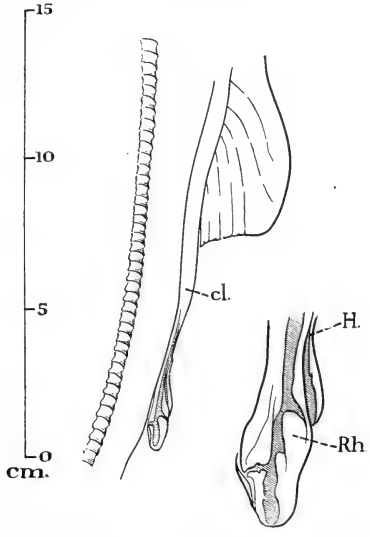


Fig. 4 Rhinobatus intermedius. *cl.*, clasper; *Rh.*, rhipidion; *H.*, possible position of hypopyle.

and are stout, affording an early example of an apparatus for the prevention of elision of the claspers from the oviduct of the female, since they curve forwards; backwards, when the clasper is bent forward in copula.

The chief point of interest is that the clasper suggests from its skeleton that it is rolled up in a scroll-like manner, recalling that of Scyllium. It is probable, therefore, that each clasper was accompanied by a clasper siphon.

RHINOBATUS INTERMEDIUS

It was not until a much later date that the skates arrived. These appear to resemble recent forms, and some are even assigned to existing genera. Figure 4, to be compared subsequently with a modern species of Rhinobatus, is drawn from the specimen 49516, from the upper cretaceous at Sahel Alma, Mount Lebanon (Lewis collection). It does not follow that the primi-

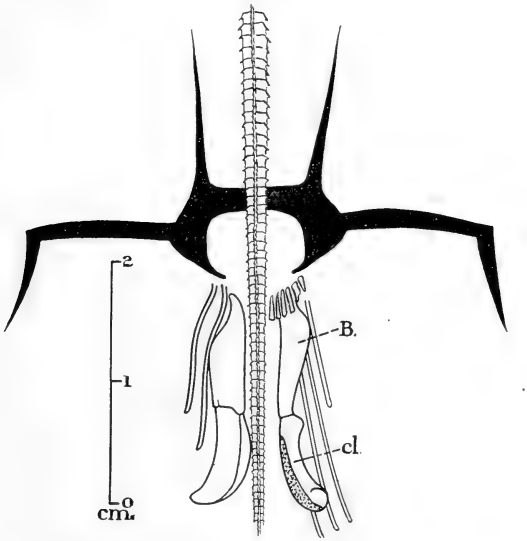


Fig. 5 Cyclobatis oligodactylus. *cl.*, clasper; *B.*, basipterygium

tive forms possessed a clasper gland as do their modern congeners, but at least such a corollary is probable. The specimen gives indications of a well-developed rhipidion.

CYCLOBATIS OLIGODACTYLUS

Also from the upper cretaceous, Hakel, Mount Lebanon, is the small and well-preserved specimen P 601 (Egerton collection). Originally described as resembling the Torpedinidae, but now known to be related to the Trygonidae, figure 5 does no more

than indicate a likeness to the skates in general as regards the claspers, which are well developed, suggesting a male of mature age.

SUMMARY

The conclusions drawn from a chronological survey of fossil forms are:

1. The oldest elasmobranchs (Cladoselache) are without claspers. It is almost certain they are without clasper siphons and clasper glands also.

2. The next fossils have a direct type of clasper. Possibly clasper siphons were not yet evolved or were present only in a rudimentary form.

3. Subsequently the scroll type of claspers appeared, suggestive of the Scylliidae. Probably these were accompanied by a clasper siphon.

4. The Lamnidae are geologically more recent than the Scylliidae, and these have progressed a stage further and evolved a clasper gland, at any rate in recent forms.

5. Later the skates arrived and resemble those of recent times.

Resumen por el autor, William H. Leigh-Sharpe.

Morfología comparada de los caracteres sexuales secundarios de los Holocéfalos y peces elasmobranquios—los órganos copuladores, sus sifones y glándulas. Memoria IV.

Chiloscyllium y Pristiurus poseen sifones semejantes a los de Scyllium. Los Holocéfalos poseen rasgos peculiares y característicos de su clase, entre los cuales pueden mencionarse un par de órganos copuladores anteriores, en adición del par encontrado comunmente, los cuales pueden retraerse dentro de bolsas; la hembra posee una bolsa en Callorhynchus; en los machos existen órganos copuladores frontales; en Chimaera existen órganos copuladores bifurcados y un cuerpo longitudinal. Los cuatro Batoideos son semejantes entre si y a Raia en lo referente a la glándula del órgano copulador, y difieren de Raia circularis en la ausencia de un tubo sifonal extendido hasta la extremidad posterior del órgano copulador. Rhinobatus se diferencia de los restantes por ser en algunos aspectos más primitivo, con una pequeña glándula pero con una sentina y una garra.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 27

THE COMPARATIVE MORPHOLOGY OF THE SECONDARY SEXUAL CHARACTERS OF HOLOCEPHALI AND ELASMOBRANCH FISHES

THE CLASPERS, CLASPER SIPHONS, AND CLASPER GLANDS

MEMOIR IV

W. HAROLD LEIGH-SHARPE
London, England

TWENTY-TWO TEXT FIGURES[1]

The preceding memoirs appeared in the Journal of Morphology as follows: Memoir I, volume 34, page 245, 1920; Memoir II, volume 35, page 359, 1921; Memoir III, volume 36, page 191, 1922. The first two contained an account of the commoner British species, Scyllium catulus, S. canicula, Acanthias, Raia, and Galeus, Mustelus, Lamna, Rhina, respectively. The third dealt exclusively with the fossil aspect of the subject. The present paper describes certain species to which I have kindly been allowed access at the Natural History Branch of the British Museum, South Kensington, London, viz.:

[1] The figures are specially drawn from the author's dissections and preparations by Miss Edith C. Humphreys, to whom best thanks are tendered.

CHILOSCYLLIUM (HEMISCYLLIUM) PUNCTATUM

The barbelled dogfish

The following description is based on the specimen numbered 55, exhibited at the Great International Fisheries Exhibition.

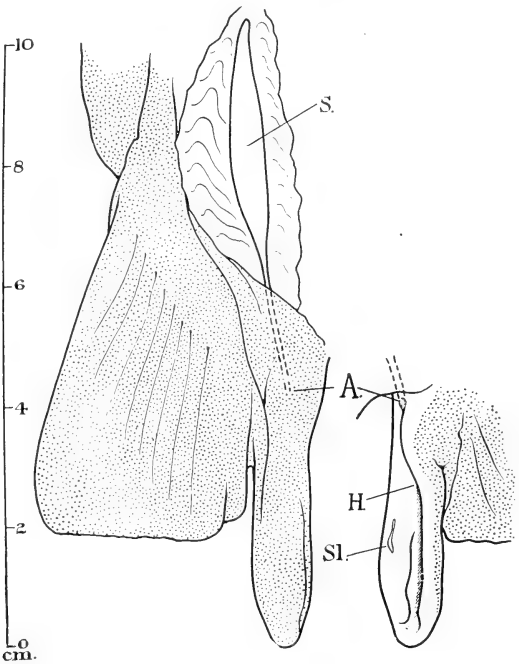


Fig. 1 Chiloscyllium punctatum. *A.*, apopyle; *H.*, hypopyle; *S.*, siphon; *Sl.*, slit.

It was taken at Singapore, was 3 feet in length and weighed 6 pounds at capture (fig. 1).

This animal possesses a siphon of the type found in Scyllium, long and narrow, about 4 cm. in length. The claspers are denticled as in Scyllium, but the closed portion of the scroll-like

clasper formed by the overlapping edges is very short. On the inner side of the dorsal surface of the clasper is a slit whose cavity leads not forwards, but slightly backwards, and hence cannot be truly regarded as a pseudosiphon.

PRISTIURUS MELANOSTOMUS

The black-mouthed dogfish

This near relative of Scyllium differs from that genus more than might be supposed. The specimen examined, one of two from an unnamed locality, measures 62 cm. in length. The siphons are long and narrow, in this case 12.5 cm., poorly developed, with but feebly muscular walls. They are proportionately longer than in Scyllium, but lie immediately under the skin as in Acanthias. The claspers terminate in a gimlet-like coil (fig. 2), which, when unrolled, as in the inset, shows slight indications of a rhipidion. The actually closed portion of the scroll-like clasper is moderately long as in Scyllium.

CHIMAERA MONSTROSA

The king of the herrings, or rabbit fish

Although the Holocephali are not strictly elasmobranchs, it is both important and essential that they should be included, since they exhibit the features which form the subject of this investigation. They possess the peculiarity of an anterior pair of claspers, or grapplers, in front of the pelvic fins in addition to the usual posterior pair. The anterior claspers are capable of being retracted into a glandular pouch, where they are usually held in retreat. They are absent in the female, but the pouch may in some cases be present in a rudimentary form. There is also a frontal clasper in the male. In Harriotta the claspers are said to be poorly developed, and the frontal clasper absent.

The specimens here described are from a batch of about nine received from Rockall, September, 1920.

In the females, the largest of which is 79 cm. long, I failed to find any vestige or rudiment of either anterior claspers or their pouch: there appears to be no trace of them, at any rate in the present species, Chimaera monstrosa.

The male examined is 68 cm. in length, the fish in each sex being measured to the extremity of the whip-like tail. The claspers are not obvious, being covered in the natural position by the pelvic fins, as seen on the (observer's) right in figure 3.

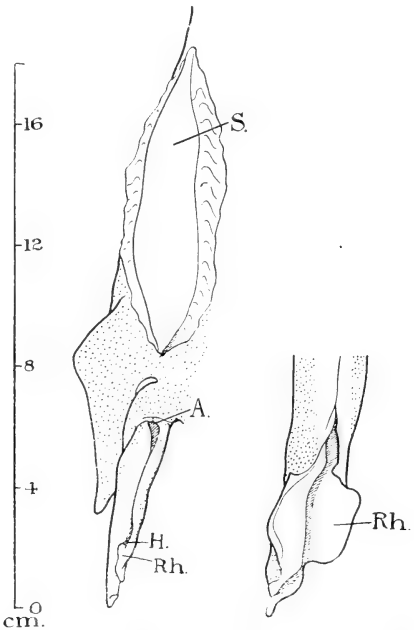


Fig. 2 Pristiurus melanostomus. *A.*, apopyle; *H.*, hypopyle; *S.*, siphon; *Rh.*, rhipidion.

They are of a type wholly different from Callorhynchus and the elasmobranchs, since each bifurcates into an external and an internal radius. At the bifurcation of the radii is the apopyle. Whilst the fish is very smooth, the clasper radii are fiercely denticled, though lacking at the tip the dermal hooklets so characteristic of the fossil Squaloraja (Memoir III, p. 193).

The external radius, which is capable of flexion outwards to prevent elision from the oviduct, is denticled at the tip on the outer border, and all along the inner border, while the more stationary internal radius is denticled all along its outer margin which is grooved, the denticles being represented by stippling in the figure. The two radii, when approximated, together form a clasper groove or tube suggestive of the elasmobranchs. The whole external radius is therefore analogous in function to the

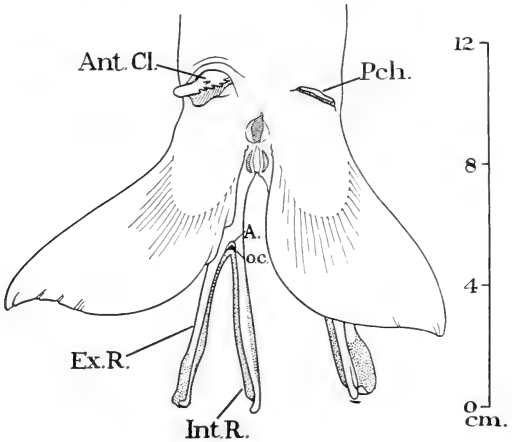


Fig. 3 Chimaera monstrosa. *Ant. cl.*, anterior clasper partly protruded; *Pch.*, pouch with anterior clasper retracted; *Ex. R.* and *Int. R.*, the external and internal radii of the posterior clasper; *O. C.*, opening of cavity; *A.*, apopyle.

spur in Acanthias, the radii being long and slender and apparently not erectile.

Leading into the apopyle is a cavity which I cannot consider the homologue of the siphon for the following reasons: 1) It does not appear to be a smooth sac with muscular walls; 2) it is situated in the swollen proximal end of the clasper, and not on the ventral surface of the abdomen; 3) part of the skeleton of the clasper (the basal portion from which the two radii originate) has to be cut through to reveal its interior, which is not the case in

any siphon I have as yet investigated; 4) it appears to have a homologue in Callorhynchus which is merely a folded portion of the clasper-tube.

Lying within this cavity is an elongated longitudinal body which is neither solid nor tubular, but rolled upon itself in a scroll-like manner (fig. 4, *L. B.*). A microscopic examination of this body in transverse section reveals (fig. 5) that it is com-

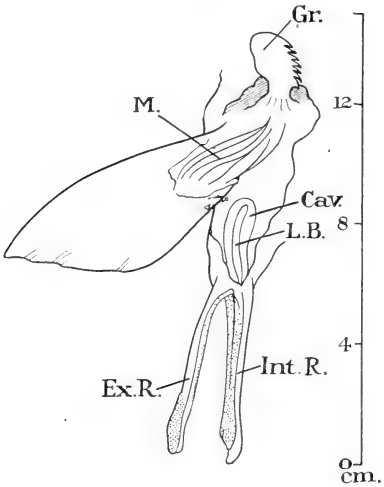


Fig. 4 Chimaera monstrosa. *Gr.*, grappler, the pouch having been dissected away; *M.*, muscle; *Cav.*, cavity; *L. B.*, longitudinal body; *Ex. R.* and *Int. R.*, the external and internal radii of the posterior clasper.

posed of compact bands of striped muscle, some spongy tissue, a supporting base of cartilage, and covered by a most curious epithelium. This epithelium consists of a single layer of extremely long, narrow, rod-like cells, with a nucleus in the centre where there is a slight dilatation to receive it. Such cells are always associated with a sensory epithelium, (e.g., olfactory) in higher types, and are probably not glandular.

The anterior claspers can each be retracted into a slit-like pouch which is placed transversely to the animal's axis (contrast Callorhynchus, where it is longitudinal); thus, in figure 3, the clasper on the (observer's) left is partly protruded, while that on the right is completely withdrawn. A reference to figure 6

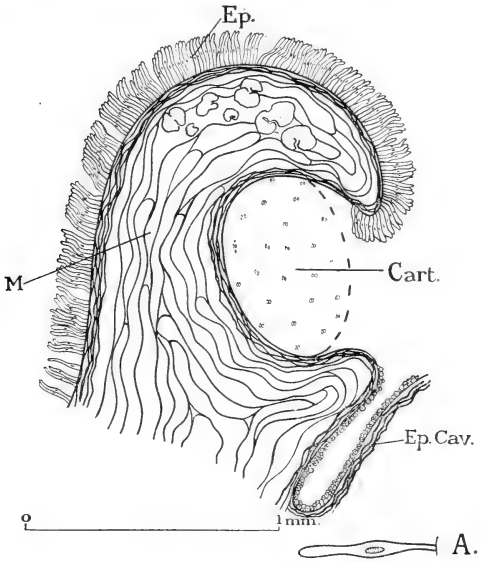


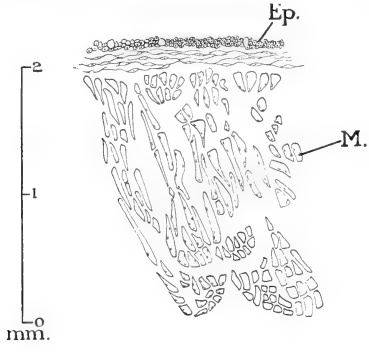
Fig. 5 Chimaera monstrosa, a transverse section of the longitudinal body (haemalum-eosin). *Ep.*, epithelium of the longitudinal body, a single cell of which is shown highly magnified in the inset *A*; *Cart.*, cartilage; *M.*, muscle; *Ep. Cav.*, epithelium of the cavity.

shows that the pouch is lined with stratified epithelium, immediately below which are fibrous connective tissue and the striped muscle bands of the derma, and that a few goblet-cells are present.

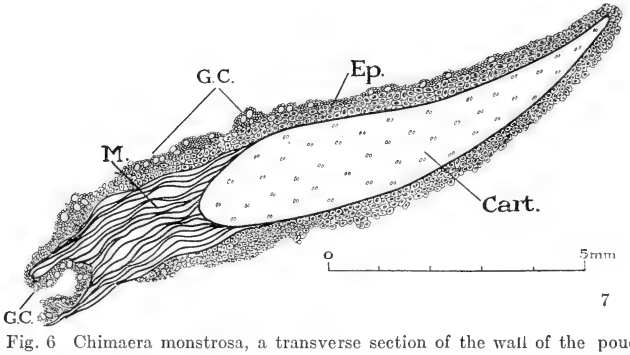
In the channel, however, formed by the continuity of the base of the pouch and the anterior clasper, numerous goblet-cells are found as indicated in figure 7, *G. C.* Goblet-cells are also present

in the epithelium of the clasper, more numerous on its concave surface. These, no doubt, secrete mucus, which fact has given rise to the expression 'glandular pouch,' although the walls of the pouch are in the main but slightly glandular.

As regards the clasper, only that portion of it which I have called the grappler in Callorhynchus, is represented here. It is composed of practically nothing but cartilage, over which is drawn



6



7

Fig. 6 Chimaera monstrosa, a transverse section of the wall of the pouch of the anterior clasper (haemalum-eosin). *Ep.*, stratified epithelium; *M.*, striped muscle.

Fig. 7 Chimaera monstrosa, a longitudinal section through the anterior clasper (haemalum-eosin). *Cart.*, cartilage; *Ep.*, stratified epithelium. *M.*, muscle; *G. C.*, goblet cells.

a normal type of stratified epithelium(fig. 7). The grappler is spoon-shaped, slightly concave, deeply serrated on its morphological inner border, but without denticles. Its function would appear to be truly a clasping or grappling one. There is no compact gland in connection with the anterior clasper as there is in Callorhynchus.

The frontal clasper (fig. 8, *A*) is a knocker-like structure on the forefront of the head, which is used for clasping, and helping to overtake, catch, and turn the female. It is limited to the male, and, from indications of scars on the females, its place of

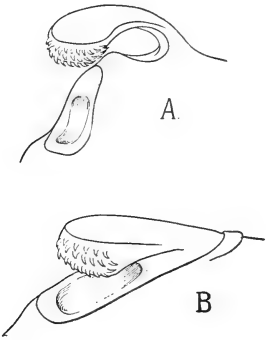


Fig. 8 Frontal claspers. *A.*, Chimaera monstrosa. *B.*, Callorhynchus antarcticus.

application appears to be near the pectoral fins. It is strongly denticled on its under surface, and, when not in action, is kept bent in a little socket-like depression in the skin, into which it just fits. In the female the position of the frontal clasper is indicated by a flat area on the skin of the forehead.

CALLORHYNCHUS ANTARCTICUS

The southern beauty

This curious member of the Holocephali agrees with Chimaera in the essential characters mentioned in the introduction to that genus, but is widely different as to details. I have examined

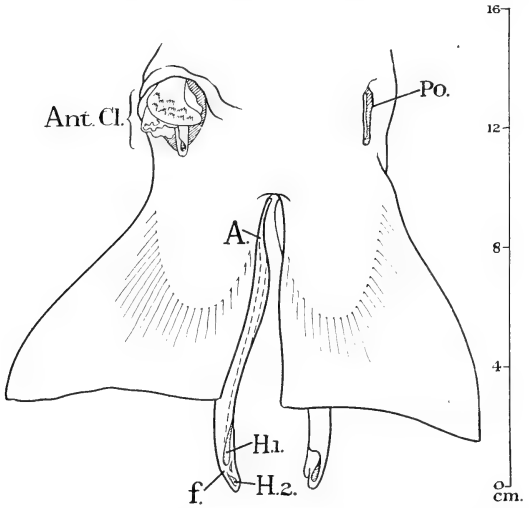


Fig. 9 Callorhynchus antarcticus. *Ant. Cl.*, anterior clasper partly protruded; *Po.*, pouch with anterior clasper retracted; *A.*, apopyle; *f.*, fused portion of clasper tube; *H1.*, true hypopyle; *H2*, false hypopyle.

an adult male from Hobart Town 66 cm. long and an adult female from Table Bay 73 cm. in length. While the posterior claspers are here again covered by the pelvic fins, they are of the ordinary scroll type with nothing specially remarkable. The closed portion of the clasper tube formed by the overlapping edges is very long, and a peculiarity is that, subsequent to the unrolling of the scroll, at the extreme tip of the clasper, is a short closed portion formed by actual fusion of the clasper edges

(figs. 9 and 10, *f*), or possibly by a fused rhipidion. This is the first recorded case in which a closed tubular passage is formed in the claspers by actual fusion. No denticles nor accessory structures are present on these claspers; neither, as far as I can at present ascertain, is there any siphon or gland to be found in connection with them.

As in Chimaera, the anterior claspers can each be retracted into a slit-like pouch which is longitudinal, or parallel to the animal's axis, not transverse as in Chimaera; thus in figure 9

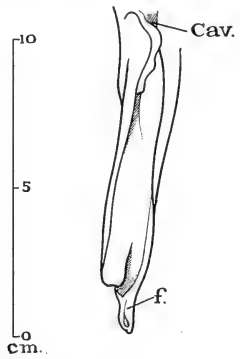
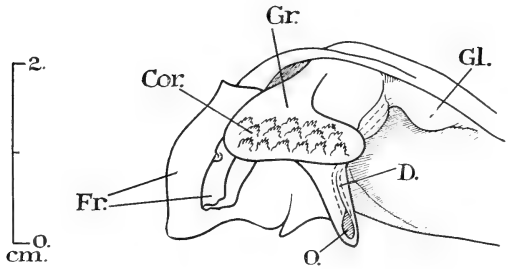
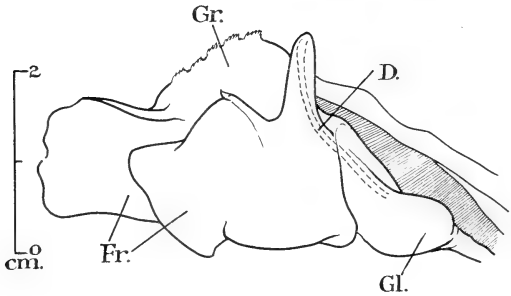


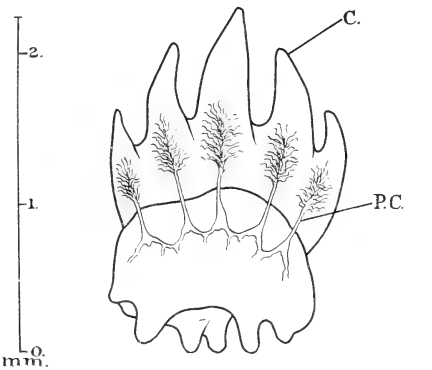
Fig. 10 Callorhynchus antarcticus, the right posterior clasper unrolled. *Cav.*, position of cavity at proximal end of clasper tube; *f.*, fused portion of clasper tube.

the clasper on the (observer's) left is partly protruded, while that on the right is completely withdrawn. The curious anterior claspers are extraordinarly complicated, and, the pouch having been dissected away, the relations between the various parts are shown in figure 11 in two positions. Fundamentally there is the grappler not unlike that of Chimaera, a spoon-shaped cartilaginous structure, slightly concave, without a serrate border, but covered on its anterior convex face with complicated dental tubercles, a type with five cusps, the coronillae (fig. 12). On the outer side of the grappler are two soft, slightly fimbriated expansions, apparently outgrowths of the walls of the pouch,





11



12

Fig. 11 Callorhynchus antarcticus, the right anterior clasper in two positions, the pouch having been dissected away. *Gr.*, grappler; *Gl.*, gland; *D.*, gland duct; *O.*, orifice of gland duct; *Fr.*, frills; *Cor.*, coronillae.

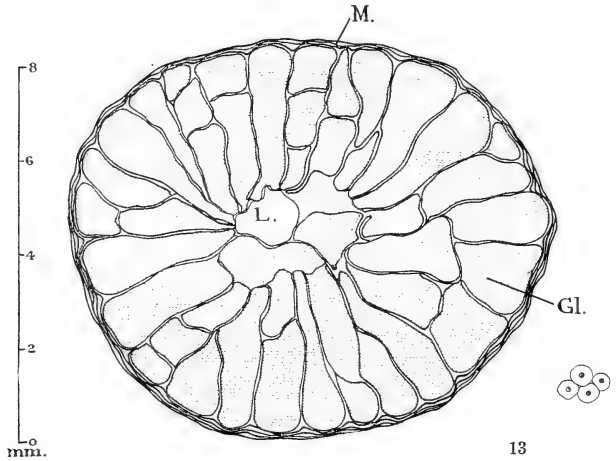
Fig. 12 Callorhynchus antarcticus, a single coronilla from the grappler. *C.*, cusps; *P. C.*, pulp cavity.

the frills, whose function is problematical. Associated with the grappler on its inner side is a globular, compact gland, with a completely closed duct, opening by a pronounced and well-defined orifice at the end of a short tube. As the whole clasper organization protrudes from the pouch, the gland orifice emerges after the grappler.

Since the gland here is associated with the anterior clasper, and none with the posterior, it is conceivable that this is morphologically the same gland shifted forwards, having taken its duct with it. Taking into consideration its absence in Chimaera and its conjunction with surrounding parts, I do not think this is likely, but that it is of independent origin. The gland does not at all resemble those of the Batoidei, as indeed there is no reason why it should, being globular instead of elongated, and completely surrounding its containing sac leaving a lumen which is very small, instead of being confined to one side of it.

Histologically (fig. 13), the gland resembles that of Lamna in being composed of compact masses of tissue, not penetrated by ducts, and separated from each other by partitions of connective tissue. The individual cells, however, are different. Instead of being elongated, they are spherical, as shown in the inset, with spherical central nuclei.

In the female anterior claspers are not present, but there is a similar and similarly situated pouch to that of the male. The pouch, the limits of whose large cavity are indicated by a dotted line in figure 14, is entered by a small sharply defined orifice, posterior to which is a directive or guiding groove, sloping down to the orifice. The material is not in a good enough state of preservation to determine the presence and nature of a gland or body at the bottom, or dorsal aspect, of the female pouch, but I am inclined to think one is present as a spongy mass. Consideration of the organs in both sexes leads to the belief that the narrow tubular duct of the gland of the male, with its strikingly penis-like extremity, is introduced into the pouch of the female, otherwise we should find the pouch of the female wide open, revealing the extremities of the large cavity, instead of guarded by a narrow orifice, *just large enough to admit and hold the penis*



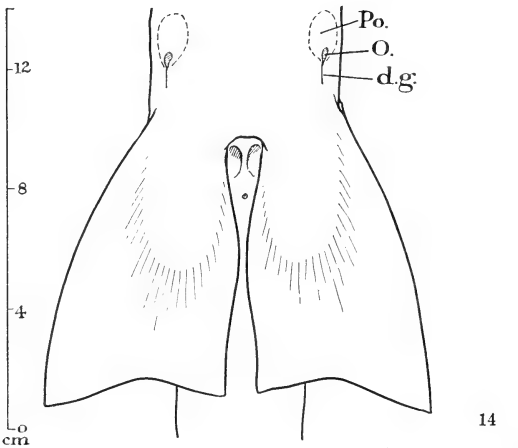


Fig. 13 Callorhynchus antarcticus, a transverse section of the gland of the anterior clasper (haemalum-eosin). *Gl.*, gland; *L.*, lumen; *M.*, muscle.

Fig. 14 Callorhynchus antarcticus, female. *Po.*, pouch, the limits of whose internal boundaries are indicated by a dotted line; *O.*, orifice of pouch; *d.g.*, directive groove.

of the male, with the directive groove to guide the latter into it. Possibly the spongy mass at the base of the female pouch, will, in the future, be revealed not as a gland for secretion, but as a body for the absorption of the secretion of the gland of the male whose function might be hedonistic.

The whole of the closed portion of the posterior clasper tube in the male and the cavity of its proximal end is filled with decomposed débris. Possibly this is what Schneider pronounced to be spermatozoa (Memoir I, p. 265) as there is no reason why it should not be, but it is not stored in what I am terming a siphon in these papers, since Callorhynchus does not possess one.

Save that it is stouter and curved in a different manner, the frontal clasper closely resembles that of Chimaera (fig. 8, *B*). It is limited to the male sex.

The material, which is nearly fifty years old, is not in a good enough state of preservation to make out the following points, which will have to be supplemented in a future memoir from fresh material. 1) The nature of the cavity and its walls in the proximal end of the posterior claspers in the male. 2) The nature of the walls of the pouch of the female. 3) The presence of a body and its nature in the pouch of the female.

TORPEDO MARMORATA

The electric ray

NOTE. In none of the Batoidei that follow in this memoir is the siphon tube carried down the clasper to open by an aperture posterior to the hypopyle as it does in Raia circularis (Memoir I, p. 260).

The largest specimen, from which figure 15 is drawn, was captured at Algoa Bay in 1891, and measures 43 cm. in length. This study has also been supplemented from other animals from Madras.

The claspers are singularly blunt and clumsy, and terminate in a double spoon-shaped conformation, suggestive of a burrowing bivalve mollusc, e.g., Solen. On their outer edge is a long slit whose cavity does not lead forward and only slightly backward. This is neither a pseudosiphon, which has been defined for Galeus

and Mustelus, nor a pera, which has been defined for Mustelus in Memoir II. On their inner edge, near the tip, is a slot whose cavity leads neither forwards nor backwards.

The glands of the Batoidei are, as far as this investigation has gone, of the same type. This has already been described in

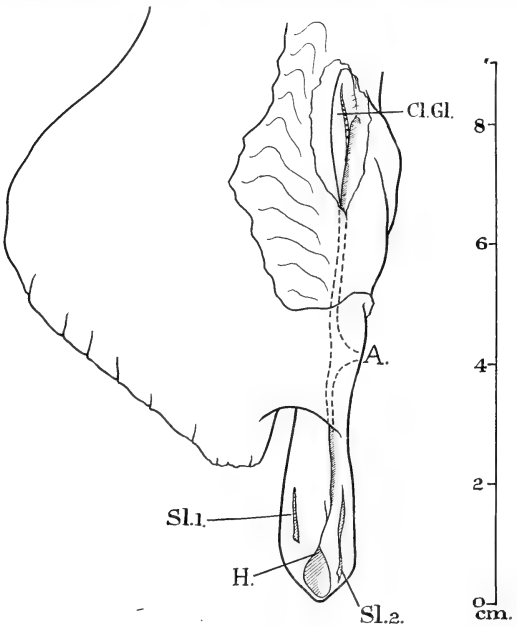


Fig. 15 Torpedo marmorata. *Cl. Gl.*, clasper gland; *A.*, apopyle; *H.*, hypopyle; *Sl. 1.*, slot; *Sl. 2.*, slit.

detail for Raia circularis, q. v., Memoir I, pp. 260 to 262. Briefly, the gland is an elongated bilobed body, with a longitudinal groove, containing a single row of papillae; superficially it resembles a date-stone and is confined to the dorsal side of the siphon sac. The duct of the siphon is thus also the duct of the gland, and debouches in this and the following genera at the

apopyle. The gland becomes more and more twisted out of one plane in each succeeding genus. A transverse section (fig. 16) reveals, in each case, that its histological structure is the same as in Raia. Owing to their larger size, the glands only are shown in section in this memoir; the relation of the gland to the siphon sac is seen in Memoir I, figures 10 and 11.

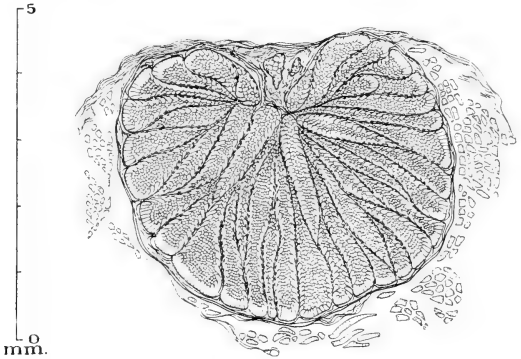
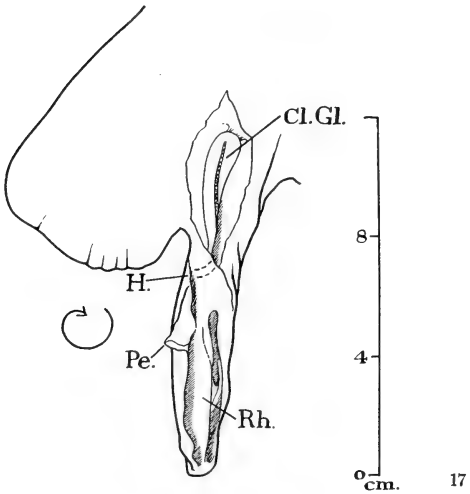


Fig. 16 Torpedo marmorata, a transverse section of the clasper gland (haemalum-eosin).

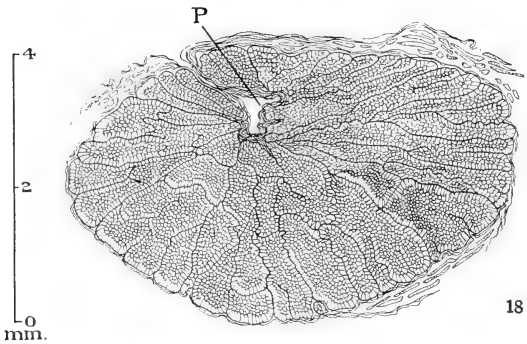
TRYGON PASTINACA

The sting-ray

The specimen considered (fig. 17) was taken in the Bay of Biscay in March, 1892. It exemplifies the two-spined stage, and measures 69 cm. in length, and 40 cm. at the greatest breadth across the pectoral fins. The flesh of the inner edge of the clasper is drawn up into a pronounced ridge-like fold, the pent. The hypopyle is very close to the apopyle, and there the clasper-gland duct debouches. The rhipidion is pronounced, and is hard and shell-like with an edge like a knife-blade, so that, in spite of an epidermis over it, it is easy to cut the finger on it. The clasper gland is as in Torpedo (fig. 18).



17



18

Fig. 17 Trygon pastinaca. *Cl. Gl.*, clasper gland; *H.*, hypopyle; *Pe.*, pent; *Rh.*, rhipidion.

Fig. 18 Trygon pastinaca, a transverse section of the clasper gland (haemalum-eosin). *P.*, papilla.

MYLIOBATIS AQUILA

The eagle-ray

The specimen investigated (fig. 19), captured at Madeira, measures 75 cm. in length and 46 cm. at the greatest breadth across the pectoral fins. The flesh of the inner edge of the clasper

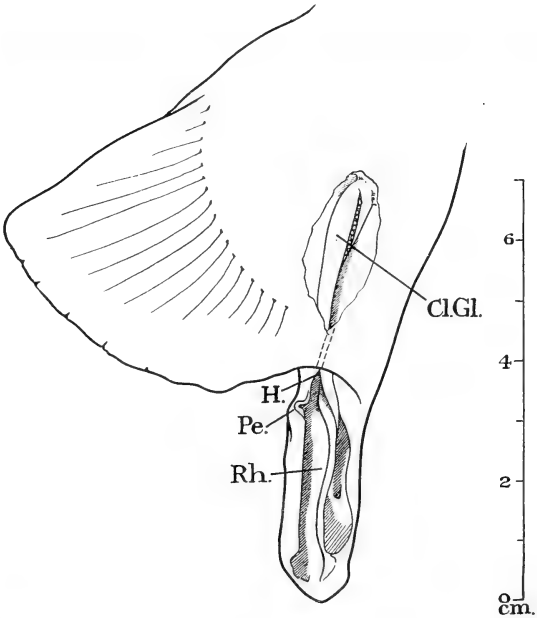


Fig. 19 Myliobatis aquila. *Cl. Gl.*, clasper gland; *H.*, hypopyle; *Pe.*, pent; *Rh.*, rhipidion.

is drawn up into a ridge-like fold, the pent, not so pronounced as in Trygon. The clasper is stouter, but otherwise resembles Trygon more closely than any other two genera approximate to each other. The rhipidion is situated more anteriorly, but resembles that of Trygon in being hard and shell-like with a knife-blade edge. The clasper gland is as in Torpedo (fig. 20).

RHINOBATUS PRODUCTUS

The long-nosed skate

This specimen (fig. 21), taken at San Diego, California, in May, 1891, measures 85 cm. in length. The first point to strike the observer is the extreme similitude of this species to its fossil forerunner, Rhinobatus intermedius (Memoir III, p. 197). The long, thin, delicate, tapering claspers with a spathe-like expansion at the extremity are unmistakable, easily distinguishing them from those of the foregoing genera. The closed portion

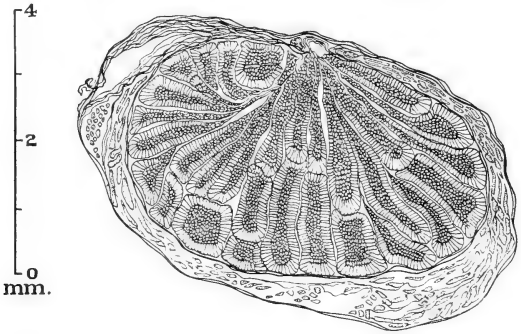


Fig. 20 Myliobatis aquila, a transverse section of the clasper gland (haemalum-eosin).

of the clasper tube is long, and towards the outer edge of the dorsal aspect of the clasper is a slit, as in Torpedo. The claspers are denticled all over, which is unusual in a skate-like form. The rhipidion is narrow and elongated, and not in the form of a fan; it is entirely concealed, unless the edge of the clasper tube is rolled back, as in the lower inset. At the place where the siphon tube debouches in Raia circularis is a well-marked sentina, into which there is no opening from the interior. The sentina is covered by an expansion resembling the web between a frog's toes, and on its outer side is a non-articulate immovable claw which does not resemble that of Acanthias. When the web

is contracted this is not seen, but by a muscular movement the web can be expanded as in the lower inset, in which case the claw protrudes, and serves to prevent elision of the clasper from the oviduct.

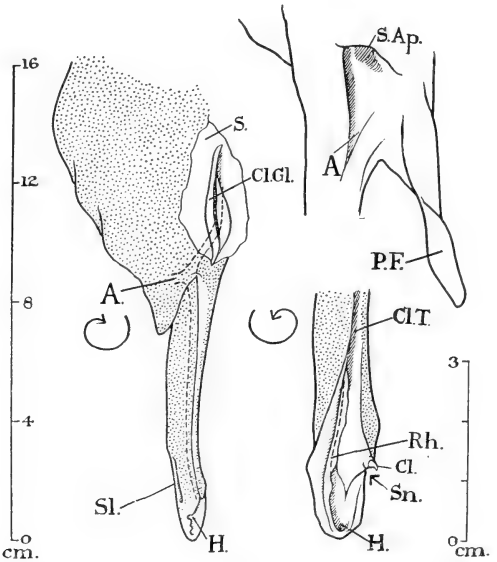


Fig. 21 Rhinobatus productus. *Cl. Gl.*, clasper gland; *S.*, siphon; *S. Ap.*, aperture of siphon tube; *A.*, apopyle; *H.*, hypopyle; *P. F.*, pelvic fin; *Cl. T.* clasper tube; *Sl.*, slit; *Rh.*, rhipidion; *Sn.*, sentina; *Cl.*, claw.

The clasper gland, while conforming to the general type as in Raia, Torpedo, etc., is the one which presents the most minor differences. It is elongated and extremely narrow, and more twisted out of one plane than the others. So small is its diameter and so small the gland components that it is possible to cut through two papillae in a transverse section (fig. 22). The origin of the groove, which ultimately becomes the siphon tube, is unusually far forward in position.

Rhinobatus appears to be a more primitive skate-like form for the following reasons: 1) The claspers are denticled all over. 2) The rhipidion is not well developed, and is not in the form of a fan. 3) The clasper gland is very small in comparison with the size of the animal. 4) A similar fossil species dates back to cretaceous times.

On the other hand, it is specialized in the following points: 1) The web expansion over the sentina with, 2) a claw.

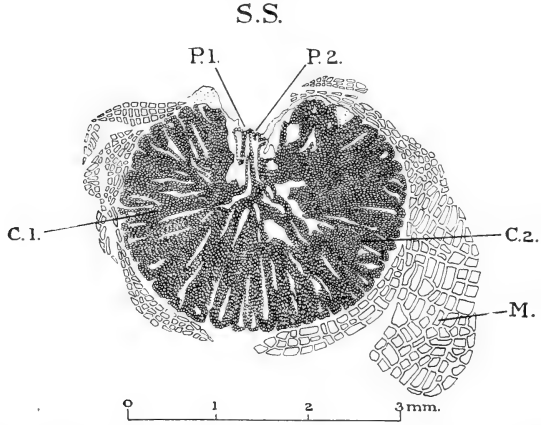


Fig. 22 Rhinobatus productus, a transverse section of the clasper gland, (haemalum-eosin). *C1.*, one component of the gland; *C2*, another component; *P1.*, papilla of the first component; *P2*, papilla of the other component; *M.*, stripe muscle; *S. S.*, siphon sac.

Resumen por el autor, William H. Leigh-Sharpe.

Morfología comparada de los caracteres sexuales secundarios de los Holocéfalos y peces elasmobranquios—los órganos copuladores, sus sifones y glándulas. Memoria V.

Notidanus posee órganos copuladores primitivos en forma de hoja, contenidos en una vaina formada por la aleta pélvica. Posee una cavidad peculiar, la cual lo mismo que la de los Holocéfalos, puede representar un estado en el proceso de formación de un sifón. Los Spinacidae poseen espinas en los órganos copuladores (las cuales tal vez falten en los jóvenes), mientras que el sifón con paredes musculares de Spinax es tal vez el más típico hallado hasta el presente. Cestracion carece de sifón; Pristiophorus posee un sifón grande y sacular. Además de estos caracteres hay notables semejanzas en los órganos copuladores de Cestracion y Pristiophorus, pero el primero posee un gancho semejante a una aguja de crochet, donde el último presenta a modo de una espuela. Rhinochimaera difiere de Chimaera por carecer de organos copuladores bifurcados y por poseer ganchitos dérmicos semejantes a los del fósil Squaloraja. Las dos especies de Chimaera descritas en el presente trabajo difieren considerablemente por sus órganos copuladores de la Chimaera monstrosa descrita en la Memoria IV. Las dos especies de Raia descritas son de tipo muy diferente al de R. circularis, y sus órganos copuladores son en extremo complicados. La inervación del órgano copulador y su saco y glándula son objeto de descripción en el presente trabajo, con los resultados de una serie de experimentos sobre la estimulación nerviosa.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 27

THE COMPARATIVE MORPHOLOGY OF THE SECONDARY SEXUAL CHARACTERS OF HOLOCEPHALI AND ELASMOBRANCH FISHES

THE CLASPERS, CLASPER SIPHONS, AND CLASPER GLANDS

MEMOIR V

W. HAROLD LEIGH-SHARPE
London, England

NINETEEN FIGURES[1]

The preceding memoirs appeared in the Journal of Morphology as follows: Memoir I, volume 34, page 245, 1920, Scyllium catulus, S. canicula, Acanthias, Raia circularis; Memoir II, volume 35 page 359, 1921, Galeus, Mustelus, Lamna, Rhina; Memoir III, volume 36, page 191, 1922; Fossil forms; Memoir IV, volume 36, page 199, 1922, Chiloscyllium, Pristiurus, Chimaera, Callorhynchus, Torpedo, Trygon, Myliobatis, Rhinobatus. The present paper deals with certain species to which I have kindly been allowed access at the Natural History branch of the British Museum, South Kensington, London, viz.:

[1] The figures are specially drawn from the author's dissections and preparations by Miss Edith C. Humphreys, to whom best thanks are tendered.

NOTIDANUS (HEXANCHUS) GRISEUS

The six-gilled shark

This primitive Protoselachian fish exhibits, as may be supposed, characters quite different from those of the species previously considered. I was fortunate enough to be able to investigate a

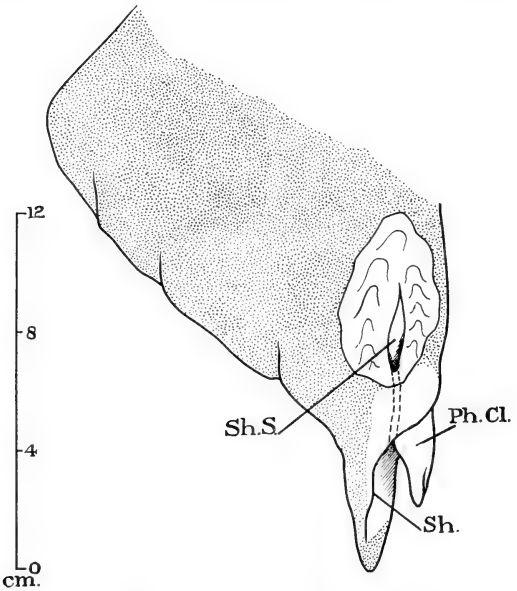


Fig. 1 Notidanus griseus. *Sh.*, prolongation of the pelvic fin forming a sheath for the clasper; *Sh. S.*, sheath sac; *Ph. Cl*, phyllaceous clasper.

large specimen measuring 77 inches, or nearly 2 meters, in length, taken in Japan, in 1905, and from this the figures are drawn. I have also examined small specimens from Nice, and in these the claspers are of a bright lemon-yellow color.

The pelvic fins are prolonged posteriorly in such a way as to form a sheath in which the claspers are effectually concealed, so that, at first sight, the small specimens may be mistaken for

females, unless the fins are parted, and, in the large specimen, the fins may be mistaken for the claspers. The claspers are obviously in a very primitive condition, and look like a duplication of the fins, resembling a rolled-up leaf, so that the closed

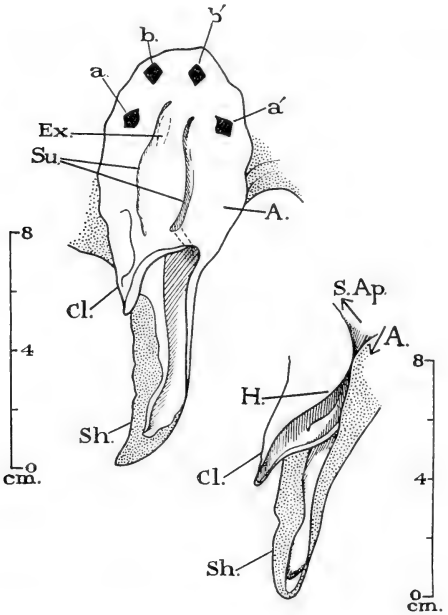


Fig. 2 Notidanus griseus. *Sh.*, sheath formed by pelvic fin; *Cl.*, clasper; *A.*, apopyle; *H.*, hypopyle; *S. Ap.*, aperture of 'siphon' tube; *Ex.*, position of the external sheath sac indicated in dotted outline; *Su.*, sulci; *a - a'*, *b - b'*, cartilages cut through in dissection.

tubular portion is brief. They are not covered with denticles, and have little or no skeletal support.

Between the outer border of each clasper and its sheathing fin is an external sac which is not merely a groove formed in the union of clasper and fin, but a space of considerable length (fig.1). In figure 2 the position of this sac is indicated in dotted outline.

Though pointing anteriorly, the sheath sac cannot be considered as homologous with a pseudosiphon.

Leading into the apopyle is a shallow cavity which appears to be homologous neither with a siphon, nor with the cavity of

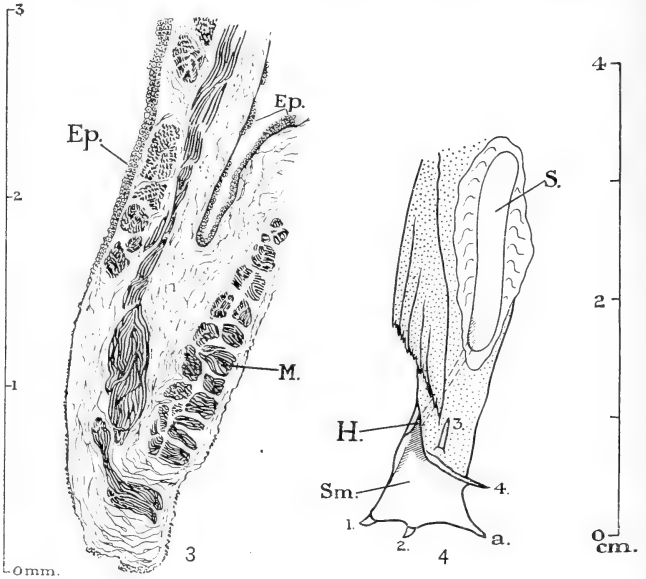


Fig. 3 Notidanus griseus. A transverse section through the party wall between the cavity and the sheath sac, in the position indicated by the dotted outline in figure 2 (haemalum - eosin). *Ep.*, stratified epithelium; *M.*, muscle.

Fig. 4 Spinax niger. *1, 2, 3, 4,* spines; *H.*, hypopyle; *S.*, siphon; *Sm.*, senticetum; *a.*, its apex.

Chimaera. It offers a homology with the former as to situation, confirmed by its being a sac with muscular walls capable of fulfilling the normal functions of a siphon.

A portion of the thin partition between this cavity and the external sheath sac, from the area bounded by the dotted outline in figure 2, was removed and sectioned, with the result that it

reveals (fig. 3) that both cavities are lined with stratified epithelium.

The apex of the cavity cannot be attained without cutting through the cartilages a - a', b - b' (fig. 2); in this it differs from a siphon, but resembles the cavity of Chimaera. On either side of the cavity is a deep sulcus leading down to the aperture of the cavity at the apopyle.

SPINAX (ETMOPTERUS) NIGER

The black dogfish

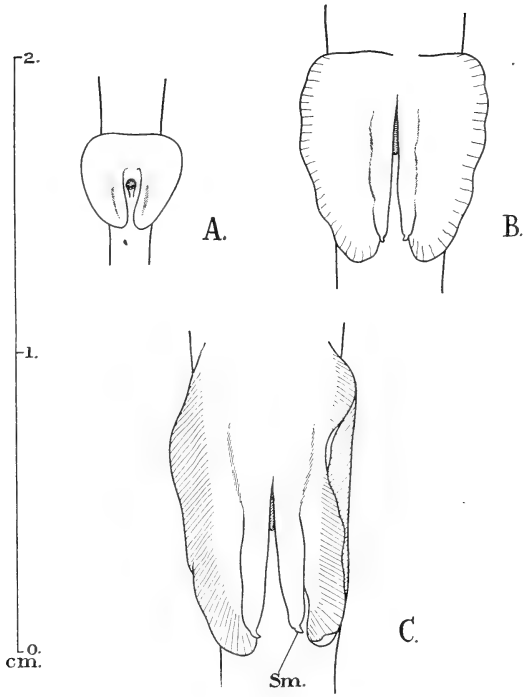
This smallest of the elasmobranchs affects deep water. I have examined specimens from 200 fathoms, from Christiansund; from 200 fathoms, from the coast of Portugal, and the specimen from which the figures are drawn was taken in 1904 at Faro, Algarve, Portugal, from 365 fathoms, and is 26 cm. long.

This memoir deals largely with types like Acanthias which depend upon movable spines rather than upon a rhipidion for fixative purposes during impregnation. These, in Spinax, are four in number, set within a senticetum which can be erected, in which case they take up the positions indicated in figure 4. One, number 3, points forward, while number 4 differs from the rest in being flat and blade-like, with a keen edge. The apex (a) of the senticetum is a tapering flap which projects over the spines, which, in a position of rest, are, according to Jungersen (b, Memoir I, p. 265), hidden by a pair of cartilaginous plates covered by skin.

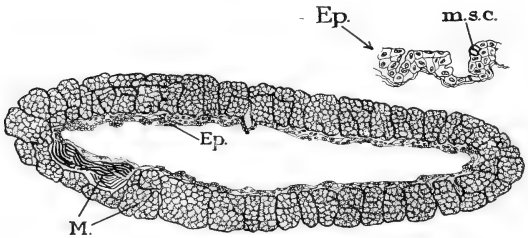
The claspers are stout, denticled all over, and adnate with the pelvic fins. There is no rhipidion.

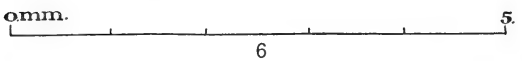
A series of embryos and young forms, taken at Bergen in 1901, and specially drawn for me by Michael G. L. Perkins at the University Museum, Cambridge, England, exhibits very clearly the development of the claspers and their state of coalescence with the pelvic fins (fig. 5).

The siphon is peculiarly stout, and may be mistaken at first sight for a gland. Sectioning, however, reveals that its solidarity is due to the extraordinary thickness of its muscular walls, which are here developed to an unusual extent (fig. 6). The



5





6

Fig. 5 Spinax niger. *A.*, embryo no. III; *B.*, embryo no. VI; *C.*, young specimen; *Sm.*, senticetum.

Fig. 6 Spinax niger. A transverse section through the siphon (haemalum-eosin). *Ep.*, stratified epithelium; *M.*, muscle; *m. s. c.*, mucus secreting cells.

epithelium lining the sac is the best example at present seen of the stratified variety, comprising four or five rows of cells, with an occasional mucus-secreting cell, as in Galeus. I should consider that in this type the siphons are the best adapted to perform the normal functions assigned to those organs.

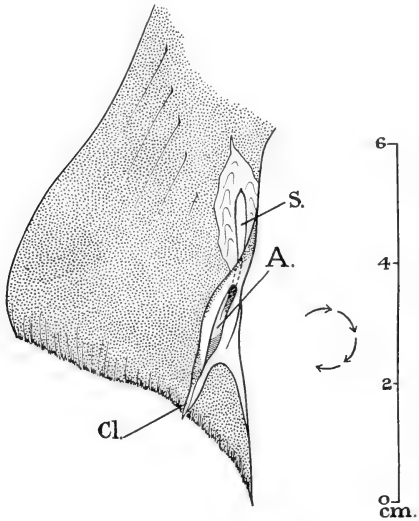


Fig. 7 Centrophorus lusitanicus. *Cl.*, clasper; *A.*, apopyle; *S.*, siphon

CENTROPHORUS LUSITANICUS

The Portuguese arreghonda

This sexually immature specimen, measuring 72 cm., taken on the coast of Portugal, shows a strong resemblance to Spinax in the following points (fig. 7):

The claspers are short, adnate with the pelvic fins; but there are no spines present in this specimen. The apopyle is wide with a tumid border.

There is no rhipidion. The siphon is short, and similarly situated to that of Spinax, but, instead of possessing thick walls, its walls are remarkably thin.

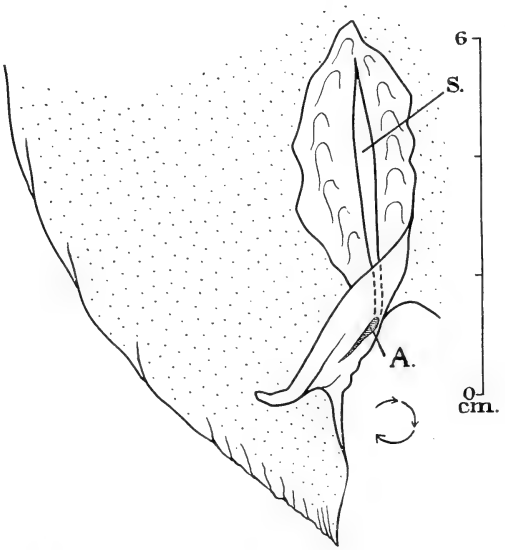


Fig. 8 Echinorhinus spinosus. *A.*, apopyle; *S.*, siphon

ECHINORHINUS SPINOSUS

The spiny or bramble shark

This species can only be, at present, described from a specimen taken at Nice, which though 85 cm. in length is immature. Figure 8 does no more than indicate a general resemblance to Centrophorus, the absence of spines and the presence of a similar siphon.

CESTRACION (HETERODONTUS) PHILIPPI

The Port Jackson or bull-head shark

An old and badly preserved specimen, taken at New Zealand, 89 cm. in length, was examined.

The claspers are long and stout, with a flexure recalling that of Mustelus (fig. 9). The apopyle, which is far removed from the cloaca, leads into a deep pouch, which is continued posteriorly in

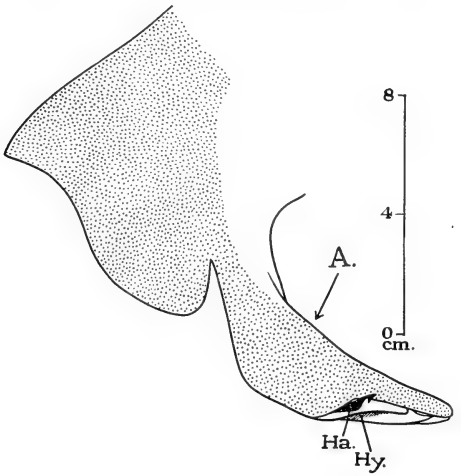


Fig. 9 Cestracion philippi. *A.*, position of apopyle; *Ha.*, hamus; *Hy.*, hypopyle.

a nearly closed tube with soft edges. A small rhipidion is present, posterior to which is a blunt, hard, movable hamus, with a recurved, crotchet-like head.

On the opposite side of the rhipidion to the hamus is a deep cavity, the crumena, not similar to any pouch previously described. There is a small thick-walled siphon present.

Notwithstanding the specialization of the claspers, as regards the development towards siphon formation, this genus appears to be one of the most primitive of recent forms, as might perhaps

be expected of a genus surviving from so geologically ancient times.

Situated in the clasper, anterior to the apopyle and leading posteriorly into it, is a blindly ending cavity whose boundaries are indicated by a dotted line in figure 10, and which is much more simple than that of Notidanus, and on about the same developmental level as that of Callorhynchus.

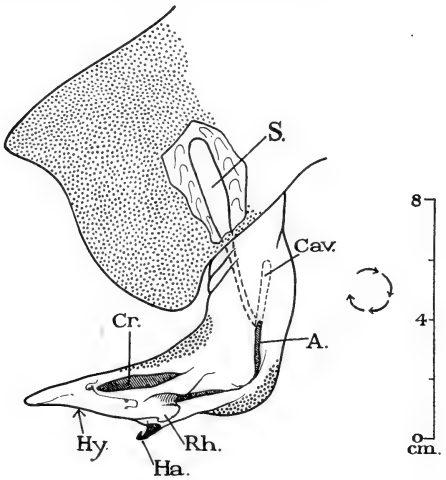


Fig. 10 Cestracion philippi. *A.*, apopyle; *Hy.*, position of hypopyle; *Ha.*, hamus; *Cr.*, crumena; *Rh.*, rhipidion; *Cav.*, boundary of cavity of the clasper indicated in dotted outline; *S.*, siphon.

PRISTIOPHORUS CIRRATUS

The saw-fish shark

A mature specimen of this species, taken at Tasmania in 1885, was examined, 109 cm. in length, a portion of the anterior end of the saw having been broken off and not included in this measurement.

As regard the claspers and their accessory structures, this animal bears a surprising likeness to Cestracion, although their

relationship is probably far apart. The apopyle, which is some distance from the cloaca, leads into a deep pouch, which is continued posteriorly in a closed tube with soft edges, all as in Cestracion (figs. 11 and 12). The small rhipidion in a similar position is so reduced as to be negligible. The hamus is replaced by a long, straight, thorn-like spur in a similar position. This

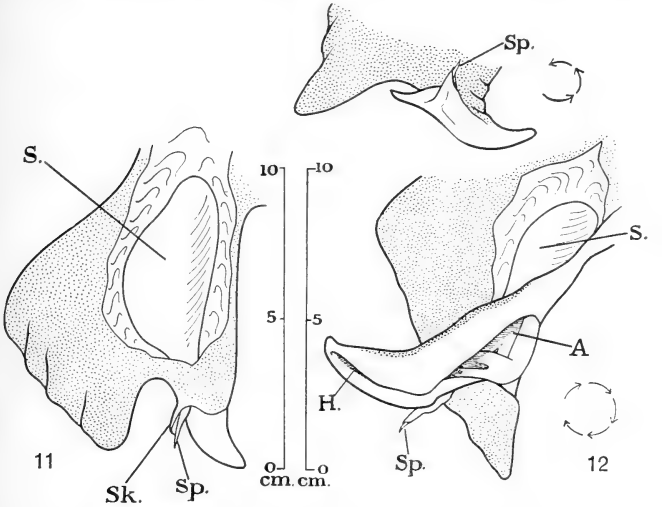


Fig. 11 Pristiophorus cirratus. *Sp.*, spur, enclosed in a sheath of skin (*Sk.*); *S.*, siphon.

Fig. 12 Pristiophorus cirratus. *A.*, apopyle; *H.*, hypopyle; *Sp.*, spur shown erected in the upper sketch; *S.*, siphon.

spur can be erected as in figure 12 upper diagram, and is suggestive of that of Acanthias save that it is perfectly straight, and when at rest lies in a sheath of skin of its own. There is, however, no crumena. The clasper tube is more perfectly closed, though still a scroll, and the hypopyle more distinctly defined. The claspers are stout and have a flexure.

The greatest difference between Cestracion and Pristiophorus is that, whereas the former has a small siphon, in the latter it is

an enormous bag. The deep pouch into which the apopyle leads is actually the wide mouth of the posterior end of this bag, so that no siphon tube is developed.

RHINOCHIMAERA ATLANTICA

It is stated that the males of Harriotta, a deep-sea member of the Holocephali, which this species closely resembles, have hitherto only been described from immature specimens. The following account of Rhinochimaera is made from a mature specimen, 84 cm. in length, taken off the southwest of Ireland in 1910.

In all its details Rhinochimaera shows a close similarity to Chimaera monstrosa (Memoir IV, p. 201), except that the claspers, instead of being bifurcated into external and internal radii as in Chimaera, are single, resembling the more slender of the radii of the other genus.

Rhinochimaera agrees with Chimaera in the following points (fig. 13): The pelvic fins are of the same conformation and cover over the claspers so as to conceal them. Leading into the apopyle is a cavity similar and similarly situated to that of Chimaera, which has already been described at considerable length. Anterior claspers are present, which can be retracted into a slit-like pouch, thus the clasper on the (observer's) left is almost fully protruded, while that on the right is almost withdrawn. The clasper is almost entirely composed of cartilage, but instead of being serrated on its morphological inner border it is beset with hooklets, like those of the pelvic claspers, which serve the same function as a serration. The histological details are the same, as far as I have been able to examine them. The clasper pouch, however, is set obliquely to the animal's axis (transverse in Chimaera, longitudinal in Callorhynchus), and the clasper is not spoon-shaped.

On account of its prolonged rostrum, inter alia, Harriotta has already been classified as showing affinities with the fossil Squaloraja polyspondyla, rather than with Chimaera; so also should Rhinochimaera. And, in the organs we are considering, there is another close resemblance, for the delicate, very flexible, posterior claspers, instead of being freely denticled, terminate in

a raspberry-shaped protuberance, grooved on the outer side of the middle line, owing to the presence of a raised external fleshy pad, and bearing attenuated, movable, dermal hooklets, as shown in the inset, strikingly like those of the fossil Squaloraja

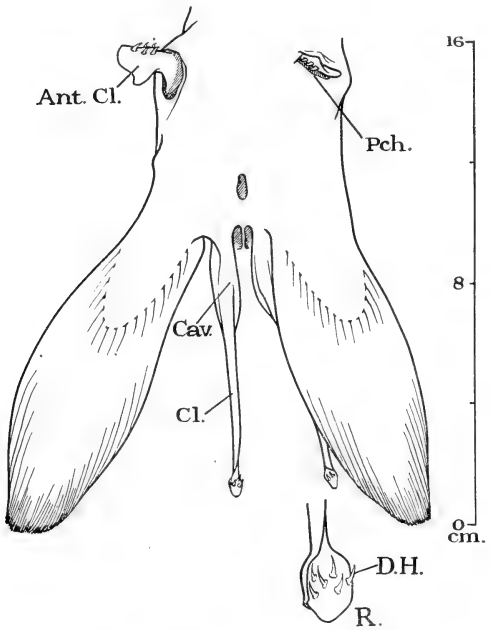


Fig. 13 Rhinochimaera atlantica. *Cl.*, posterior clasper; *Cav.*, position of cavity of clasper; *Ant. Cl.*, anterior clasper; *Pch.*, pouch of anterior clasper; *D. H.*, dermal hooklets; *R.*, enlarged view of the posterior (distal) end of the right clasper.

(Memoir III, fig. 3). The hooklets, which point anteriorly, and, when not erected, lie flat against the protuberance, appear to be more numerous than in Squaloraja, there being at least six principal ones on each side of the groove, besides other minute subsidiary ones which grade into scale-like forms; doubtless, some are not preserved in the fossil. There is no frontal clasper.

CHIMAERA (HYDROLAGUS) COLLIEI

This small Pacific species, taken near Monterey, California, measuring 49 cm. in length, differs from Chimaera monstrosa (Memoir IV, p. 201) in the following particulars: The anterior

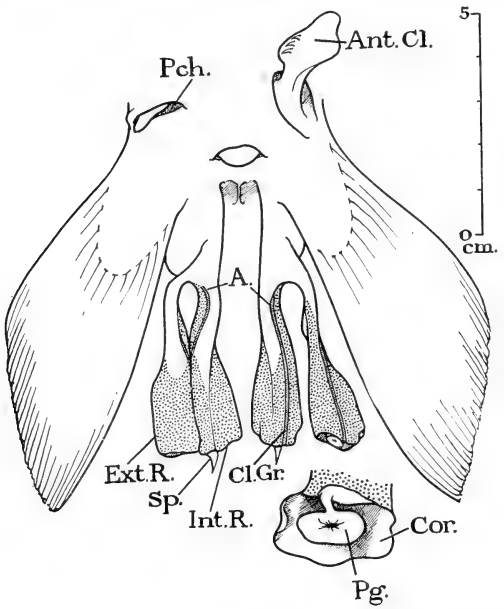


Fig. 14 Chimaera colliei. *Ext. R.* and *Int. R.*, the external and internal radii of the posterior clasper. *Cl. Gr.*, clasper groove; *A.*, apopyle; *Ant. Cl.*, anterior clasper; *Pch.*, pouch of anterior clasper; *Sp.*, spike; *Pg.*, peg; *Cor.*, corolla of skin, an enlarged view from the posterior aspect of the distal end of the left external radius.

claspers bear but 3 spines on their morphological inner border and their pouch is more widely open. The pelvic fins, of similar shape, do not so completely cover the posterior claspers, which are markedly different from those of the other species (fig. 14). Both the external and the internal radii are grooved, so that,

when the radii are apposed, as indicated on the (observer's) left, a temporarily closed passage is formed. The denticles are larger and differently situated, surrounding the posterior half of each radius. Posterior to, and unconnected with the hypopyle,

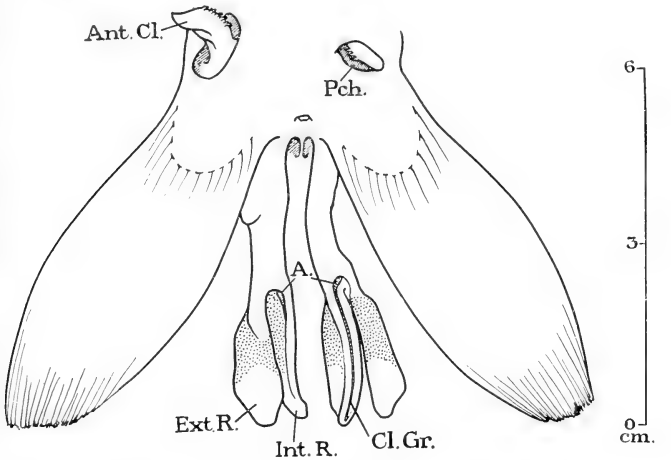


Fig. 15 Chimaera mirabilis. *Ext. R.* and *Int. R.*, the external and internal radii of the posterior clasper; *Cl. Gr.*, clasper groove; *A.*, apopyle; *Ant. Cl.*, anterior clasper; *Pch.*, pouch of anterior clasper.

the internal radius ends in a cartilaginous spike, and the external radius in a peg, having a hole in its center from which all tissue, if any, has disappeared, and surrounded by a foxglove-like corolla of skin, as shown enlarged in the inset. The proximal curvature of the external radius is very striking. Leading into the apopyle is a very minute cavity, entirely embedded in cartilage, whose nature could only be investigated by sectioning. The frontal clasper is huge in proportion to the size of the animal and the spikes on it larger.

CHIMAERA MIRABILIS

A male and a female of this small species, each measuring 77 cm., were examined from the west coast of Ireland. They rival Chimaera monstrosa in length on account of their extremely long tails. They differ from that species in the following particulars: The anterior claspers have five spines on their morphological inner border; their pouch is more widely open and set at a slightly oblique inclination to the animal's axis. The posterior claspers are small and their division into an external and an internal radius does not take place until half way down their length. They are slightly grooved, and approximation of the radii forms a narrow tube as in Chimaera colliei, but the denticles are but small. The apopyle is half way along the internal radius and no cavity leads into it (fig. 15). There are neither anterior claspers nor their pouch present in the female. The frontal clasper of the male is small.

RAIA

TYPE: RAIA CLAVATA

The thornback

How far various species of the same genus differ from one another must form the subject of a future memoir; meanwhile, no two genera could present more differences than the two types of Raia here discussed: R. circularis (Memoir I, p. 260) and R. clavata. Unfortunately, erection is not the same in the two cases, for, while in the former it consists in the diametrical expansion of a soft clasper by a suffusion of blood, here it is effected by the unfolding of the clasper edges and the protrusion of complicated structures by muscular contraction, from the position of rest shown in figure 19A to the condition shown in figure 16. The clasper gland, though similar in structure, is situated more posteriorly in the lobe of the pelvic fin, and its duct, really that of the containing sac, is not carried down the clasper as a closed tube to open posterior to the hypopyle, as in R. circularis, but debouches at the apopyle, as in Torpedo, Trygon, etc. (Memoir IV). A deep sentina is present as in Rhino-

batus (Memoir IV, p. 218), while its inner border is raised into a prolonged spike having a strong cartilaginous support to prop the oviduct open. The rhipidion is elongated and not in the

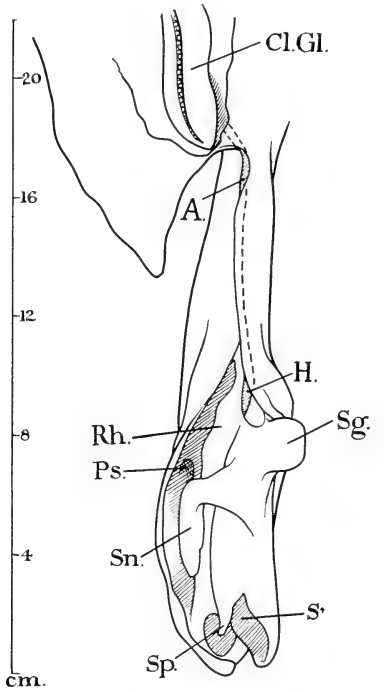


Fig. 16 Raia clavata. *Cl. Gl.*, clasper gland; *A.*, apopyle; *H.*, hypopyle; *Rh.*, rhipidion; *Sg.*, signal; *Sn.*, sentinel; *Ps.*, pseudosiphon; *Sp.*, spike; *St.*, sentina.

form of a fan; owing to its being twisted out of one plane, its function is very effectively performed. Posterior to the hypopyle, at the level of the commencement of the rhipidion, is a soft, fleshy pad, the signal, resembling the 'foot' of a bivalve mollusc, which, during erection, turns laterally through an

angle of 180°, and whose apex, from pointing inwards, becomes directed outwards, also propping the oviduct open, and distending it. Posterior to the signal, but remaining on the opposite face, is a knife-blade-like structure, the sentinel, so keen is whose edge, that, in spite of an epidermis over it, I repeatedly cut my finger on it. The oviduct is thus distended and stimulated in three directions. A pseudosiphon is present on the inner border of the concavity, serving partly as a sheath for the sentinel, when not in the erect condition. A shield is present, not so well developed as in R. blanda, and covered by the signal in figure 16.

The greater part of the year 1920 was spent in the electrical stimulation of pithed skate. Various species were used, including both R. circularis and R. clavata, but the results in the latter case were more conclusive owing to less difficulty in isolating the nerve twigs.

Previous practice in dissection revealed (fig. 17) that the siphon (*S*) and its contained gland are innervated by a posterior branch of the 52nd[2] spinal nerve, and that the clasper is innervated by a posterior branch of the 54th spinal nerve.

The animal figured is 84 cm. long. The spinal cord was severed just behind the medulla and the brain destroyed; it was also necessary to divide the cord again just anterior to the 50th spinal nerve to prevent any electrical reflux agitating the pectoral fins. The results point to the conclusion that the suggestions tentatively put forward in Memoir II, page 371, are not tenable for this species. This does not directly prove any point, since erection is of a different type in the two species, and the circumstance that the claspers of R. circularis taken immediately after copulation were both in a state of erection may well point to the fact that that phenomenon is there due to a chemical stimulus.

The results of electrical stimulation were as follows:

1. Stimulation of the posterior branch of the 52nd spinal nerve causes, first, secretion from some or nearly all of the papillae of the gland followed by a slow undulating contraction of

[2] The basis of this count is that of fish having thirteen spinal nerves in the anterior group of the brachial plexus.

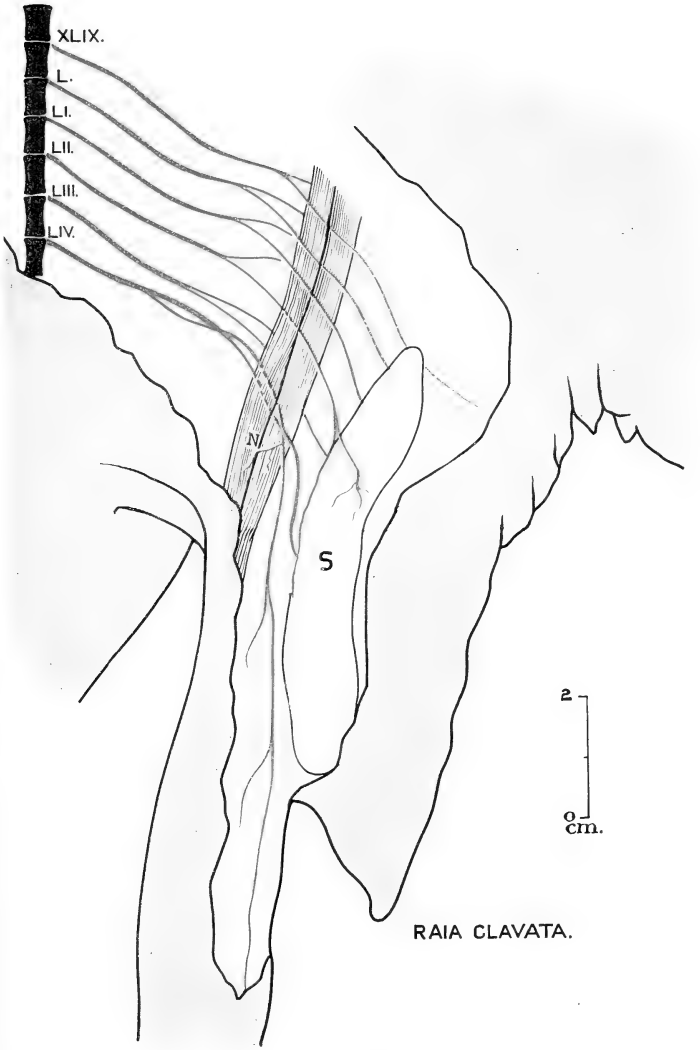


Figure 17

the containing siphon sac (*s*) in an anteroposterior direction, but no erection of the clasper.

2. A series of successive stimuli to the posterior branch of the 52nd spinal nerve causes prolonged spasmodic secretion, and the sudden activation of secretion from hitherto dormant papillae, but no contraction. During sustained stimulation contraction appears to be inhibited. On the cessation of stimulation a similar undulating contraction of the siphon sac follows. After prolonged stimulation there may be from two to as many as six such contractions following one another at gradually lengthening periods.

3. Stimulation of the posterior branch of the 54th spinal nerve causes erection of the clasper which continues for fifty seconds, the clasper continuing to remain erect during, and for some time after, a series of successive stimuli, but no secretion or sac contraction.

4. If the posterior branch of the 52nd spinal nerve be severed, and the common portion of this spinal nerve be stimulated, movements of the pelvic fin follow, but no secretion or sac contraction.

5. If the spinal cord be stimulated between the origin of the 52nd and 54th spinal nerves (and, indeed, at other places, in which cases other responses occur), secretion, followed by sac contraction, and clasper erection supervene simultaneously; wherefore, in order to obtain perfect results in cases 1 and 3, it is better to sever the spinal cord between the origin of the 52nd and 54th spinal nerves.

6. Besides being distributed to the accessory structures of the clasper, such as the signal, etc., the posterior branch of the 54th spinal nerve also gives off prominent branches to the muscles that work the whole clasper, such as the one indicated at *N* (fig. 17). If these branches are not cut, stimulation of the whole posterior branch causes not only erection, in the sense of that word as previously used, but also flexion of the clasper in an anterior direction, exactly as would be required in copulation. If these secondary branches be severed, stimulation of the main branch still causes erection, but in not so complete and perfect a

manner as when the other branches are left intact, so that anteroflexion of the clasper is brought about. In fine, the anteroflexion helps to originate the erection and is normally part of it, though, on nerve severance, erection alone can still be induced by stimulation.

Secretion of the clasper gland takes the form of the protrusion of long, glairy, transparent strings of substance from the papillae, suggestive of the squeezing out of an artist's oil colors from their

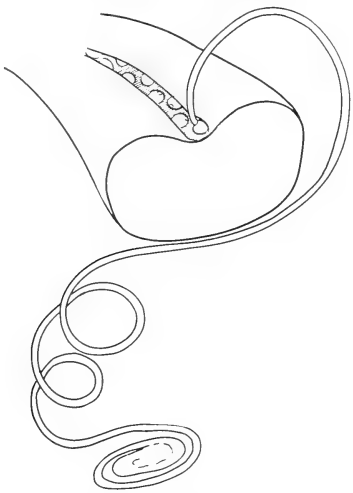


Fig. 18 Raia clavata. A diagrammatic representation of the secretion of a single string of albumin from a papilla of the clasper gland.

containing tubes. For the sake of simplicity, only one such string is shown diagrammatically in figure 18. This substance exhibits all the chemical and physical properties of albumin; hence the clasper gland must in the future be considered an albuminous gland. Long strings of albumin in an undisintegrated state were found lying in the siphon sacs of fresh skates dissected for practice which had died a 'natural' death, without resort to electrical or other stimulation. The secretion of many papillae at once is very striking to observe. Since all do not secrete

together, but here and there two or three papillae are passive, I gather that all the gland components are not in a state of activity simultaneously. I regret I have not obtained such results as yet in R. circularis.

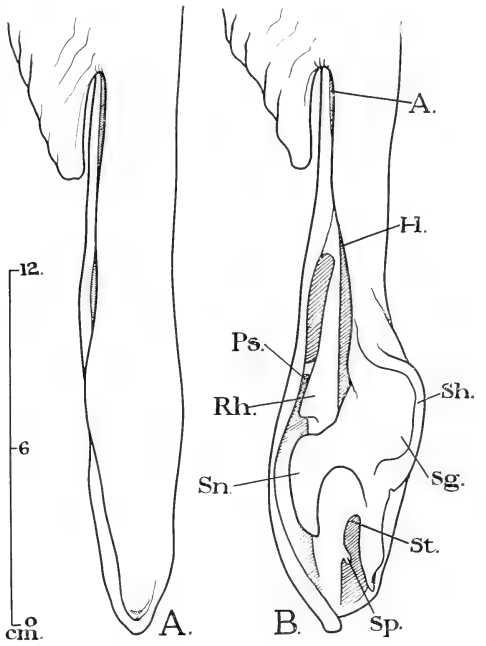


Fig. 19 Raia blanda, the right clasper in, *A.*, the normal position, *B.*, the erect position. *A.*, apopyle; *H.*, hypopyle; *Rh.*, rhipidion; *Ps.*, pseudosiphon; *Sg.*, signal; *Sh.*, shield; *Sn.*, sentinel; *Sp.*, spike; *St.*, sentina.

I have not yet met with skates in copula, but a reliable authority informs me that in these larger species only one clasper is inserted at a time.

The specimens of R. clavata were from Weymouth, Plymouth, and other parts of the English Channel.

RAIA BLANDA

The blonde ray

This skate is of the same type as R. clavata, and all the accessory structures of the clasper are similar, save that the spike is smaller and the shield parallel with and covering and protecting the signal, and of the same hard constituency as the sentinel, is larger, as is also the signal (fig. 19). The clasper gland is as in R. clavata.

Resumen por el autor, Walter N. Hess.

Orígen y desarrollo de los órganos luminosos de Photurus pennsylvanica De Geer.

El primer indicio de la formación de los órganos luminosos de la larva en el embrión se manifiesta a la edad de quince dias. En este momento un grupo de células adiposas, con sus grandes glóbulos de grasa que se tiñen en negro por el ácido ósmico, emigran ventralmente en el segmento octavo y vienen a situarse en la región ocupada por los futuros órganos luminosos de la larva. Los órganos luminosos del adulto comienzan a desarrollarse unas pocas horas antes de la formación de la ninfa, en cuyo momento un gran número de esferas grasosas, en el sexto y séptimo segmento abdominal, se desintegran y las células adiposas así liberadas, con sus glóbulos grasosos teñidos en negro, emigran a la región de los futuros órganos luminosos del adulto.

Durante unos dias, tanto en el embrión como en la ninfa, no existe diferencia perceptible alguna en ninguna de las células del órgano luminoso, pero finalmente tiene lugar una diferenciación en ellas, transformándose en las capas reflectora y fotogénica. Aún después de ser perceptibles ambas capas, los glóbulos de grasa coloreados en oscuro pueden a menudo observarse en sus células. La presencia de glóbulos grasos oscuros en todas las células de los órganos luminosos en vías de desarrollo, lo mismo en la larva que en la ninfa, junto con el método de formarse estas células, conduce al autor a la conclusión de que los organos luminosos de Photurus pennsylvanica son de orígen enteramente mesodérmico. Los órganos luminosos larvarios cesan de funcionar durante el segundo dia de la vida adulta, a cuyo tiempo son fagocitados.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, FEBRUARY 20

ORIGIN AND DEVELOPMENT OF THE LIGHT-ORGANS OF PHOTURUS PENNSYLVANICA DE GEER

WALTER N. HESS

Department of Entomology, Cornell University

FIVE PLATES (SEVENTEEN FIGURES)

CONTENTS

INTRODUCTION

In spite of the fact that the light-organs of fireflies have been the object of much study during the past century, comparatively little has been done on their development. During the past few years, however, this phase of the subject has received more attention, though as yet the question of their origin is not settled.

Shortly after the publication of the two brief conflicting reports, regarding the origin and development of the light-organs in insects, by Vogel ('13) and Dubois ('13), the author undertook a study of the development of these organs. During the winter of 1916 to 1917 there appeared two more articles on the origin of these organs (Williams, '16, and Dahlgren, '17), but as these contradicted one another, the question was considered still unsettled. At that time the present study, which had been largely devoted to the development of the light-organs in the embryo, was interrupted by the war, so a brief preliminary report was published. Upon resuming this

work during the past year, a careful study was made of the development of the light-organs in both the embryo and the pupa. This has resulted in the modification of the tentative views expressed in the preliminary report.

The author is indebted, especially, to Dr. William A. Riley, under whose supervision the greater part of this study was made, also to Dr. O. A. Johannsen for his helpful suggestions and criticisms.

MATERIALS AND METHODS

Eggs were obtained, for a study of the embryonic development of the light-organs, by confining ripe females in jars that had been partly filled with earth and moss. Since oviposition occurred very readily in captivity, it was easy to obtain a complete series of eggs by removing the insects to different jars each day.

The different larval and pupal stages were obtained by collecting second-year larvae in April and confining them in jars with earth and moss. By observing them each day, desired stages could be selected.

Two complete sets of eggs, late larvae, and pupae were obtained as described above. The eggs were collected at intervals of twenty-four hours, from the time of oviposition until hatching. The pupae were collected at twenty-four-hour intervals from the time of pupation until emergence. Several stages of late larvae were obtained previous to pupation. One set of this material for histological purposes was fixed by heating in water at a temperature of 80°C. for four minutes, after which it was transferred to alcohol. These eggs were punctured with a fine needle immediately after heating, in order to insure good preservation. The chorion of each egg was later dissected off to expose the embryos. The other set of material was fixed in Flemming's fluid (strong formula). These eggs were punctured and allowed to remain for twelve hours in the fixer, after which the chorions were removed and the eggs were returned to the fixer for another twelve hours. The first twelve hours of fixation was sufficient to harden the embryos so that their chorions could be dissected off, while the last twelve hours insured good fixation. In the

case of the larvae and pupae, the posterior four segments were removed by scissors and placed in the fixers.

The material was all imbedded in paraffin. In the case of the embryos, a binocular microscope was used to insure proper orientation for sectioning. Sections of the larvae and pupae were cut from 6 to 10μ in thickness, while those of the embryos were cut 3 and 4μ thick. Heidenhain's iron hematoxylin was used for staining all sections.

HISTORY OF EMBRYOLOGICAL WORK ON THE LIGHT-ORGANS OF INSECTS

There are, in general, three conflicting views regarding the origin of the light-organs in insects. One view is that they are derived by a proliferation of the hypodermis and hence are ectodermal; another, that they are formed from both ectoderm and mesoderm and, lastly, that they are formed from fat-cells and hence are of mesodermal origin.

Two views were suggested by early workers concerning the possible ectodermal origin of these organs. The one of these that is least generally accepted was advanced by von Kölliker ('57) and Lindemann ('63), who maintained that the structures are nervous in origin. Von Kölliker compared these organs with the electric organs in fishes, which, together with the fact that the light-organs of insects are under the control of the nervous system, led him to conclude that they are of nervous origin. Lindemann considered the organ a definite part of the nervous system. The other of these views was supported by Owsjannikow ('68), Heinemann ('86), Dubois ('98, '13), and Marchal ('11), who upheld the idea that these organs arise from the hypodermis by a proliferation of its cells. Owsjannikow considered the organ in the nature of a gland and hence ectodermal in origin. Heinemann, who worked on the light-organs of the elaterid beetles, considered that those organs, as well as the light-organs in the Lampyridae, were derived from the hypodermis. Marchal was also of the opinion that they are formed from ectoderm. The observations of all these workers, unless it is Dubois, are of little value, as they studied only the adult organs.

The question of the origin of these organs can be settled only by a study of the development of the photogenic tissues. With the exception of three recent papers, Vogel ('13), Williams ('16), and Dahlgren ('17), this has not been done by any one except Dubois. Dubois ('98) studied the embryology of both Lampyris noctiluca and Pyrophorus noctiluca. He believed that he was able to follow the development of the photogenic organs through the different stages, from the beginning of segmentation to the adult insect. He discovered a close histological resemblance between the blastoderm cells and the photogenic cells of the larva, pupa, and adult. Furthermore, he concluded that the cells of the hypodermis multiplied, and by proliferation formed directly the photogenic organs of the larva. For some reason Dubois' work has attracted little attention, and is practically ignored in general discussions of the subject. Moreover, he misinterpreted the normal structure of the body wall in Lampyris, and for this reason his conclusions have been severely criticised by the few who have discussed them.

Wheeler and Williams ('15), in their study of a mycetophilid fly of the New Zealand caves, found that the light-organs were a modified portion of the malpighian tubules. These structures, as is well known, are of ectodermal origin, and this furnishes a clear instance of light-organs from the ectoderm in insects.

Dahlgren ('17) studied the development of the adult light-organs in the pupa of Photurus pennsylvanica. He states that the light-organs are formed by a proliferation of cells from the ventral abdominal hypodermis, which multiply and later differentiate into the two layers of the light-organs. He does, however, express some doubt as to the possible origin of the reflector layer.

Two different workers upheld the view that the light-organs arise from both ectoderm and mesoderm. Gegenbauer ('74) advanced the idea that the non-luminous, or reflector, layer of the light-organs is derived from the fat-body, while the photogenic layer is formed from the hypodermis. Wielowiejski ('90), in opposition to Dubois, states that the so-called reflective, or urate, layer of the photogenic organs is derived from fat-cells, while the light-giving, or luminous layer, on the contrary, is

composed of cells derived from oenocytes, and is, therefore, ectodermal. Here again the conclusions were based on a study of the mature organs, and hence are not conclusive.

With the exception of the two previously mentioned papers by Vogel and Williams, all authors who favor the theory of fat-cell origin have based their conclusions only on a study of the adult organs. Many of these, including Peters ('41), Leydig ('57), Schultz ('65), Emery ('84), Seaman ('91), Wheeler ('92), Bongardt ('03), and Berlese ('09), seem to be of this opinion, largely because the structure of the mature organ resembles somewhat that of fat-cells, and also because certain cells of the photogenic organs secrete urates and other products, comparable with fat-cells.

Recently two important papers have appeared, which were based upon a definite study of the developmental tissues; one by Vogel ('13) and the other by Williams ('16). Vogel studied the embryonic development of this organ in Lampyris noctiluca, beginning with a stage in which the organ was already clearly differentiated. He made a comparable histological study of its cells with those of the neighboring tissues, from which he concluded that the cells of the photogenic organ, in this stage, agree with the neighboring fat-cells in shape, size, and relations of their nuclei. On this he based his conclusions that the light-organs arise from fat-cells.

Williams ('16) worked upon our native species, Photurus pennsylvanica and Photinus consanguineus, and apparently confirmed the work of Vogel. Although he studied the development of both the larval light-organs and the light-organs of the adult, his observations were devoted almost entirely to the adult light-organs. In the case of the adult he found that the fat-spheres broke down, liberating their cells, and that these became distributed along the hypodermis in the region where the light-organs were to form. He also found that the cells of the early organs formed a gradual gradation from the rather dark pigmented cells lying against the hypodermis, to those on the side towards the fat-cells and continuous with them.

Buchner ('14) and Pierantoni ('14) both consider the light-organs a symbiotic structure in which there are found luminous bacteria, or fungi, that cause the light. Buchner showed a very close similarity between the granules in the photogenic layer of the light-organs and the symbiotic bacteria of the homopteron, Aphrophora spumaria.

LOCATION AND STRUCTURE OF THE LIGHT-ORGANS

The light-organs of all the luminous Lampyridae were found lying next to the sternal side, in the eighth abdominal segments of larvae, and in one or both of the sixth and seventh abdominal segments of the adults. In the larvae the organs appear as two small elliptical discs (fig. 1). In an adult male of Photurus pennsylvanica the light-organs cover the entire sternites of the sixth and seventh abdominal segments (fig. 2), while in the female of this species the organs occupy only about two-thirds of the corresponding sternites (fig. 3). During the first one or two days of adult life, the larval light-organs can be seen to emit light from the eighth abdominal segment (fig. 3). The phagocytosis of these organs however, is usually so far advanced by the end of the second day of adult life that they no longer emit light.

In the species studied, the arrangement of these organs, in the larva, is shown diagramatically by figure 4. The photogenic layer (*P*) lies next to the ventrolateral sternite of the eighth abdominal segment. In the adult male (fig. 5) the photogenic layer (*P*) extends entirely across the sternite next to the hypodermis, while the reflector layer (*R*) completely covers this layer on its dorsal side in both the larva and the adult.

The mature light-organs whether in the larva (fig. 9) or in the adult (fig. 17), are of the same general structure. Both are composed of two layers of cells, the inner reflector layer (*R*) and the outer photogenic, or luminous layer (*P*). The former is composed of fairly regular polygonal cells in which are located a large quantity of crystals of urate salts. This layer in life is opaque and chalky in appearance. The ventral layer is composed of two parts: the tracheal structures (*T*) and the photo-

genic cells (*P*). The photogenic cells, whose walls are often indistinct, contain numerous granules of a non-urate composition, called photogenic granules. Tracheae penetrate both layers of the light-organs, and in the region of the photogenic layer they are profusely branched. These branches each end in a tracheal end-cell, which sends its tracheal capillaries, or tracheoles (*T C*), in among the photogenic cells. The photogenic layer is overlaid on the ventral side by a thin layer of hypodermal cells, which in the region of the light-organs secretes a non-pigmented cuticula.

ORIGIN AND DEVELOPMENT OF THE LARVAL LIGHT-ORGANS

The eggs of Photurus pennsylvanica, in this climate, require an average of about twenty-six days to complete their embryonic development. This period, however, is influenced largely by weather conditions.

Since the embryonic light-organs are of such minute size, it was often difficult to locate the structure in cross-sections, hence, for most of this work only sagittal sections were used.

Up to and including the thirteenth day of incubation, the embryos were found to be bent backwards so that they nearly formed a circle. On the fourteenth day the embryos began to turn, and at the head end there was a slight indication of their coiling up. The posterior end, however, was still turned slightly backward. The embryos on the fifteenth day showed more evidence of coiling up than on the fourteenth day, for now the posterior end was also turned slightly forward. Sagittal sections of the lateral portions of the eighth abdominal segment (fig. 6) show the presence of many fat-cells, as well as a clearly defined hypodermis surrounding the body. Those embryos which were killed in Flemming's fluid and stained in Heidenhain's iron hematoxylin show a very clear differentiation between the fat-cells and those of the hypodermis, since the fat-cells contain many fat-globules which are colored dark by osmic acid. No such globules were found in any of the hypodermal cells. As the embryos begin to coil up, the abdominal segments become larger, forming a space of considerable size in the region of the future

body cavity. This space, in the fifteen-day embryos, is largely filled with fat-cells.

The earliest indication of the formation of the light-organs was found in the fifteen-day embryos. At this time some of the large fat-cells with their dark colored globules, located in the eighth abdominal segment, were collecting together and becoming closely applied to the hypodermis in the region of the future light-organs (fig. 6). This section, which was prepared as described in the preceding paragraph, shows a very clear differentiation between these cells and those of the hypodermis. In fact, the cells of the early light-organs are larger than those of the hypodermis, and in addition they contain the dark colored globules which are so characteristic of the fat-cells. The nuclei of the fat-cells are also larger than those of the hypodermis. At this time the cells of the light-organs were found to be continuous with the fat-cells of the body proper. The dark colored globules, in the cells of the light-organ that were located nearest to the hypodermis, were smaller and fewer in number than those of the fat-cells in the body dorsal to the light-organ. In fact, there appeared to be a gradual gradation in the size and amount of these globules from those cells that were found nearest to the hypodermis, where they were smaller and fewer in number, to the cells dorsal to the light-organ where the globules were larger and greater in number. There was no indication, at this time, of the two layers which are so characteristic of the mature light-organs.

A large cylindrical group of tracheal epithelial cells (*T*) was found just dorsal to the light-organ, but as yet it had not secreted any chitin.

In the sixteen to seventeen-day embryos the light-organs are entirely laid down so far as the contribution of fat-cells is concerned (fig. 7). In fact the organs at this time are regular in outline and their cells do not appear to be so closely applied to the hypodermis. The individual cells of the light-organs, at this stage, appear similar to those of the fifteen-day embryo, except that they are now closer together; their cell boundaries are less distinct, the dark colored fat-globules are smaller in size and

fewer in number. So far as could be determined, the cells of the entire mass are alike in size and structure and give no indication of a differentiation into two layers. The similarity between the light-organ cells and the neighboring fat-cells is very evident even at this stage. The only difference in structure that could be determined between these two groups of cells is in respect to the size and abundance of the fat-globules. These globules appear slightly larger and a little more abundant in the fat-cells than in the cells of the light-organ.

The group of tracheal cells, dorsal to the light-organ, have now secreted a lining of chitin and have become connected to the light-organ.

At the age of about twenty days there occurs a differentiation of the cells of the light-organs into the two layers: the photogenic layer (*P*), which lies next to the hypodermis, and the reflector layer (*R*), which surrounds the cells of the photogenic layer, except in the region of the hypodermis (fig. 8). At this time the cell walls of all the cells that compose the light-organs are rather indistinct. Those, however, of the reflector layer appear slightly more distinct than those of the photogenic area. The cells of the two layers resemble one another in shape and size, but in structure they appear much different. The cells of the reflector layer seem to be considerably vacuolated and less granular, while those of the photogenic layer give a much denser appearance due to denser granulation. The fat-cells at this time are much vacuolated. They no longer appear similar to the cells of the photogenic layer, but they do resemble very much those of the reflector area. The dark osmic acid colored globules, so characteristic of the early light-organs and the fat-cells were not noticeable in the preparations of this period. Their absence, however, may be due to fixation, since these globules appear in the preparations of a corresponding period in the development of the adult light-organs in the pupa.

At the end of twenty-two days the embryos begin to emit light from the light-organs. At this time they are capable of moving about within the chorion, through which membrane the light-organs appear as two minute spots of light. The two layers of

the light-organs are now well differentiated, although considerable change takes place subsequently.

The entire light-organ appears to be attached to the hypodermis by a delicate, non-cellular membrane,

The mature light-organ, in a larva one year old (fig. 9), shows very distinctly the arrangement of the two layers, namely, the reflector layer (*R*) and the photogenic layer (*P*). The cells of the photogenic layer contain many rather small granules, yet in the cells of this layer of the adult light-organ, except along their margins, these granules are very large. The cells of the reflector layer are much less granular. With the fixers and stains used the cell walls of the photogenic cells are rather indistinct, while those of the reflector cells are noticeable. As will be observed by comparing the larval light organ with the mature adult light-organ, the cells of the two structures are similar in appearance, yet the reflector layer, in the larva, is thinner than in the adult organ in proportion to the thickness of the photogenic layer in the two stages. One large trachea sends its branches to the cells of the larval light-organ. The hypodermal cells in the body wall are now much reduced in size in comparison with those of the embryo. These cells are especially small on the ventrolateral side of the light-organ. Here they secrete a rather thick cuticula, but, due to its transparency, the light from the organ easily penetrates it.

ORIGIN AND DEVELOPMENT OF THE ADULT LIGHT-ORGANS

Since the adult light-organs of insects are developed during the pupal stage, a large number of larvae of Photurus pennsylvanica were collected during April, for the purpose of obtaining material for the study of the development of this organ. About the 25th of May certain of these larvae built their pupal chambers, and in about five days they transformed to pupae.

Several of the active larvae were taken about a week or ten days before their normal pupation period, and sections were made of the sixth and seventh abdominal segments, to determine the nature of the various histological structures. A transverse section (fig. 11) shows a normal layer of hypodermal cells (*H*),

just dorsal to which are large fat-spheres (*F*) containing many large fat-cells. Certain sections show these spheres closely appressed together and lying upon the hypodermis. There were often two layers of these fat-bodies lying near the hypodermis, although some sections showed only one. The cells of these fat-spheres near the hypodermis are destined to form the light-organs. They contain many dark colored fat-globules which are characteristically colored by the osmic acid. Williams called them the photogenic fat-spheres. At this stage several large and small leucocytes are scattered about among the fat-masses.

Williams, in his discussion of the early stage in the development of the light-organs, places considerable emphasis upon the small leucocytes which he terms fat 'haemocytes.' According to this author, there occurs at this time a partial investment of the photogenic fat-spheres by a band of yellowish-brown material. He does not think that it is a secretion of the fat-spheres, but that it is formed from material in the blood, which, together with certain haemocytes, have been attracted to one side of the fat-body. He finds the small leucocytes almost always in contact with this investment and not applied elsewhere to the fat-spheres. He then concludes that the investing cap, as well as the small leucocytes, seems to be instrumental in breaking up the fat-body. This is described as taking place by the inflection of the cap in which the fat-sphere is squeezed, or constricted, until the thin membrane opposite the envelope can no longer stand the strain and ruptures. This pressure often serves to distort the nuclei. He finds the investment only on the side of the fat-spheres next to the alimentary canal, which make it possible for the fat-cells, as soon as they are liberated to migrate immediately to the body wall and there form the photogenic layers.

Although considerable attention was given to the nature of the fat-spheres just previous to the formation of the light-organs, no such investment membrane could be made out with any degree of certainty. In certain cases there appeared what seemed like a little denser mass of insect blood about these fat-bodies, but

in no case was there definitely evident a semicircular band-like structure on the intestinal side of the fat-spheres that appeared to be functioning in breaking down these globular masses of cells. It is true that there were a few small leucocytes present at this stage and as the light-organs began to develop, they seemed to increase slightly in numbers. They were not, however, found attached to the fat-spheres as Williams described them. Whether they may function in connection with the early formation of the light-organs was not determined. Because of the apparent increase in numbers, it is possible that they help in breaking down the fat-bodies.

Since there is a considerable variation in the stage of development of the light-organs in different larvae and pupae, at the same age with respect to the time of pupation, it is difficult exactly to correlate stages in the development of the light-organs with definite periods preceding and following pupation. Among the different series of the developmental stages studied, of the same age, a difference of fully one day is frequently noted in the development of these organs.

A typical larva, taken about one-half day before pupation, appears sluggish and distended with blood. On sectioning, the light-organ cells usually appear as is shown in figure 12. Certain of the large fat-spheres have ruptured and their cells are being distributed along the hypodermis next to the basement membrane. In certain instances, where the fat-spheres are found lying near and closely appressed against the basement membrane, they are flattened and their cells distributed along the hypodermis, without apparently leaving the fat-spheres. A little later, however, these fat-spheres liberated their cells. A regular gradation in the cells of the fat-spheres in such instances can be seen; those next to the basement membrane are rather flattened, their nuclei larger, their fat globules smaller and fewer in number, and their cell walls more distinct than the cells farther from the hypodermis. As these cells spread out in this manner, the old coverings of the fat spheres disappear, due undoubtedly to the action of the leucocytes.

The fat cells, when they first leave the fat-spheres, are large and nearly circular, though somewhat irregular in outline. Their nuclei are large and fairly distinct, though they are often more or less concealed by the fat-globules which are colored dark by osmic acid. Their nuclei are not elongated and distorted, as Williams described. The cytoplasm of the cells show a more or less vacuolated condition. Soon after the fat-cells are liberated from the fat-spheres and become distributed along the hypodermis, their cells divide rapidly, and it is not uncommon, in sections of this stage, to find them in mitosis. Occasionally the fat-cells can be observed dividing before leaving the fat-spheres, but in all such cases, the spheres have ruptured and are lying against the basement membrane.

It is true that there are numerous leucocytes present about the fat-spheres and the newly liberated fat-cells, though the enveloping membrane of the fat-spheres described by Williams does not appear to be present. The large leucocytes are especially abundant, and it is possible that they function in breaking down the fat-bodies. The small leucocytes are also present in considerable numbers, and it seems possible that they may also have a similar function. Some of these leucocytes are undoubtedly functioning in breaking up some of the fat-masses for the actively developing tissues. Neither the small nor the large leucocytes are observed among the cells of the light-organ, though they are often found lying near, or in the region of, these cells.

The hypodermis at this stage also shows evidence of considerable activity, for its cells are much elongated (fig. 12) and some show evidence of division. Sections of some of the larvae at this stage do not show these elongated hypodermal cells, though as a rule they appear to be attenuated to a considerable extent. It seems possible that the stage of their elongation may not correspond with the early origin of the light-organs, so the two structures may not appear the same at the same stage in different specimens.

Branches from the larger neighboring tracheae make their appearance very early, and at this stage these smaller tracheae

(*T*) may be seen extending down among the fat-spheres in the region of the undifferentiated cells of the light-organ (*U*). They do not extend to the hypodermis at this stage. Their cells often show evidence of active mitosis. It is not until late in the development of the light-organs that these tracheal cells form the mature trachea and tracheoles.

A section of the light-organs of a typical pupa taken one-half day after pupation (fig. 13) shows a considerable modification of that of the previous figure. The fat-spheres are no longer observed in a ruptured condition, and it seems very probable that all of those destined to function in the development of the light-organs have liberated their cells. The undifferentiated cells of the early developing light-organ (*U*) at this stage are about three cells deep and they are entirely undifferentiated, so far as any evidence of a differentiation into two layers is concerned. These cells now appear considerably different from those of the early liberated fat-cells shown in figure 12. Their nuclei are larger and they contain a denser chromatin mass. Their cytoplasm appears to contain a fine network of granular protoplasm. The large dark colored fat-globules are much less numerous, although a few of them can be seen in the cytoplasm of all these cells. It seems very probable that they function as a reserve supply of food, and are used up during the increased activity of these cells while they are forming into the new light-organs. There is still evidence of mitotic division among these undifferentiated light-organ cells.

The hypodermis presents some complicated, yet interesting, conditions at this time. Its cells no longer show the attenuated condition of figure 12, but, instead, they lie along the cuticula. They present a very irregular appearance, for many of them appear as if they might be wandering up among the undifferentiated cells of the light-organ. Their cytoplasm is of a fine granular nature and resembles rather closely that of the light-organ cells at this period. Their nuclei are rather large and also resemble very closely the nuclei of the light-organ cells. The size of the two groups of cells varies very little, although as a rule those of the hypodermis are smaller. To add to the difficulty of inter-

pretation at this stage, the basement membrane has largely broken down, so that no very definite line of separation between the two groups of cells could be found. Since modifications of a similar character were observed in the hypodermis remote from the light-organ, it was concluded that these peculiarities are associated with the normal metamorphosis of this tissue.

The dark colored globules which were present in the fat-cells are still present, in a small number in all the undifferentiated cells of the light-organ, but none are present in the cells that are definitely known to be hypodermal. The presence of these fat-globules in the fat-cells and the cells of the developing light-organ, but not in the hypodermal cells, leads one to conclude that these undifferentiated light-organ cells are derived entirely from fat-cells.

The tracheae by this time have penetrated the light-organ cells at frequent intervals, and at many places they have extended their cells to the hypodermis. Frequent mitotic divisions are still observed among these cells. Some of the tracheal cells, which appear to be grouped in masses, are frequently observed lying just dorsad of the light-organ cells. As a rule, several of the cells from these larger masses, extend down between the cells of the light-organ. These tracheae, while in an immature stage of development, grow down among the light-organ cells at more or less regular intervals and they resemble in location those of the mature light-organs.

A slightly later stage than figure 13, which was taken of a pupa one day after pupation, is represented by figure 14. Even at this stage the cells of the light-organ show no evidence of a separation into the two layers, but, on the other hand, they all appear to have the same general characteristics and resemble very closely those of the undifferentiated light-organ cells shown in figure 13. It seems evident, however, that their cytoplasm is slightly more granular and the fat-globules are slightly less abundant, but, aside from the fact that they are now four or five cells in depth, there is little real difference. Emphasis here should be placed upon the fact that all the undifferentiated cells of the light-organ (*U*), at this stage, appear to be alike histologi-

cally and that they all contain the dark colored fat-globules. If certain of these cells had been proliferated from the hypodermis, it seems very probable that there would be two different types of cells present. The hypodermis, which was so irregular and indefinite in outline in the previous stage, now shows its cells all arranged in a regular manner along the cuticula, except for an occasional cell.

In a pupa four days old, the cells of the future light-organs show a decided advance in development (fig. 15). The cells of the two layers can be fairly clearly distinguished, though they still appear to intergrade to a certain extent. Those of the photogenic layer (*P*) are larger, nearly spherical, and more regularly arranged than those of the reflector layer (*R*). Their cytoplasm is of a nearly uniform dense granular nature, except for an occasional dark colored fat-globule, around which there appears to be a lighter area. Their nuclei are larger, but their chromatin content does not appear as dense as formerly. The cells of the reflector layer (*R*) are smaller, rather irregular in outline, and their cytoplasm is made up of a fine granular network. The fat-globules are still present in the cells of both layers.

The hypodermis with its basement membrane is now represented by a narrow border of cells lying next to the cuticula.

The developing tracheae (*T*) show little advance over those of the previous stage, except that they have enlarged, and their cells now rest firmly upon the hypodermis. Their cells do not appear to be dividing at this stage.

A little later stage, represented by a pupa five days old (fig. 16), shows the two layers of the light-organ clearly differentiated. The photogenic layer (*P*) is composed of much enlarged cells, which, except for their larger size and semirectangular nature, appear much the same as the photogenic cells of figure 15. The cells of the reflector layer are of the same general appearance as they were in the previous stage. The cells of both layers still retain some of the dark colored fat-globules.

The development of the light-organs from the stage represented by figure 16 to the mature organ requires a period of about one week. The most noticeable change takes place in the tracheal

cells, from which mature tracheae with their tracheal end-cells develop. These tracheae (fig. 17, *T*), like the tracheal end-cells, are formed from the tracheal epithelium. The boundaries of these end-cells are not distinctly seen, yet their nuclei (*E C N*) appear much the same as the nuclei of ordinary tracheal epithelium. The large tracheal branches in the region of the photogenic layer gives off many smaller branches which divide and often redivide, each branch finally ending in a tracheal end-cell. In the region of the photogenic layer the tracheal epithelium is much thicker, and it is here that the tracheal end-cells are formed. Since these cells are very abundant, they form a contiguous mass, arranged in the form of a cylinder about the large tracheal branches, and applied closely to the neighboring photogenic cells. The tracheal branches bear taenidia, but the capillary tubules, or tracheoles, which arise from the tracheal end-cells and extend among the cells of the photogenic layer, do not, although they are chitinous. Where these tracheoles enter the photogenic mass there are little depressions, which probably are located in the divisions between the cells of this structure.

The cells of the photogenic layer are found to contain, except along their peripheral boundaries, much larger granules than those represented in the previous figure. These are called photogenic granules. The cells of the reflector layer, even in the mature organ, closely resemble in general outline and appearance fat-cells. Those of the photogenic layer, on the other hand, show little similarity. The large fat-globules, which are present in the cells of both layers, during the early stages in the development of the light-organs, disappear shortly before the organs reach maturity.

The pupae continue to emit light from the larval light-organs throughout the pupal period, but the adult light-organs do not begin to function until one or two days before the end of the pupal period. It requires from sixteen to eighteen days for the completion of the pupal period.

Thus, in the development of the light-organs, groups of fat-cells become localized in the regions of the future light-organs, which for a considerable period after they become so localized

all show the same general characteristics. These cells later become differentiated into the photogenic and reflector layers, but both before and after they become so differentiated all these cells contain the dark fat-globules which are so characteristic of fat-cells after treatment with osmic acid. These observations lead me to conclude that all the cells of the light-organs are derived from fat-cells, and hence are mesodermal in origin.

PHAGOCYTOSIS OF THE LARVAL LIGHT-ORGANS

The larval light-organs of Photurus pennsylvanica begin to show evidence of breaking down soon after the pupa changes to an adult, and from this time on their light becomes fainter, until it finally disappears about forty-eight hours after the emergence of the adult. At the end of the second day of adult life, just before the luminosity disappears, a cross-section of the organ has the appearance of that shown in figure 10. At this stage the cells of the reflector layer (*R*) are still intact and their structure appears normal. Those of the photogenic layer, on the other hand, show definite evidence of breaking down. They are no longer together in a mass, but are separated into different groups. The structure of their cell walls is very indistinct and their granules are less prominent. Surrounding the cells of the photogenic layer, and to a certain extent intermingled among them, are many large leucocytes (*LL*). Whether they have a phagocytic action was not determined, yet their presence in such numbers suggests very probably that they are functioning in the destruction of the light-organ. No other blood cells are found in the neighborhood of the light-organ in sufficient numbers to make it seem possible that they are functioning in the destruction of this structure. Anglas ('00) apparently found similar cells during metamorphosis in Vespa. He did not attribute to them a phagocytic function. A section of this organ taken at the end of the third day of adult life shows very little evidence of the light-organ cells. Numerous large leucocytes are present at this time in the region of the old larval organ, but the cells of this structure are indistinct and most of their walls have broken down. This indicates that the destruc-

tion of the light-organ is very rapid as soon as luminescence ceases and that the leucocytes are probably the chief agents in destroying it.

SUMMARY

1. The first indication of the formation of the light-organs, in the embryo, is noticeable at the age of fifteen days, just as the embryo revolves from its backward-turned position and starts to coil up.

2. At this time groups of fat-cells, with their large globules which are colored dark by osmic acid, migrate ventrally in segment eight and come to lie in the region of the future light-organs. These undifferentiated light-organ cells are now continuous with the groups of fat-cells dorsal to them.

3. As soon as the fat-cells become localized in the region of the future light-organs, their dark colored globules become smaller in size and fewer in number. In fact, in the fifteen-day embryos there appears to be a gradual gradation from the cells lying next to the hypodermis, which contain smaller and fewer of these globules, to the fat-cells near the central part of the body, which contain more and larger globules.

4. In the sixteen- and seventeen-day embryos the light-organs are regular in outline, and they have become separated from the other fat-cells. The fat-globules are now smaller and fewer in number than on the fifteenth day. All cells that compose the light-organ are apparently now of the same histological structure.

5. At the age of twenty days there begins to take place a differentiation of the cells of the light-organs into the photogenic and reflector areas.

6. At the age of twenty-two days the light-organs become functional and appear as two minute spots of light.

7. The larvae emerge on about the twenty-sixth day of incubation.

8. These larvae require nearly two years (about twenty-two months) to reach maturity, at which time they pupate.

9. In mature larvae, about one-half day before pupation, the cells of the fat-spheres, which lie near the hypodermis in

the ventral part of the sixth and seventh abdominal segments, are liberated and become distributed along the hypodermis. These cells contain numerous fat-globules, which appear dark after treatment with osmic acid.

10. The fat-cells, which are liberated from the fat-spheres during the last day of larval life and the first one or two days following pupation, compose a layer about three cells deep above the hypodermis. Sections of the light-organs at this stage show some of these cells in mitosis.

11. The undifferentiated cells of the light-organs, at this stage, are all of the same general histological appearance, which suggests a common origin.

12. The cells of the photogenic and reflector layers, in the five-day pupae, are clearly differentiated. At this time the cells of both layers still contain some of the dark colored fat-globules.

13. Tracheal epithelium, by the rapid division of its cells, now extends from the region of the body cavity down between the cells of the light-organs at regular intervals. It later gives rise to the trachea of the light-organs, together with their tracheal end-cells and tracheoles.

14. Shortly before the light-organs become mature, in both the embryo and the pupa, the fat-globules disappear and the organ takes on its characteristic adult structure.

15. The light-organs of both the larva and the adult are formed from fat-cells which become differentiated into the photogenic and reflector layers of the mature light-organs. Hence the light-organs are entirely mesodermal in origin.

16. In the breaking down of the larval light-organs, which occurs about forty-eight hours after the emergence of the adults, the cells of the photogenic layer become separated into small groups, soon after which their cell walls and cytoplasmic contents become indistinct. Soon after the cells of the photogenic layer break down the cells of the reflector layer meet the same fate. Numerous large leucocytes are found surrounding the cells of the breaking down light-organs at this period. It seems probable that they are the chief agents in the destruction of these organs.

BIBLIOGRAPHY

Anglas, J. 1900 Note préliminaire sur les métamorphoses internes de la guêpe et de la l'abeille la lyocytose. C. R. Soc. Biol., LII, pp. 94–97.

Berlese, A. 1909 Gli insetti; loro organizzazione, sviluppo, abitudini e rapporti coll'uomo. Milano.

Bongardt, J. 1903 Beiträge zur Kenntnis der Leuchtorgane einheimischer Lampyriden. Zeit. f. wiss. Zool., Bd. 75, S. 1–45, pls. 1–3.

Buchner, P. 1914 Sind die Leuchtorgane Pilzorgane? Zool. Anz., Bd. 45, S. 17–21.

Dahlgren, U., and Kepner, W. A. 1908 A text-book of the principles of animal histology. New York.

Dahlgren, U. 1916 Investigations of the light organs of arthropods. Anat. Rec., vol. 11, pp. 481–483.

1917 The production of light by animals—The fireflies or Lampyridae. Jour. of Franklin Inst., vol. 183, pp. 323–348.

1917 The production of light by animals—Histogenesis and physiology of the light-tissues in Lampyrids. Jour. of Franklin Inst., vol. 183, pp. 593–624.

Dubois, R. 1886 Contribution a l'étude de la production de la lumière par les êtres vivants. Les Elétérides lumineux. Bull. Soc. Zool. France, T. IX, pp. 1–275, pls. 1–9.

1898 Leçons de physiologie générale et comparée. Paris, pp. 301–331.

1900 Sur le méchanisme de la Biophotogénèse. C. R. Soc. Biol. Paris, T. 52, pp. 569–570.

1911 Sur la photobiogenese ou la production de la lumière par les êtres vivants. C. R. Assoc. Franc. Av. Sci. XXXIX, T. 2, pp. 194–195.

1913 Sur la nature et la développement de l'organe lumineux du *Lampyre noctiluque*. C. R. Acad. Sci., Paris CLVI, pp. 730–732.

Emery, C. 1884 Untersuchengen über Luciola italica L. Zeit. f. wiss. Zool., Bd. 40, S. 338–355., pl. 19.

Gegenbaur, C. 1874 Grundzüge der vergleichenden Anatomie. Leipsig.

Geipel, E. 1915 Beiträge zur Anatomie der Leuchtorgane tropischer Käfer. Zeit. f. wiss. Zool., Bd. 112, S. 239–290, pls. 7, 8.

Harvey, E. N. 1914 On the chemical nature of the luminous material of the firefly. Science, vol. 40, pp. 33–34.

Heinemann, C. 1886 Zur Anatomie und Physiologie der Leuchtorgane mexikanische Cucujo's. Arch. mikr. Anat., Bd. 27, S. 296–382.

Henneguy, L. F. 1904 Les Insects. Paris.

Hess, W. N. 1917 Origin and development of the photogenic organs of Photurus pennsylvanica De Geer. Ent. News, vol. 28, pp. 304–310.

1918 Orgin and development of the photogenic organs of Photurus pennsylvanica. Science, N. S., vol. 47, pp. 143–144.

Kölliker, A. von 1857 Ueber die Leuchtorgane von Lampyris. Verh. d. phys. med. gesell. Würzburg, Bd. 8, S. 217–224.

Langley, S. P., and Very, F. W. 1890 On the cheapest form of light, from studies at the Allegheny Observatory. Am. Jour. Sci., ser. 3, vol. 40, pp. 97–113, pls. 3–5.

LEYDIG, F. 1857 Lehrbuch der Histologie des Menschen und der Thiere. Leipzig, s. 342–344.

LINDEMANN, C. 1863 Anatomische Untersuchungen über die Structur des Leuchtorganes von Lampyris splendidula. Bull. Soc. Imp. Nat. Moscow, Bd. 36, S. 437.

LUND, E. J. 1911 On the structure, physiology and use of photogenic organs, with special reference to the Lampyridae. Jour. Exp. Zoöl., vol. 11, pp. 415–467, pl. 3.

MARCHAL, P. 1911 Physiologie des Insectes, in Richtet. Dictionnaire de Physiologie. Paris.

OWSJANNIKOW, P. 1868 Ein Beitrag zur Kenntniss der Leuchtorgane von Lampyris noctiluca. Mem. Acad. Sci. St. Petersb., Bd. 11, No. 17, S. 12, Pl. 1.

PETERS, W. 1841 Ueber das Leuchten der Lampyris italica. Arch. f. Anat., Jahrg. S. 229–233.

PIERANTONI, H. 1914 La luce degli insetti luminosi e la simbiosa ereditaria. Rendic Accad. Sci. Napoli, 20, pp. 15–21.

SCHRÖDER, CHR. 1913 Handbuch der Entomologie Bd. 1. Jena.

SCHULTZE, MAX 1865 Zur Kenntniss der Leuchtorgane von Lampyris splendidula. Arch. mikr. Anat., Bd. 1, S. 124–137, pls. 2.

SEAMAN, W. H. 1891 On the luminous organs of insects. Proc. Amer. Soc. Microscopists, vol. 13, pp. 133–162, pls, 1–5.

TARGIONI-TOZZETTI, A. 1866 Come sia fatto L'organo che fa lume nella lucciole volante. Mem. della. Soc. Ital. di Sci. Natural. Milano.

1870 Sull' organe che fà lume nelle lucciole volanti d'Italia (Luciola italica). Bull. Soc. Ent. Ital. Anno. 2, pp. 177–189, pls. 1, 2.

TOWNSEND, A. B. 1904 The histology of the light organs of Photinus marginellus. Amer. Nat., vol. 38, pp. 127–151.

VOGEL, R. 1913 Zur Topographie und Entwicklungsgeschichte der Leuchtorgane von Lampyris noctiluca. Zool. Anz., Bd. 41, S. 325–332.

WHEELER, W. M. 1892 Concerning the 'blood tissue' of the Insecta. Psyche, vol. 6, pp. 253–258, pl. 7.

AND WILLIAMS, F. X. 1915 The luminous organ of the New Zealand glow-worm. Psyche, vol. 22, pp. 36–43, pl. 3.

WIELOWIEJSKI, H. R. VON 1882 Studien über die Lampyriden. Zeit. f. wiss. Zool., Bd. 37, S. 354–428, pls. 23, 24.

1889 Beiträge zur Kenntniss der Leuchtorgane der Insecten. Zool. Anz., Bd. 12, S. 594–600.

1890 Contributions a l'histoire des Organes Lumineux chez les Insectes. Bull. Sci. Fr. Belg., T. 28, pp. 145–207.

WILLIAMS, F. X. 1916 The photogenic organs and embryology of lampyrids. Jour. Morph., vol. 28, pp. 145–207, pls. 1–10.

PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Larva, ventral view of abdomen. *LO*, larval light-organ, located on the eighth abdominal segment.

2 Adult male, ventral view of abdomen. *AO*, adult light-organ, located on the sixth and seventh abdominal segments.

3 Adult female taken immediately after emergence, ventral view of abdomen. *AO*, adult light-organ; *LO*, larval light-organ.

4 Larva, cross-section to show position of the light-organs. *R*, reflector layer; *P*, photogenic layer.

5 Adult male, cross-section to show position of the light-organs. *R*, reflector layer; *P*, photogenic layer.

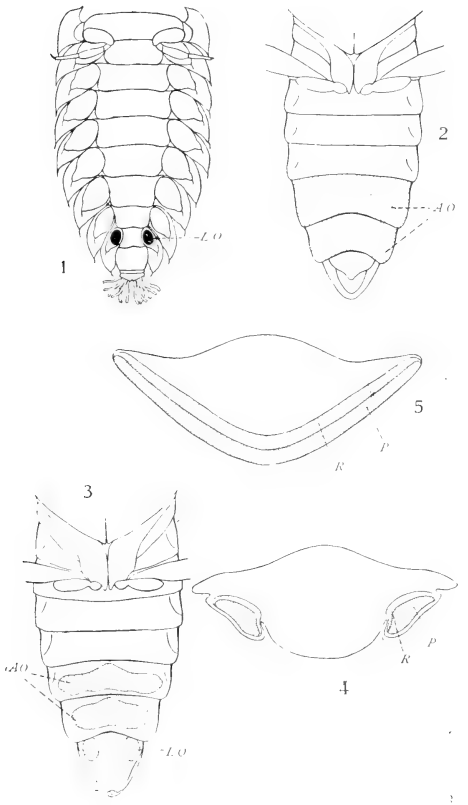

1
LO
2
AO
5
P
R
3
AO
LO
4
P
R

PLATE 2

EXPLANATION OF FIGURES

6 Sagittal section of the light-organ of a fifteen-day embryo, which shows the light-organ in a very early stage of development. *F*, fat-cells; *FG*, fat-globules; *H*, hypodermis; *T*, trachea; *U*, undifferentiated cells of the light-organ.

7 Sagittal section of the light-organ of a seventeen-day embryo. *C*, cuticula, *F*, fat-cell; *H*, hypodermis; *M*, muscle; *T*, trachea; *U*, undifferentiated cells of the light-organ.

8 Sagittal section of the light-organ of a twenty-day embryo, which shows a beginning of the differentiation of the cells into the two layers of the mature light-organ. *C*, cuticula; *F*, fat-cell; *H*, hypodermis; *P*, photogenic layer; *R*, reflector layer.

Note. All the material that was used for the histological preparations that are illustrated on this plate and the three following plates was fixed in Fleming's fluid and stained in Heidenhain's iron hematoxylin.

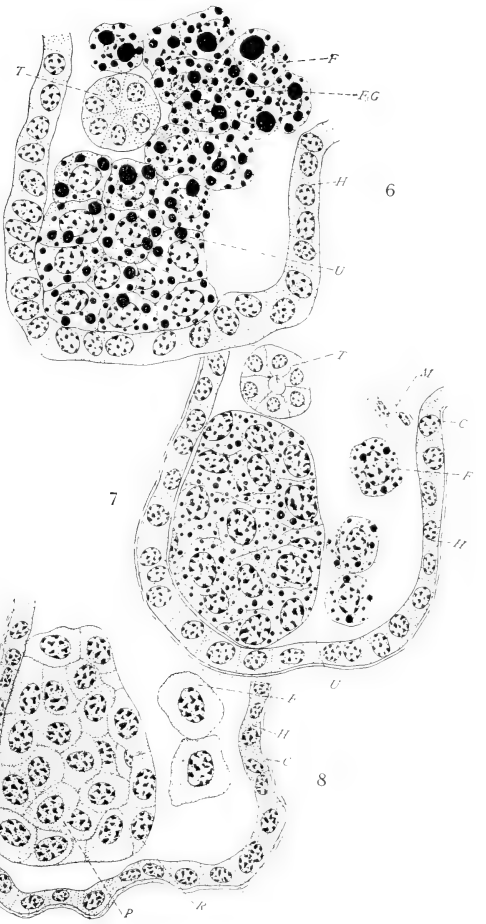

T
F
F.G
H
U
6
T
M
C
F
7
H
U
F
H
C
8
P
R

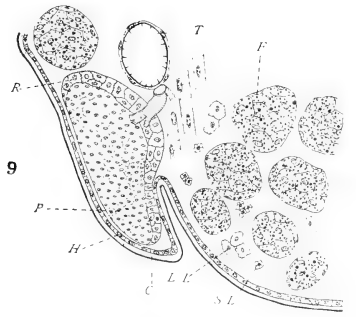
PLATE 3

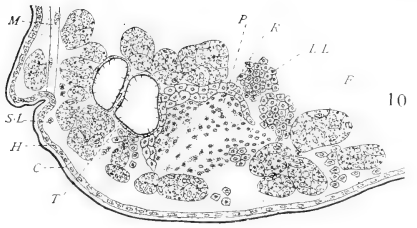
EXPLANATION OF FIGURES

9 Cross-section of the light-organ of a one-year-old larva. *C*, cuticula; *F*, fat-sphere; *H*, hypodermis; *LL*, large leucocyte; p, photogenic layer of light-organ, R, reflector layer of light-organ; *SL*, small leucocyte; *T*, trachea.

10 Cross-section of the larval light-organ, forty-eight hours after the emergence of the adult insect. *C*, cuticula; *F*, fat-spheres; *H*, hypodermis; *LL*, large leucocyte; *M*, muscle; *P*, photogenic layer of larval light-organ; *R*, reflector layer of larval light-organ; *SL*, small leucocyte; *T*, trachea.

11 Portion of a cross-section of the seventh abdominal segment of a larva, taken about one week before pupation, to show the arrangement of the fat-spheres and hypodermis. *B*, basement membrane; *F*, fat-sphere; *FG*, fat-globules *H*, hypodermis; *LL*, large leucocyte; *M*, muscle; *O*, oenocyte; *PC*, primary cuticula; *SC*, secondary cuticula; *SL*, small leucocyte; *T*, trachea.


T
F
R
9
P
H
C
L L
S L


M
P
R
L L
F
10
S L
H
C
T

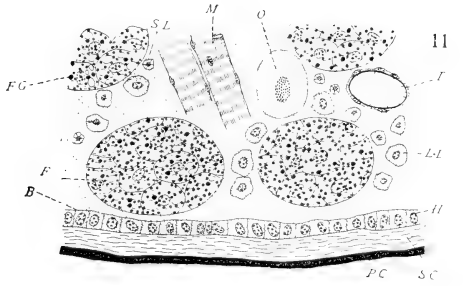

M
O
S L
11
F G
I
L L
F
B
H
P C
S C

PLATE 4

EXPLANATION OF FIGURES

12 Portion of a cross-section of the seventh abdominal segment of a larva, taken one-half day before pupation. *B*, basement membrane; *F*, fat-sphere; *H*, hypodermis; *LL*, large leucocyte; *PC*, primary cuticula; *SC*, secondary cuticula; *SL*, small leucocyte; *T*, trachea; *U*, undifferentiated cells of the light-organ.

13 Portion of a cross-section of the seventh abdominal segment of a pupa, taken one-half day after pupation, to show a little later stage than figure 12 in the formation of the light-organ. *C*, cuticula; *F*, fat-sphere; *H*, hypodermis; *LL*, large leucocyte; *M*, muscle; *SL*, small leucocyte; *T*, trachea; *U*, undifferentiated cells of the light-organ.

14 Portion of a cross-section of the seventh abdominal segment of a pupa, taken one day after pupation. It represents a slightly later stage than figure 13. For labels see figure 13.

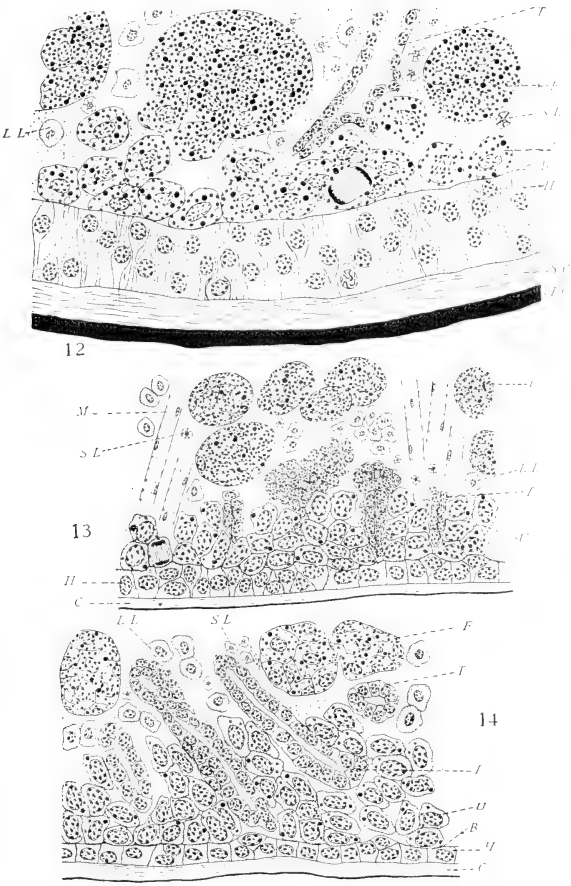

L L
12
M
S L
13
H
C
L L
S L
F
I
14
I
U
B
U
C

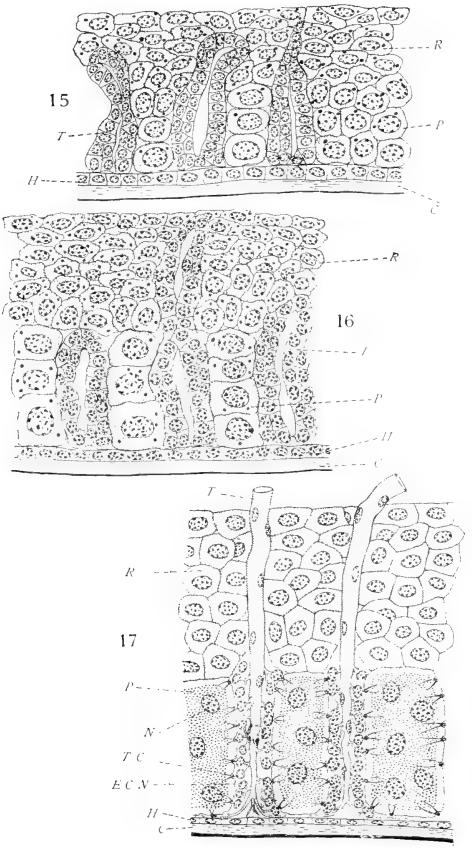
PLATE 5

EXPLANATION OF FIGURES

15 Cross-section of the fourth stage in the development of the adult light-organ, taken four days after pupation. It illustrates an early stage in the differentiation of the light-organ into two layers. *C*, cuticula; *H*, hypodermis; *P*, photogenic layer of light-organ; *R*, reflector layer of light-organ; *T*, trachea.

16 Cross-section of the fifth stage in the development of the adult light-organ, taken five days after pupation. This represents a stage in which the cells of the two layers of the light-organ are definitely differentiated. The dark fat-globules, so characteristic of fat-cells after osmic-acid fixation, are distinctly visible in all the cells of the light-organ at this stage, as well as in the four previous stages. For labels see figure 15.

17 Cross-section of the adult light-organ fully developed. *C*, cuticula; *ECN*, nucleus of tracheal end-cell; *H*, hypodermis; *N*, nucleus of photogenic cell; *P*, photogenic layer; *R*, reflector layer; *T*, trachea; *TC*, tracheole.


15
R
T
P
H
C
16
R
I
P
H
C
17
T
R
P
N
T C
E C N
H
C

Resumen por el autor, Sante Naccarati.

Contribución al estudio morfológico de la glándula tiroides de Emys europea.

En Emys europea la tiroides es un órgano medial impar, de forma esferoidea y color rojizo, situado encima del corazón en la cavidad del arco formado por el tronco innominado. El volumen y peso de este órgano presentan considerable variaciones, que dependen en su mayor parte de la edad y tamaño del animal; el peso medio es 0.025 gramos y su longitud media 5 mm. La irrigación sanguínea de la glándula se lleva a cabo mediante dos arterias tiroideas superiores y otras dos inferiores y el mismo número de venas. Variaciones y anomalías en el número y distribución de los vasos tiroideos son bastante frecuentes. La inervación tiene lugar mediante el vago y el simpático.

La tiroides de Emys no difiere esencialmente en estructura histológica de la de los demás vertebrados, incluso el hombre. En la cápsula de tejido conectivo fibroso existen cromatóforos esparcidos Las células del epitelio son generalmente cuboideas, menos frecuentemente cilíndricas o aplanadas y están en contacto directo con el coloide. El núcleo ocupa siempre la parte basal de la célula; es distintamente vesícular, bastante grande, provisto de gránulos cromáticos y sin nucleolo. Entre los alveolos adyacentes existen escasas fibras elásticas delicadas derivadas de las ramificaciones de la red elástica más grosera que cubre la superficie de la glándula. Los gránulos de secreción son más grandes y menos numerosos que los gránulos de grasa y las mitocondrias. Son claramente fuchsinófilos con el método de Galeotti. El coloide intravesicular no difiere del que se halla en la tiroides humana.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

CONTRIBUTION TO THE MORPHOLOGIC STUDY OF THE THYREOID GLAND IN EMYS EUROPAEA

SANTE NACCARATI

Department of Histology, Royal University, Rome

FIVE COLORED FIGURES

NOTES ON THE EMBRYOLOGY OF THE THYREOID

The thyreoid, together with the thymus, the postbranchial or suprapericardial bodies, the carotid gland, the thymous lobules, and the parathyreoids (also called epithelial corpuscles), belongs to the group of organs called branchial derivatives.

The first embryogenetic researches on the thyreoid go back to Huschke ('26), Rathke, Remak ('55), and Götte ('67) and, with many others which followed not long after, were conducted almost exclusively on mammals. They served to establish that the thyreoid is derived from a thickening and hollowing of the ventral wall of the pharynx at the level of the second pair of branchial arches.

His ('68) established that the thyreoid is derived from two equal lateral rudiments in the pharyngeal wall.

Müller ('71), Sessel ('77), and Kölliker ('79) found that in mammals the thyreoid is derived from a single medial rudiment in the form of a hollow diverticulum (Müller and Seessel) or of a solid bud (Kölliker) from the ventral wall of the pharynx, with which it remains in temporary connection.

Born ('83) and Fischelis ('85) found that in the pig embryo the thyreoid comes from three rudiments, originally independent, a middle thyreoid rudiment arising from the ventral wall of the pharynx at the level of the second pair of branchial arches, and two lateral thyreoid rudiments, supposed to be formed from the epithelium of the fourth entodermic branchial pouches.

Other researches were conducted by Wölfer ('80), Stieda ('81), Dohrn ('87), Götte ('75), DeMeuron ('86), Maurer ('85), Balfour ('78), Kastschenko ('87), Piersol ('88), Prénant ('94–'99), Van Bemmelen ('85–'93), Platt ('96), Simon, Soulié ('97), Verdun, Jacoby ('94, '96, '97), Verson ('07), and others more recently, too many to be quoted here, with resulting confirmation of the origin of the thyreoid from three distinct and originally independent rudiments, i.e., one middle and two lateral thyreoid rudiments, in mammals.

According to Maurer ('99) Echidna, and according to Symington ('97, '98) the Edentata and Marsupialia are exceptions to this rule, in that they retain the independence, of the three rudiments, having, in addition to a two-lobed thyreoid, two organs developed from the lateral rudiments, homodynamic with the postbranchial or suprapericardial bodies, by which name they are called.

Livini ('02) made a careful study of the embryology of the organs of the thymus-thyreoid system in Amphibia urodela, and found the thyreoid arises as a single medial solid epithelial bud from the caudal wall of an entodermic spur of the pharyngeal floor which enters in intimate contact with the ectoderm. He considers this spur as a rudiment of the hypobranchial groove of the Tunicata. However, the level at which the thyreoid arises cannot be established because when its bud is already recognizable, neither the branchial pouches nor the cartilages of the branchial arches have yet been differentiated.

NOTES ON THE COMPARATIVE ANATOMY OF THE THYREOID

Cyclostomata. In the lamprey the thyreoid gland is but little developed in the adult. In the larvae (Ammocoetes) it remains in open communication with the buccal cavity, at a level between the third and the fourth branchial slit, in such a manner that it may be considered as a diverticulum of the ventral wall of the pharynx. With the coming of the metamorphosis it becomes a glandular organ of vesicular structure and is isolated from the pharynx. The arrangement in the larvae recalls a homologous relation between the thyreoid in the

Cyclostomata and the hypobranchial groove of Amphioxus, on the one hand, and between the latter and the ventral furrow of the branchial basket in the Tunicata on the other. This homology is based on the following considerations:

I. The thyreoid of Petromyzon in the Ammocoetes stage is a sort of muciparous gland, which, if it in some respects differs from the endostyle of the Tunicata, nevertheless presents such points of resemblance to it that the two formations may be regarded as homogeneous (Dohrn, '80).

II. During the development this gland is transformed into an organ corresponding to the thyreoid of other vertebrates.

In the Elasmobranchii the thyreoid is a single medial organ sometimes spherical, sometimes cylindrical, sometimes triangular or pear-shaped; its volume and weight differ in different animals; sometimes (as in Scyllium catulus) it is cranially located at the angle of bifurcation of the branchial artery; sometimes it is located in the vicinity of the tongue, between the coracohyoideal and coracomandibular muscles (as in Acanthias vulgaris and in Mustelus laevis); sometimes it is immediately under the skin (as in Squatina angelus).

Within the thyreoid of the Elasmobranchii there exist, according to Thompson ('10), masses of small solid cells, partly epithelial, partly adenoid, which have been thought to indicate a homology with the parathyreoids and the thymus.

In the Teleostei, according to Maurer ('85), the thyreoid is a single organ until, at a certain age, it divides into an accumulation of follicles which surround the branches of the branchial artery on every side. In Amiurus, according to Thomson, the thyreoid consists of a number of vesicles scattered here and there, enclosed in the matrix of the connective tissue; the cells which line the vesicles are cylindrical, very low, and in some cases almost flat.

In the Urodela the thyreoid is a double organ, yellowish in color, spherical or egg-shaped; its largest diameter is less than 1 mm.; it is slightly flattened, and very superficially located, between the mylohyoid and sternothyreoid muscles, in the immediate vicinity of the jugular vein.

In the Anura, also, the thyreoid is a double organ (single in the embryo), lateral, egg-shaped, pinkish in color; each half is 4 mm. in diameter; both are located in the ventral side of the animal, at the posterior horns of the hyoid bone, in front of the jugular veins, to which they are closely adherent.

In the Ophidia the thyreoid is a single discoidal organ located in the median line at the base of the heart, between the two carotids in the young animal it is at the lower extremity of the thymus. Its diameter is about 3 mm. and its weight about 2 mgm.; by its grayish-white color it is easily distinguished from the two thymous lobes, noticeable for their brighter color, whose medial margins cover it.

In the Lacertilia also (aside from a few genera like Monitor, in which it consists of two lobes located at the side of the neck in front of the carotids) the thyreoid is a single medial organ just under the skin on the ventral side of the trachea, along which it lies with its greatest diameter transverse to the diameter of the trachea. It is dark gray; fusiform, largest in the middle, the two extremities narrowing until, as they reach the lateral extremities of the neck, they terminate in a fine filament, often bifurcated. In the Lacerta viridis it reaches a length of 1 cm.

My research on the thyreoid in the Squamata, on the species Zamenis viridiflavus, Tropidonotus natrix, Lacerta viridis, and Lacerta agilis, confirming this statement, will form the subject of another paper.

In the Aves the thyreoid consists of two rounded or oval lobes, pink in color, varying in size with the animal, and located at the sides of trachea near the syrinx, attached to the ventral side of the carotid, generally at the level of the vertebral artery. In the pigeon, for example, the thyreoids occur in the anterior wall of the thorax, near the junction of the thorax and neck. They are ovular in form, with their longest diameter lengthwise of the body; their lowest point is slightly above the point where the main branches of the carotid artery divide. In front of them are the jugular veins on the outside and the oesophageal arteries on the inside.

Mammalia. Aside from the human thyreoid, the detailed description of which may be found in any treatise on anatomy, it may be remarked that, in general, this organ in mammals consists of two lateral lobes at the sides of the trachea, between the first and the ninth tracheal rings—the exact position depending on the animal; the weight and volume also vary with the animal.

In general the two lobes are joined to each other by an isthmus, usually thin, as in the dog, cat, rabbit, rat, and guinea-pig; in old animals it may atrophy or disappear. Thus in the donkey the isthmus is easily seen in the young animal, while in the old it is reduced to a slight atrophic filament without glandular structure. In the horse and sheep, on the other hand, the isthmus is so thin that often it cannot be distinguished; this has led some to believe that in these animals it occurs only exceptionally. Aberrant nodes of thyreoid tissue have been described in non-human mammals; in these, however, it has not been possible to recognize the pyramid of Lalouette or the appendix of Morgagni.

PERSONAL INVESTIGATIONS

My researches on the thyreoid of Chelonia were carried out on the two Italian species, Emys europaea and Testudo graeca. There is very little difference in the macroscopic aspect and no difference at all in the microscopic appearance of the thyreoid in these two species. I will give the description of the thyreoid of Emys europaea and refer to Testudo graeca for the main differential points.

Macroscopic anatomy. In Emys europaea the thyreoid is a single medial organ of spheroid form and pinkish color, located in the cavity of the arch formed by the truncus innominatus. From the anatomic-topographic point of view, in order to reach the thyreoid by trepanning it is necessary to apply the point of the instrument half a centimeter above the point of union of the hyoplastral with the hypoplastral plates. Removing the bone, and taking pains to hold the animal's forepaws well apart so as to withdraw from the operative field the two scapuloclavicular ligaments (with the animal's neck extended), one finds a small

rounded body, easily recognized by its pink color, across the adipose tissue and suprapericardial connective tissue, larger or smaller than a pea according to the size of the animal.

In the classic treatise of Bojanus (1819–21) in which are reported with clearness and precision all the characteristics of the macroscopic anatomy of Emys europea, the thyreoid is taken for the thymus. The latter, when it exists, is a long double organ of a light gray color, located in front of the carotids, with which it is in close contact, at the point of conjunction of the neck with the thorax. It is strange that so able an anatomist should have fallen into such an error. The pink color of the thyreoid is due to the blood which it contains, the amount of which is very considerable (according to Tschuovsky, 560 cc. of blood pass each minute through 100 grams of human thyreoid tissue). When the excess of blood in the thyreoid of the tortoise is eliminated, it acquires the appearance of an opalescent lens.

Volume and weight. The volume and weight of the thyreoids of Emys europaea are very variable. The most noteworthy variations are due to the size and age of the animal. With the purpose of establishing as exactly as possible the average weight, I have weighed the thyreoid of thirty Emys and found that in adults weighing about 275 gm. the thyreoid has an average weight of 0.025 gm. In general, 100 gm. of body weight corresponds to about 10 mgm. of thyreoid. For man this proportion is about five times as big. If the weight of the Emys is taken without its carapace and plastron, which averages about 40 per cent of the total, according to my measurements of thirty animals, the proportion is 16 mgm. of thyreoid to 100 gm. of the animal's weight. There are great individual variations from the average. In another paper I have prepared, in tabulated form, the weights of the thyreoid and other glands in groups of several species of reptiles, including Emys europaea.

As regards the volume of the thyreoid in Emys europaea, what I have said regarding the weight holds good, namely, that it varies within very wide limits, according to the size of the animal. In general it may be stated that in an animal of 300 gm. weight, the maximum diameter of the gland is about 5 mm.

Topographical relations. As I have said above, the thyreoid in Emys europaea lies within the large upward-curving arch formed by the truncus innominatus, just above the heart. As this arch leans slightly toward the right, the gland is not absolutely in the middle, but is a little to the right. In front (on the animal's ventral side), the thyreoid is separated from the thoracic wall by a lamellar connective, transparent and fairly tough, consisting of several layers, continuous below with the pericardium and surrounded above by the large vessels of the neck. The vascular arch along which the thyreoid lies is closely connected with it, both by means of the vessels and by means of the connective tissue. The rear wall of the gland (toward the animal's dorsal side) is in front of the trachea, with which it is not in contact. It must be noted that in Emys europaea the trachea divides into the two bronchi a little above the thyreoid, while in Testudo graeca the division occurs much higher, near the base of the tongue.

Circulation and innervation. As in a man, the thyreoid in these Chelonia is highly vascularized. The blood flows to it through the two superior and the two inferior thyreoid arteries. The inferior pair are short, but very capacious; they issue from the truncus innominatus, and penetrate the gland at right angles, passing through its outer inferior margin. Regarding the behavior of the large vessels as they leave the heart, it should be remembered that, whereas the left aorta reaches the left bronchus without branching, the right aorta on the contrary, before curving, sends off a large but very short trunk (truncus innominatus) which forms a superior concavity and then divides into the right and left carotid and subclavian arteries, after sending off the inferior thyreoid arteries and the oesophageal arteries.

The superior thyreoid arteries are longer but thinner; they branch from the carotids and turn downward and inward, issuing in the outer superior margin of the thyreoid gland. These arteries (unlike the inferior thyreoid arteries which are always present) are sometimes missing.

It must be noted that the division of the truncus innominatus into subclavian and carotid sometimes occurs a little higher on

the right than on the left; when the animal's neck is extended, the point of bifurcation of the two carotid arteries and the right subclavian is in a line with the right forepaw.

In connection with the thyreoid arteries it must be noted that there are many variations, especially of the superior pair, which often, instead of penetrating the gland directly, join the inferior pair, thus entering the gland as a single trunk. When this occurs, the superior thyreoid artery turns downward immediately after leaving the carotid and follows a course of about 1 cm., while the inferior artery, turning slightly upward, follows a very short course. The trunk which results from their union is so short and thick that it resembles an arterial sinus.

At other times the superior thyreoid artery is missing, and is replaced by three or four small arteries forming a network around the upper tip of the gland. At still other times there may be a median artery which arises from one of the two carotids near the hyoid bone and turns downward along the median line of the neck, reaching the upper tip of the gland.

The ramifications of these arteries, finely divided, form a plexus around the fibrous capsule which surrounds the gland, and penetrate the parenchyma, where they form a very fine capillary network interwoven with the thyreoid vesicles, which they enclose, passing through the intervesicular septa. The musculature of these vessels is very distinct.

The veins which originate in the form of fine branchlets traversing the vesicles compose on the surface of the gland a thick venous network, a large plexus from which issue the principal veins (inferior thyreoid); the latter unite with the accessory pectoral veins and empty into the subclavian vein formed by the confluence of the jugular and axillary veins.

The fine perivesicular veins are without musculature and appear as little tubes with endothelium alone, traversing the interlobular connective tissue. Fine elastic fibers passing through this tissue seem to provide a kind of support for the larger vessels. The lymphatics are also very numerous; as in human thyreoid, they arise as small vacuoles between the cells lining the vesicles; these unite to form intervesicular canals, and those in

turn join to form larger trunks (the interlobular canals). These last follow the course of the arteries, veins, and nerves till they reach the external surface of the gland, where they form a dense network, from which emerge the larger branches through which the lymph is emptied into the lymphatic ganglia of the neck.

The innervation of the thyreoid is by the sympathetic. The fine non-medullated fibers accompany the arterial ramifications in the gland. The vagus also sends two fine branchlets into the gland through the laryngeal nerves, but their distribution is not constant.

HISTOLOGY

For the microscopic study of the thyroid of Emys europaea I have made use of specimens preserved in—

1. Formalin, from 5 per cent to 10 per cent aqueous solution.
2. Mercuric chloride
3. Zenker's fluid
4. Flemming's fluid
5. 96 per cent alcohol
6. Müller's fluid

The sections were stained in different ways. For the general study of the thyreoid tissue, preservation in 10 per cent formalin and staining with Ehrlich's acid-haematoxylin and the aqueous solution of eosin gave good results. Fixation in Flemming and staining with ferric haematoxylin (Heidenhain) and eosin permitted greater accuracy in studying the delicate structure of the cellular elements. Safranin and carmine have been very useful in delicate cytological study. For studying the elastic fibers fucselin and Weigert's fluid were used combined as follows:

a. Fucselin.

b. Fucselin-Van Gieson: Weigert's fluid.

c. Weigert's fluid; borax carmine, alcoholic solution of the Naples Zoological Station.

d. Weigert's fluid safranin.

e. Safranin, picric acid, Weigert's fluid.

There is no substantial difference in structure between the thyreoid of Emys europaea and Testudo graeca and that of the

other vertebrates, including man. It presents externally a fibrous connective-tissue capsule in which, here and there, are scattered pigmented cells (chromatophores). From this capsule issue numerous connective-tissue septa, which, gathering on the inside of the gland, form a network enclosing the vesicles. These vesicles, called also follicles or alveoli, are irregularly rounded, from 50 μ to 300 μ in size, and are lined with simple epithelium, the cells of which are mainly cubical, less often cylindrical or flat, and are in direct contact with the interior of the vesicular cavity, in which is contained the colloidal fluid, an amorphous, homogeneous substance presenting under the microscope transverse streaks or fissures and staining with acid stains; for example, it stains pink with haematoxylin-eosin and yellow with Van Gieson or with safranin and picric acid. The interior surface of the epithelial cells, namely, the surface looking in the lumen of the vesicles, is not clearly defined, but it has a broken appearance, recalling that of the colloidal substance, and probably, since it is not constant, due to the latter's remaining adherent to the cells. The protoplasm is homogeneous and contains fine grains. The nucleus always occupies the basal part of the cell, is well marked, vesicular, rather large, and provided with chromatin granules and does not have a nucleolus. The limits between the cells are quite clear, and in the cellular walls, which are in contact with the connective tissue limiting the alveoli, there is a basal membrane, not always, however, well differentiated. In specimens colored with safranin and picric acid there are cells having a nucleus which contains granules colored red (chromatin) noticeable against the brighter background of the rest of the nucleus, and cells whose nucleus is entirely colored red (fig. 3). These two kinds of cells correspond to the two types, principal and colloidal, described by Langendorff, who interpreted them as different aspects assumed by the same cell at different functional periods. This interpretation seems very probable because the aspect and the disposition of the cells in the different alveoli is so variable that they suggest many functional phases from the beginning elaboration to the complete secretion of the colloid.

The gland is subdivided into lobules by larger connective-tissue septa derived from the external fibrous-connective capsule, and the lobules in their turn are subdivided into alveoli by thinner septa of the same nature. The blood vessels, the lymphatics, and the nerves run into the intervesicular and interlobular septa, where they form a highly complicated network. In the small thyreoid arteries I have not found it possible to demonstrate those thickenings or buds (Schmidt's 'Zellknospen') of which Kölliker ('02) speaks. The intervesicular substance is rather scanty, and is formed of areolar connective tissue, extremely rich in blood vessels, which constitute a capillary net surrounding the alveoli and extending its finest branches into the epithelium. Between each alveolus and the next are scanty delicate elastic fibers which accompany the blood vessels and are derived from the ramifications of the coarser elastic network covering the surface of the gland. The elastic fibers are numerous and well demonstrable only in the external connective capsule. Toward the interior of the gland they grow thinner and scarcer till they disappear entirely in the walls of the most central of the alveoli. Elastic fibers are more frequent in the thyreoid of young animals.

Under the microscope the intravesicular colloid does not differ essentially from that of the human thyreoid. In the interior of the alveoli there are, at times, free epithelial cells, detached from the alveolar walls, as if some cellular desquamation had occurred (fig. 3). This condition noted in the thyreoid of individuals suffering from Basedow disease was at first given a pathological significance; later it was seen that it was a normal phenomenon, a form of holocrine desquamation of certain thyreoid cells (Pende, '18).

The granules of secretion, as in the cells of the human thyreoid, appear larger and less numerous than the granules of fat and the mitochondria. They stain distinctly red (fuchsinophile) with the method of Galeotti. This method, proposed by Galeotti for the study of the granules of secretion, is of the utmost importance for finer cytologic researches and should never be omitted. The fixative for the employment of this method is either Flemming's

or Hermann's fluid. The sections must be very thin, about 4 or 6 μ.

The technique is as follows:

1. The section is stained from five to ten minutes at the temperature of 50°C. with a freshly prepared saturated solution of fuchsin in aniline water.

2. Wash in water for about thirty seconds.

3. Transfer to a semisaturated solution of picric acid in 50 per cent alcohol for twenty or thirty seconds.

4. Prolonged wash in water until the section does not yield any more picric acid.

5. Staining for four or five minutes with a $\frac{1}{2}$ per cent solution of methyl green in 90 per cent alcohol.

6. Rapid transfer to grades of alcohol, during which the sections yield much stain.

7. Transfer to xylol and mount.

Sections thus prepared show the following characteristics: The nuclear chromatin, the centrosomes, and the granules of secretion are bright red, the protoplasm and the connective green. The strongly basophile substances, such as mucin and chondrin, take also a green, but more intense stain. The picric acid, acting as a mordant on the methyl green, renders it a plasma dye. In good sections the plasma takes an emerald green stain. If it takes a yellowish-greenish stain, the section can be utilized, provided that the fuchsinophile granules take a distinctly bright red stain. Sometimes (either because of a much prolonged action of the picric acid or for other reasons) the section does not stain at all with methyl green. It is advisable to repeat this method several times until a good section is obtained.

Figure 4, showing only a part of the epithelium of the vesicle, gives the appearance of a few cells stained with the method of Galeotti.

BIBLIOGRAPHY

Balfour, F. M. 1878 A monograph on the development of elasmobranch fishes. London.

Bojanus 1819–21 Anatome Testudinis europeae. Vilna.

Born, G. 1883 Ueber die Derivate der embryonalen Schlundbogen und Schlundspalten bei Säugethieren. Arch. mikr. Anat., Bd. 22.

Dohrn, A. 1886 Studien, usw. VIII. Die Thyroidea bei Petromyzon, Amphioxus und den Tunicaten. Mitth. Zool. Sta. Neapel, Bd. 6.

1886-7 Studien, usw. XI. Thyreoidea und Hypobranchialrinne. Mitth. Z. S. Neapel, Bd. 7.

Fischelis, Ph. 1885 Beiträge zur Kenntnis der Entwickelungsgeschichte der Glandula thyreoidea und Gl. Thymus. Arch. mik. Anat., Bd. 25.

Goette, A. 1867 Beiträge zur Entwickelungsgeschichte des Darmkanals des Hünchens. Tübingen.

1875 Die Entwickelungsgeschichte der Unke. Leipzig.

His, W. 1868 Untersuchungen über die erste Anlage des Wirbelthierleibes. Leipzig.

1880–85 Anatomie menschlicher Embryonen. Leipzig.

1889 Schlundspalten und Thymusanlage. Arch. Anat. u. Physiol., Anat. Abth.

1891 Der Tractus thyreoglossus und seine Beziehung zur Zungenbein. Arch. Anat. u. phys., Anat. Abth.

Huschke, E. 1826 Ueber die Umbildung des Darmcanals und der Kiemen der Froschquappen. Oken's Isis, Bd. 1.

Jacoby, M. 1894 Ueber die mediane Schilddrüsenanlage bei Säugern (Schwein). Anat. Anz. Bd., 10.

1896 Ueber die Entwicklung der Nebendrüsen, der Schilddrüsen und der Carotiddrüsen. Anat. Anz., Bd. 12.

1897 Zur Entwickelung der Nebendrüsen. Anat. Anz., Bd. 13.

Kastschenko, N. 1887 Das Schicksal der embryonal Schlundspalten bei Säugethieren Arch. f. mikr. Anat.,, Bd. 30.

1887 Das Schlundspaltengebiet des Hünchens. Arch. Anat. u. Entw., Anat. Abth.

Kölliker, A. 1879 Entwickelungsgeschichte des Menschen und der höheren Thiere. Leipzig.

1902 Handbuch der Gewebelehre des Menschen. Leipzig.

Livini, F. 1902 Organi del sistema timotiroideo nella Salamandrina perspicillata. Arch. Ital. Anat. e Embriol., T. 1

Maurer, F. 1885 Schilddrüse und Thymus der. Teleostier. Morph. Jahrb., Bd. 11.

1888 Schilddrüse, Thymus und Kiemenreste der Amphibien. Morph. Jahrb., Bd. 13.

1898 Der Derivate der Schlundspalten bei der Eidechsen. Verh. Anat. Gesellsch., Bd. 12.

1899 Schlundspalten Derivate von Echidna. Verh. Anat. Gesellsch., Bd. 13.

1899 Die Schilddrüse, Thymus und anderen Schlundspalten Derivate bei der Eidechse. Morph. Jahrb., Bd. 27.

DE MEURON, P. 1886 Développement du Thymus et de la glande thyreoïde. Arch. d. Sci. Phys. et Nat., Geneve, T. 14.

MÜLLER, W. 1871 Ueber die Entwickelung der Schilddrüse. Jena. Zeitsch., Bd. 6.

PENDE, N. 1918 Endocrinologia. Milano.

PIERSOL, G. M. 1888 Ueber die Entwickelung der embryonal Schlundspalten und ihrer Derivate bei Säugethieren. Stzber. phys. med. Gesellsch., Würzburg.

PLATT, J. 1896 The development of the thyroid gland and of the suprapericardial bodies in Necturus. Anat. Anz., Bd. 11.

PRÉNANT, A. 1894 Contribution a l'étude du développement organique et histologique du thymus, de la glande thyreoïde, et de la glande carotidienne. La Cellule, T. 10.

1896 Élements d'embryologie de l'homme et des vertebrés. Paris.

1896 Sur le développement des glandes accessoires de la glande thyroïde et celui de la glande carotidienne. Anat. Anz., Bd. 12.

1898 Sur les dérivés branchiaux des Reptiles. Bibliog. Anatom., T. 6, fasc. 5.

1899 Rectification au sujet de la communication de M. Maurer: "De Schlundspalten Derivate von Echidna. Anat. Anz., Bd. 16.

Les dérivés branchiaux chez l'orvet. Arch. Anat. norm. et. path., Ve. Ser., T. 8.

RATHKE, H. 1828 Ueber die Entwicklung der Athmungswerkzeuge bei den Vögeln und Säugethieren. Nova Acta, 14 (quoted by Wölfler).

REMAK, R. 1850–55 Untersuchungen über die Entwicklung der Wirbelthieren. Berlin.

SEESEL, A. 1877 Zur Entwickelungsgeschichte der Vorderdarms. Arch. Anat. u. Physiol.

SOULIÉ ET VERDUN 1897 Sur les premières stages du développement de la thyroïde mediane. C. r. Soc. Biol. de Paris.

STIEDA, L. 1881 Untersuchungen über die Entwicklung der Glandula thymus, Thyreoidea und carotica. Leipzig.

SYMINGTON, J. 1897 Ueber Thyreoidea, Glandulae parathyreoidea und Thymus beim dreizehingen Faultier. Arch. Anat. u. Physiol., Anat. Abt.

SYMINGTON, J. 1898 The thymus gland in the Marsupialia. Jour. Anat. and Physiol., vol. 32.

THOMPSON, F. D. 1910 The thyroid and parathyroid glands throughout vertebrates. Phil. Trans. R. Socy., 201 B.

VERDUN, P. 1896 Sur les glandes satellites de la thyroïde du chat les kystes qui en dérivent. C. r. Soc. Biol. Paris, T. 48.

1897 Sur les dérivés de la quatrième poche branchiale chez le chat. ibid., T. 49.

1898 Sur les dérivés branchiaux du poulet. Ibid., T. 50.

1898 Glandules branchiaux et corps post-branchiaux chez les Reptiles. Ibid., T. 50.

1898 Dérivés branchiaux chez les vertebrés superieurs. Toulouse.

Évolution de la quatrième poche branchiale et de la thyroïde laterale chez le chat. Jour. Anat. et Phys., T. 34.

VAN BEMMELN, J. F. 1885 Ueber vermuthliche rudimentäre Kiemenspalten bei Elasmobranchiern. Mitth. Zool. Sta. Neapel., Bd. 6.
1893 Ueber die Entwickelung der Kiementaschen und der Aortabogen bei den Seeschildkröten untersucht an Embryonen von Chelonia viridis. Anat. Anz., Bd. 11.

VERSON, S. 1907 Contributo allo studio della ghiandolo tiroide ed annessi. Arch. Sci. Mediche, Torino, T. 31.

WÖLFLER, A. 1880 Ueber die Entwicklung und Bau der Schilddrüse. Berlin.

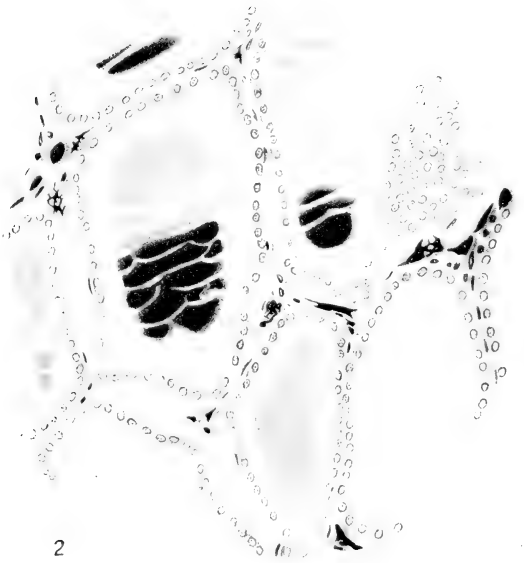
PLATE 1

EXPLANATION OF FIGURES

1 Thyreoid of Emys europaea. Fixation: alcohol 96 per cent. Staining: alcoholic carmine solution of the Zoological Station of Naples, Weigert's fluid picric acid. 8* objective, 4 ocular. The colloid is stained yellow, the cellular nuclei red, the elastic fibers blue. A portion of the external capsule has been left in place. Note how numerous are the elastic fibers in the capsule and how extremely scarce are they in the intervesicular connective.

2 Same. Fixation: Flemming's fluid. Staining: Heidenhain's haematoxylin. 7* objective, 4 ocular. In some places the intervesicular connective has given way and the alveoli appear detached.

1



2

PLATE 2

EXPLANATION OF FIGURES

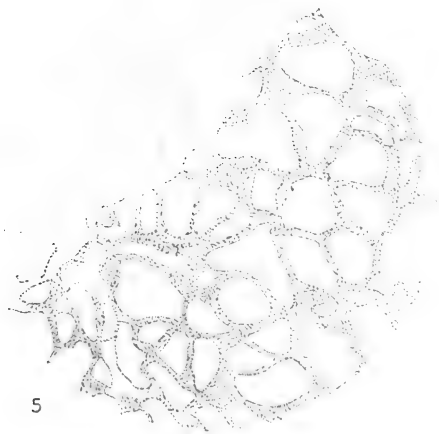
3 Same. Fixation: Flemming's fluid. Staining: Safranin-picric acid. 1/15 imm., ocular 4. The two different aspects of the thyreoid cell, viz., the principal and the colloidal cells, are distinctly shown. Some cells detached from the epithelium are shown in the colloid.

4 Same. Fixation: Flemming's fluid. Staining: Galeotti's method. 1/15 imm., ocular 4. Only a part of the section has been drawn, in order to demonstrate the granules of secretion within the cell bodies.

5 Same. Fixation: Formalin. Staining: Haematoxylin and eosin. Objective, Zeiss BB, ocular 4. An island of thymus substance was found in the thyreoid of this animal and is shown in this section.

3

4



5

Resumen por el autor, Charles E. Johnson.

Derivados branguiales en las tortugas.

El tema de este trabajo es el desarrollo de los derivados branquiales de las tortugas, representadas por las formas Chelydra serpentina, Chrysemys marginata y Trionix sp. El timo persistente se origina en la porción dorsal de la tercera bolsa visceral, mientras que un brote transitorio aparece en conexión con la porción dorsal de la segunda bolsa visceral. Una paratiroides se desarrolla en la porción ventral de la tercera bolsa. La cuarta bolsa no produce ningún órgano persistente y las puebas de la existencia de estructuras transitorias son dudosas. La quinta bolsa origina una paratiroides persistente y, aunque faltan pruebas directas, es posible que produzca algunas veces un timo rudimentario.

En estados jóvenes halló el autor con gran constancia un divertículo ultimobranquial bien desarrollado, situado a cada lado del cuerpo, pero el del lado derecho está generalmente destinado a formar una estructura sumamente pequeña, cuando se compara con la del lado izquierdo, y en algunos casos dicha estructura parece faltar por completo en estados más avanzados. Las bolsas cuarta y quinta y el divertículo ultimobranquial se diferencian a expensas de lo que al principio es una sola evaginación de la pared faríngea lateral. La quinta bolsa es de naturaleza rudimentaria y durante un periodo considerable mantiene conexiones celulares con la vesícula ultimobranquial. Esta se caracteriza por la forma vesicular voluminosa que a menudo exhibe. Al llegar la época de salir el embrión del heuvo ha adquirido la estructura de un órgano linfoide.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, DECEMBER 27

BRANCHIAL DERIVATIVES IN TURTLES[1]

CHARLES EUGENE JOHNSON
Department of Zoology, University of Kansas

FIVE PLATES (TWENTY-FOUR FIGURES)

INTRODUCTION

Studies on the branchial derivatives of reptiles have been confined chiefly to lizards and snakes. The most recent work on these groups is that of St. Remy et Prenant ('03–'04). In the former group, represented by the genera Anguis and Lacerta, these authors found that a thymus body is formed by the second and third gill pouches only. The derivative of the second pouch is of variable size; whether it persists into the adult stage or not they were unable to determine. The third pouch gives rise also to a persisting epithelial body or parathyreoid. The fourth pouch gives origin only to a transitory epithelial body. The fifth pouch is of very rudimentary nature; it attains the form of a small blind pocket which soon disappears without giving rise to derivatives of any kind. A right and a left ultimobranchial evagination is present in the early stages, but the left one alone is destined to develop into a glandular organ; the right, as a rule, very soon disappears entirely, but in one instance a rudimentary ultimobranchial body was found on this side in an embryo Anguis of 6 cm. length.

In snakes, represented by the genera Coluber and Tropidonotus, a somewhat different condition was found. In this group the first and second gill pouches give rise to rudimentary, transitory thymus bodies, that of the second being the larger. The third pouch likewise produces a transitory thymus bud very similar to that of the first or the second pouch, but in addition

[1] Technical assistance for a part of the present work was made possible through the research fund of the University of Kansas.

there is formed a persisting parathyreoid body. The persisting thymus is developed from the fourth and fifth pouches. In Coluber, moreover, the fourth pouch gives origin to a persisting parathyreoid, while a very rudimentary transitory body of this kind, as a rule, is formed by the fifth pouch. In Tropidonotus, on the other hand, the fifth pouch only exceptionally gives rise to a parathyreoid, which likewise is of transitory nature. The right ultimobranchial evagination does not disappear, as in the lizards, but, like the left, undergoes progressive development into a glandular organ. The two are symmetrically situated in Coluber, but in Tropidonotus the position of the right one is somewhat variable.

For the turtle group very little work appears to have been done in connection with the branchial derivatives. A brief account by van Bemmelen ('93) has reference to Chelonia viridis. According to this account, the earlier phases of the development of the gill pouches correspond with the conditions in lizards and in snakes, but in later stages there is greater similarity with the processes in birds than with those of reptiles. Five pairs of visceral pouches are recognized, of which the first three become perforate, as does probably the fourth pair also. From the second pouch arises an epithelial bud which develops into the anterior lobe of the thymus; but the pouch itself becomes, as in snakes, an isolated vesicle which is destined to disappear, as in the case of the corresponding pouch in birds. The third pouch becomes an expanded epithelial vesicle provided with numerous secondary evaginations. It separates from the epidermal and the pharyngeal epithelium, and the secondary evaginations give rise to the thymous tissue, in the midst of which the central epithelial cyst persists as a homologue of the 'carotid body' of lizards. The fourth and fifth visceral pouches arise simultaneously with the 'suprapericardial' (ultimobranchial) evagination, from a lateral 'blinddarmförmigen Falte' at the caudal end of the pharynx (recessus praecervicalis), in the same way as in snakes. These outpouchings soon become separated from the pharynx and form a complex of three connected vesicles. If these vesicles, says van Bemmelen, in their further development

were to proceed in the same manner as in the case of the serpents, then the first two, which represent the fourth and fifth pouches, must develop into thymous tissue, while the third and hindmost remains epithelial. But this does not occur; all three maintain an epithelial character, and even in much later stages are found in this condition, situated between the aorta and the pulmonary arch.

MATERIAL AND METHODS

The present study is based upon a series of sectioned embryos varying in size from 4 mm., greatest length, to newly hatched specimens. The stains employed were borax carmine and Lyon's blue. Wax-plate reconstructions of the structures involved were made from specimens of Chrysemys of 10 mm., greatest length, and of 8 mm. carapace length (c. l.); and from embryos of Chelydra of lengths of 5 mm., 9 mm., and 9.5 mm.

I am indebted to Dr. B. M. Allen for material to supplement certain stages in my own series.

The earlier part of the work unfortunately was undertaken with very inadequate material and resulted in the erroneous conclusion that the body closely associated during its development with the ultimobranchial body was a derivative of the fifth visceral pouch instead of the fourth. In the meantime there appeared the work of Shaner[2] whose excellent models, especially of a 9.5–mm. Chrysemys picta, leave no doubt as to the origin of the body in question. While a corresponding stage is lacking in my own material, I have a somewhat older specimen of Chelydra which proves the correctness of Shaner's results.

THE VISCERAL POUCHES

The account of the earlier stages in the development of the visceral pouches is based upon embryos of Chelydra and of Chrysemys. The conditions in the two genera are essentially similar, and selection is made from one or the other accordingly

[2] R. F. Shaner, The development of the pharynx and aortic arches of the turtle, with a note on the fifth pulmonary arches of mammals. Am. Jour. Anat., Nov., 1921.

as the more favorable stages are at hand to illustrate the successive steps in the developmental processes. For the later stages a number of specimens of Trionyx also are available. As in lizards, the first gill pouch does not give rise to any parts of the organs under consideration and may therefore be omitted from further reference in this connection.

In an embryo Chrysemys of 4 mm. the first three visceral pouches are clearly differentiated. Just behind the third pouch is a fourth conspicuous evagination from the lateral pharyngeal wall. In form this diverticulum is more rounded than the preceding pouches. Its lateral wall is flattened and has rather broad contact with the ectodermal epithelium; and on its dorsal, ventral, and posterior walls constrictions occur in the sections by which the limits between the diverticulum and the pharyngeal wall proper appear clearly defined, but anteriorly these limits may be recognized only in a general way. The size and form of this evagination readily distinguish it from the typical visceral pouch and in its walls appear no differentiations that might indicate, as such, the developing fourth and fifth visceral pouches or the ultimobranchial diverticulum. In this specimen the first pair of the associated aortic arches is complete, but the second and third pairs are visible in their dorsal portions only.

A somewhat more advanced condition is shown in a 5-mm. Chelydra (fig. 1). The first three pouches have increased in depth. The first and second are already perforate and the third is nearly so. The second aortic arches are conspicuous, but reach only about half way to the ventral aorta. The third arches are now complete, as are also a very slender pair of fourth arches.

The diverticulum behind the third pouch has grown very considerably and two distinct areas or divisions in its wall are now discernible: an anterior larger part which is elongated in the dorsoventral direction, parallel with the third visceral pouch, and a second smaller part which appears as a diverticulum from the first, pushing out from its posterior wall. The former is in close contact with the ectoderm nearly throughout its length; and on its inner surface, along this line of contact, it presents a conspicuous furrow. In the light of subsequent stages, the

larger of these two secondary diverticula represents the developing fourth visceral pouch; the smaller one represents an early stage in the differentiation of the diverticulum destined to give rise to the ultimobranchial body. A fifth pouch cannot as yet be positively identified (fig. 1, V, p. 5?), though potentially present.

At this stage there is on each side, between the pharynx proper and the diverticulum just mentioned, a slender blood vessel which inosculates with both the fourth aortic arch and the aortic root; it ends at the boundary line between the fourth visceral pouch and the ultimobranchial diverticulum. This vessel is shown by later stages to be the fifth aortic arch.

The next available specimen is an embryo Chrysemys of 6.5 mm. (figs. 2, 3, and 13). In this embryo the fourth gill pouch also is perforate. The first three pouches, except for an increase in size, reveal no new features requiring comment. The pharyngeal outpouching, which in the preceding developmental stages embodied in one the fourth and fifth pouches and the ultimobranchial diverticulum, is now for the first time distinctly differentiated into its three component parts. Furthermore, the fifth visceral arch has arisen, interposing itself so as to separate the fourth pouch anteriorly from the other two components of the original vesicle. At its pharyngeal end the fourth pouch is broadly continuous with the remaining portion of the vesicle. The latter now consists largely of the ultimobranchial diverticulum, the fifth pouch appearing to be merely an anterior, somewhat laterally projecting, secondary outgrowth. The complex of diverticula as a whole has been constricted from the pharynx so as to open into it through a short but still relatively wide passage formed by the confluence of the mouth of the fourth pouch with a very short common opening of the fifth pouch and the ultimobranchial diverticulum. The ultimobranchial pocket has a depth equal to about two-thirds the dorsoventral extent of the fourth pouch. It is somewhat elliptical, flattened lateromedially, and its walls are thick. The fifth pouch is small and that of the left side is developmentally more advanced than its fellow. While the fifth pouch, as before remarked,

appears to be a secondary diverticulum from the ultimobranchial pocket, this is evidently the result of the early differentiation of the ultimobranchial vesicle, involving as it does a relatively large area on the primary pharyngeal outpouching, one in which the diminutive fifth pouch is unable, as it were, to express itself until at a somewhat later stage. The short common passage or stalk previously referred to, by which the fifth pouch and the ultimobranchial diverticulum join the fourth pouch in opening into the pharyngeal cavity, evidently represents originally a portion of the pharyngeal wall proper, and the relations existing are consequently of secondary nature. The rudimentary fifth pouch had been carried bodily out from the pharynx by the ultimobranchial evagination.

In the fifth visceral arch at this stage there is a complete aortic arch, which, about one-fifth of its distance from the dorsal aorta, gives off a more slender posterior branch, the sixth aortic arch; this, after making a loop about the fifth visceral pouch, rejoins the fifth arch. The sixth aortic arch of the right side is incomplete ventrally. The sixth aortic arch lies in the angle formed by the ultimobranchial diverticulum and the fifth pouch, the former being wholly medial to the vessel.

The fifth visceral pouch is in intimate contact with the ectoderm along its lateral edge. The ultimobranchial outgrowth nowhere touches the outer germ layer; its basal or proximal end is opposite the origin of the trachea, and the distal end is directed ventrally, parallel to the long axis of the fourth pouch.

In an embryo Chelydra of 7.5 mm. and one of 9 mm., the second and third pouches are still perforate, and in the latter specimen the fourth also is open. The pouches are much flattened anteroposteriorly and their dorsoventral axes have increased considerably in length. Because of the greatly narrowed ectodermal and entodermal connections, the dorsal and ventral portions of the second and third pouches appear in the sections as closed vesicles and the posterior wall of the dorsal extensions of these two pouches is now much thicker than the anterior wall. A more pronounced advance, however, is apparent in connection with the posterior complex of diverticula (figs. 4 and 5) where the

fifth visceral arch, by its increase in depth, has separated the fourth visceral pouch more widely from the associated fifth pouch and ultimobranchial body. Also a second process of separation, proceeding simultaneously, is well under way, namely, the pinching off of the complex as a whole from the pharynx by the constriction of the common connecting stalk.

The fifth visceral pouches in these stages appear to attain their full development as such. In the larger specimens the right pouch is distinctly larger than the left, but in the smaller the two are of about equal size. Contact with the ectoderm is still maintained, but is more restricted than in the preceding stage. On each side of the body a neck-like stalk connects the fifth pouch with the ultimobranchial diverticulum. While, as remarked, the right pouch in the larger specimen of Chelydra is larger than the left, other specimens of this genus as well as of Chrysemys indicate that there is considerable variation in the comparative size of right and left pouches in different embryos. In the 9-mm. Chelydra the long, or dorsoventral axis, of the larger right pouch is about one-fourth that of the fourth visceral pouch.

The ultimobranchial body, beyond an increase in length and the clearer demarcation noted above, exhibits no important changes.

A notable feature in connection with the aortic arches at this stage is that the middle segment of the fifth arch, or that which forms the anterior limb of the loop, is exceeded in caliber, although slightly, by the posterior limb or that which represents the sixth aortic arch. In another embryo Chelydra of 7.5 mm., which in other respects is in a corresponding stage of development, the sixth aortic arch is already much larger than the fifth. In both specimens the pulmonary artery is now present as a branch of the sixth arch immediately above its junction with the fifth.

In a 9.5-mm. Chelydra, the second visceral pouch has lost its connection with the ectoderm; its dorsal portion shows a thickening of the epithelium which probably represents a transitory thymus bud, disappearing with the closure of the pouch. The ectodermal duct is a very much attenuated tube, but has a longitudinal cellular ridge projecting into its lumen from its medial wall (fig. 6).

The third pouch also has severed its connection with the ectoderm and appears as an elongate, rather thick-walled longitudinal vesicle, extending from the tip of the anterior horn of the hyoid to a point opposite the middle of the posterior horn. The cephalic end of the pouch lies medial to the anterior horn, while the caudal end is lateral to the posterior horn. In length, the left pouch extends through nineteen sections (285μ), the right through seventeen sections (255μ). A very short pharyngeal stalk or entodermal duct, now closed, extends through the sixth to the eighth sections, inclusive, on the left and through the fifth to the seventh on the right. On each side the pouch is crescentic in cross-section (fig. 7), but anterior to the pharyngeal stalk the convex side is ventral while posterior to the stalk it is dorsal. The walls of the vesicle are generally of uniform thickness anterior to the pharyngeal attachment, but here and there the epithelium shows a tendency to fold, and at the anterior end solid buds of cells have formed; likewise on the ventrolateral surface of the vesicular wall there is a conspicuous ridge, formed evidently by local proliferation, extending from the anterior end of the pouch to its pharyngeal stalk. This ridge is symmetrical on the two sides of the body and, together with the cell proliferation noted on the anterior wall of the pouch, is apparently the beginning of thymus formation. Caudal to the pharyngeal stalk the ventral wall of the pouch is decidedly thicker than the dorsal, and from the dorsolateral wall there projects outwardly a solid cellular peg which evidently represents the point of separation from the ectoderm.

The fourth visceral pouch is detached and far removed from the surface epithelium. It is a small, more or less rounded vesicle, with irregular surface contour and with slit-like cavity. The ventrolateral wall is thickened, especially in its middle portion. The entire vesicle extends through eight sections (120μ). It is attached to the ultimobranchial vesicle by a short, narrow stalk which contains the last traces of a cavity. The two sides of the body exhibit practically identical conditions. A differentiation into thymus and parathyreoid portions is not with certainty recognizable.

Regarding the fifth pouch, the gap in my series between the present stage and the preceding is too great to indicate what has taken place in the meantime. In the present specimen there is a small mass of cells lying between the fourth pouch and the ultimobranchial vesicle, just behind the point of connection between these two; the mass has the appearance of undergoing degeneration, and it is possible that it represents the remnants of the fifth pouch.

The ultimobranchial body of the left side is typical for the stage under consideration—an elongate tube lying lateral to and parallel with the trachea. It is largest in its middle portion and tapers more or less towards the ends. The walls are of uniform thickness and the enclosed cavity is sharply defined. Proximally, the vesicle narrows rapidly in approaching its connection with the fourth pouch, and from this point on it becomes merely an attenuated pedicle connecting the two vesicles as a unit with the pharynx. Close to the entodermal wall this stalk is about to be constricted off, but within it a pinhole cavity is visible.

The next step is based upon a 10.5-mm. Chelydra, a 6-mm. Chrysemys, and a 9-mm. Trionyx. In Chelydra the third visceral pouch has been transformed into an elongate, compact mass. The anterior two-thirds is considerably larger than the caudal third and it contains a vestige of the original cavity, around which the innermost cells retain in slight degree their epithelial character. Anteriorly, and to a less extent in other parts, the mass sends out a number of solid mounds of cells, which give it a somewhat lobular appearance. The smaller caudal mass is a continuation of the medial part only of the anterior mass. It is cylindrical and, like the anterior part, contains a trace of the earlier lumen. In brief, the conditions just described simply mean that the third pouch at this stage shows definite differentiation into an anterior thymus body and a posterior parathyreoid body, representing, respectively, dorsal and ventral portions of the original visceral pouch. In the specimens of Chrysemys and of Trionyx the third pouch is developmentally slightly more advanced, but otherwise it presents conditions similar to those just described.

The fourth pouch has by this time also developed into an almost entirely solid body, club-shaped in form, the tapering end directed forward and slightly marked off from the posterior part, as if it represented a rudimentary thymus. The pouch extends through eleven sections (175μ), the three middle sections alone containing evidence of the former cavity. The caudal end is in close proximity to the ultimobranchial body from which it apparently has just become separated. The ultimobranchial vesicle of the left side (fig. 9) shows a very considerable increase in size and is expanded so as to be nearly circular in cross-section, but it has the same smooth-walled appearance as in preceding stages. In greater part the wall shows three or four tiers of nuclei, but in some places there is only one. Its anterior extremity bears a small cellular peg which evidently fixes the point of separation of the fourth visceral pouch.

The conditions of the right side in this embryo deserve notice in that there apparently is complete absence of the ultimobranchial body; it is the only instance in my series where this occurs. A slender cellular stalk, similar to that of the left side, extends from the pharynx to the fourth pouch, to which it furnishes a short pedicle, and then ends only three sections beyond this point, without discernible evidence of an ultimobranchial vesicle. However, the limits between what constitutes the ultimobranchial vesicle proper and the part which represents more or less of the drawnout portion of the pharyngeal wall cannot in any case be exactly determined, and therefore, in view of the conditions found in subsequent stages relative to the point of connection between the fourth pouch and the ultimobranchial vesicle, it is still possible that the latter is potentially present, though in a very rudimentary form, in the distal portion of the entodermal stalk.

The embryo Chrysemys, in corresponding stage of development, shows a condition of the branchial derivatives similar to that of Chelydra, with minor variations. The second visceral pouches have identical tube-like extensions (ectodermal ducts), but these are without cellular buds or areas of proliferation. The third pouch is somewhat more advanced. Its anterior portion is a solid mass of more or less lobular appearance, the original

cavity having been obliterated as far back as the pharyngeal stalk. Caudal to this point the pouch has still a conspicuous lumen, but it becomes solid again in the posterior half. In its entirety the third pouch does not exhibit such clear conditions as in Chelydra, and it is uncertain from available material whether or not any particular portion of its wall may be considered as initiating the process of organ formation, such as appears to be the case in Chelydra. In sections through the region of its pharyngeal connection the vesicle has the same crescentic form as in Chelydra and, on one side at least, the lateral wall is noticeably thicker, but, because of the solidification of the pouch anteriorly, the original relation or the significance of this thickening cannot be determined. The fourth visceral pouch has a broader connection with the ultimobranchial vesicle and the latter is well developed on both sides of the body, although that of the left is by far the larger.

In another 10.5-mm. Chelydra a variation in connection with the fourth pouch and the ultimobranchial body should be noted. On the right side a relatively large ultimobranchial vesicle is present. It is spindle-shaped and extends through fifteen sections, having a diameter in its widest part of approximately one and a half times that of the trachea; its walls are thick and the lumen clear-cut. Its anterior end lies just outside the mesenchymal coat of the oesophagus and reaches the level of the parathyreoid III. With this ultimobranchial body the fourth pouch derivative as yet maintains a slender cellular connection (fig. 11), but, instead of being situated at the anterior end of the vesicle, where it is found in most cases, it here lies at the posterior end. How this relation may have been brought about is not evident, but it possibly may be accounted for by assuming that, after the ultimobranchial vesicle had separated from the pharynx, that portion of its neck proximal to the junction of the fourth pouch, in which the limits of the ultimobranchial vesicle proper are indefinite, continued to develop, while the part distal to the junction suffered regression or had, perhaps, been rudimentary from the outset. On the left side of the body the relations are of the usual kind. The ultimobranchial

vesicle extends through twenty-five sections; while its walls are thicker than in the preceding embryo—indicative, as a rule, of an earlier stage— it shows a more advanced condition in that they bear a number of secondary evaginations of various sizes as well as numerous solid protrusions or sprouts. Both kinds are especially large and conspicuous about the anterior end of the vesicle, while minor ones occur somewhat distal to its middle section.

In a 6-mm. Chrysemys, representing approximately the same developmental stage as the foregoing embryo, a further variation with respect to the fourth visceral pouch and the ultimobranchial vesicle occurs. The left fourth pouch has been converted into a compact cellular mass with even surface contour and without trace of lumen, and is attached in the usual manner by a solid stalk near the anterior end of the ultimobranchial vesicle. The last named, except for its smaller size, is similar to that of the 10.5-mm. Chelydra. The right fourth pouch derivative is much longer than the left (330μ as against 240) and its middle portion is expanded into a vesicle of nearly the same diameter as the ultimobranchial vesicle itself (fig. 18), into which it opens by a passage extending through five sections; and the ultimobranchial vesicle is unusually large for this side, being somewhat more than half the length and width of the left one.

A 9-mm. Trionyx is the youngest specimen of this genus n my possession. In general development it agrees well with the preceding specimen of Chrysemys. The derivatives of the third visceral pouch reveal no noteworthy differences from those of corresponding stages of Chrysemys or Chelydra; but the fourth pouch derivative and the ultimobranchial vesicle show distinct variations from the conditions in those genera. On the left side the two bodies in question have the usual position relative to each other and have a cellular connection, but in form they are of somewhat different type. The ultimobranchial vesicle is much more advanced in development than that of either Chrysemys or Chelydra of corresponding age in that a large portion of it has already been transformed into solid cord-like cell-clusters, while elsewhere it bears spherical, hollow outgrowths from its

walls. These growth processes have been most active in the anterior portion of the vesicle, but are present in varying degree throughout its length. In the midst of the proliferating mass, however, the walls are sufficiently intact to show what had been the general form and size of the vesicle at the height of its development, and in these respects it bears closer resemblance to Chrysemys than to Chelydra, as it evidently attains neither the large size nor the thin-walled condition of the latter. The right ultimobranchial vesicle is a thin-walled tubular structure whose epithelium consists of one or two layers of flattened, loosely arranged cells, evidently in process of retrogression. The fourth pouch derivatives are both characterized by a highly vesicular condition, quite in contrast to the usual solid cellular mass in corresponding stages of the other two genera, but a tendency toward which was seen in the 6-mm. Chrysemys. The walls of these vesicles retain, in part, their early sharply defined epithelial form, in part contain foldings and thickenings due to cell proliferation. The tendency of the fourth pouch derivative in Trionyx to assume a vesicular form occurs in later stages and appears to be a distinctive feature of this genus.

Figure 14 represents a wax-plate reconstruction of the branchial derivatives of the left side of an embryo Chelydra of 9.5-mm. carapace length. The thymus and the parathyreoid III maintain their earlier linear arrangement and partly encircle the carotid artery. The fourth pouch derivative, still attached to the ultimobranchial vesicle, lies medial to and occupies the interval between the systemic and the pulmonary arch (the latter omitted in the model). The ultimobranchial vesicle has attained relatively enormous proportions, the maximal in my series, having a diameter approximately one-half that of the oesophagus. Only on its anterior and anterodorsal surfaces do the sections reveal cellular outgrowths and extensions from the otherwise smooth wall of the vesicle. Its fellow of the opposite side is relatively insignificant and the fourth pouch of this side is also much inferior in size and is furthermore completely detached from the ultimobranchial body.

An embryo Chrysemys and one of Trionyx of 8-mm. and 9-mm. carapace length, respectively, show a general developmental stage corresponding to the preceding embryo Chelydra. In Chrysemys the derivaties of the third visceral pouch together form a more or less rounded three-lobed mass, partly encircling the carotid artery from the dorsal side (on the left), or from the medial side (on the right). Two larger anterior lobes constitute the thymus, while the third lobe, smaller and situated posteriorly, is the parathyreoid body, the two still having cellular continuity. The parathyreoid here is lateral to the thymus instead of caudal, as in Chelydra, possibly due to a growth or shifting caudad of the thymus. The fourth pouch derivative and the ultimobranchial body have the same relative positions as in Chelydra. The latter body here likewise attains its maximal size as a vesicle, but is relatively and absolutely much smaller and has the general form of a cylindrical tube. The vesicle of the opposite side is rudimentary.

In Trionyx the thymus and its associated parathyreoid III have the same tandem arrangement as in Chelydra. The fourth pouch derivative may lie against the medial side of the systemic arch, or between this vessel and the pulmonary arch, opposite the bifurcation of the trachea. On both sides of the body the walls of this derivative are somewhat thickened, but maintain an even epithelial arrangement about a relatively large central cavity, as in the earlier 9-mm. stage. The right ultimobranchial vesicle is very rudimentary; the left one is even smaller than that of Chrysemys, and is profusely covered with cellular excrescences, especially in its posterior portion.

LATER DIFFERENTIATION

In the well-advanced embryos just described the various branchial derivatives have been identifiable, largely or entirely by their respective histories and place relations. Actual structural differences in the thymus, the parathyreoids, and the fourth pouch derivatives are, even in the oldest of these embryos, wanting or at least uncertain in the sections. The form of the dominant ultimobranchial vesicle renders this organ unmistak-

able, but, especially in Trionyx, on the side where the vesicle is rudimentary, the fourth pouch derivative may at times assume a very similar form, so that the two may be distinguished with certainty chiefly by their relative position.

The following account is based upon an embryo Chrysemys of 11-mm. carapace length, one of 15-mm. carapace length, and one at hatching; two embryos of Trionyx of carapace length of 9 mm. and 13 mm., respectively, and two of Chelydra of carapace length of 15 mm. and 16 mm., respectively.

The thymus and parathyreoid bodies are now readily distinguishable from each other, both as to structure and staining properties. The thymus has taken on the characteristic lymphoid appearance and stains deeply. The parathyreoid, on the other hand, exhibits its usual cord-like, epithelial cell masses, with invasions among them of mesenchymal tissue; these features together with the relatively greater amount of cytoplasm in the cells and their less deeply staining nuclei contrast this organ sharply with the thymus. In regard to the relation of the thymus to the carotid artery, Chrysemys and Chelydra are in accord and differ from Trionyx. In the former two the artery is situated laterally, having changed from an earlier, more ventral position. In Trionyx the vessel courses along the medial surface of the gland, but in an earlier stage it was near the ventral surface. In both groups, if a large series were examined, a considerable amount of variation would no doubt be found in the degree of rotation of the thymus about the artery. The parathyreoids are apparently also quite variable, within certain limits, as to their position in the later stages. In Trionyx, where they are somewhat less advanced than in the other two forms, the organ of the left side lies on the ventromedial, while that of the right lies on the ventrolateral surface of the thymus, slightly anterior to its caudal end. In Chrysemys the parathyreoid III is on the medial side of the thymus, more or less deeply imbedded and separated from the carotid by a considerable mass of thymous tissue; in Chelydra its situation is lateral or dorsolateral upon the thymus adjacent to the carotid in the younger specimen of this genus, but in the older it is found to have been shifted somewhat and

has become partly imbedded in the thymus (figs. 21, 22). Regarding the growth changes in the parathyreoid in these later stages my series is too small to furnish definite answer, but from measurements in Chrysemys it seems that, while the thymus increases greatly, the parathyreoid III suffers a cessation or retardation of growth in size between the stage of 15-mm. or 16-mm. carapace length and that of hatching.

In the embryo Chrysemys of 11-mm. carapace length the fourth pouch derivative shows structural and staining characteristics identical with those of the parathyreoid III. It lies somewhat isolated from the derivatives of the third pouch and I find no evidence of thymus tissue in connection with it on either side of the body. The derivative of the fourth pouch, therefore, at least from the evidence in this case, is a parathyreoid body only, but it is quite possible that a thymus sometimes is developed also. In the present specimen the parathyreoid IV has suffered little if any change in position from that of this derivative of earlier stages, being situated upon the dorsolateral surface and slightly caudal to the anterior end of the ultimobranchial body; lateral to it appears the posterior tip of the thymus III. The parathyreoid IV of the right side, which is somewhat larger than its fellow, still has the rudimentary ultimobranchial body attached to its ventral surface. As stated in connection with the 9-mm. Trionyx, the fourth pouch derivative was inclined to be more vesicular than in the other two genera during the early stages, and the same tendency appears in the older embryos now concerned. In the smaller of these (9-mm. c. l.) it has an appearance not unlike that of the ultimobranchial vesicle, but is smaller. The body of the right side especially is large and thin-walled (fig. 17) and caudally has developed three secondary out-pouchings from the main vesicle, giving to the whole still more the character of an ultimobranchial body. In the older embryo (13-mm. c. l.) the bladder form is even more pronounced, but here this feature may involve only a part of the entire organ. Thus, on the left side, the fourth pouch derivative consists of a ventromedial solid mass and a dorsolateral bladder-like portion in which the wall is extremely thin and apparently in process of

disintegration, while on the right side there is a single much enlarged cyst in which the dorsal and posterior walls alone bear thickenings or proliferating cell masses (fig. 19). The walls of these bladder-like expansions of the fourth pouch derivative at this stage do not, as a rule, possess the clear-cut epithelial arrangement of their cells nor the smooth even contour of their inner and outer surfaces which characterized the earlier stages. The cells are notably crowded and jumbled, with here and there dissociated cells intruding into the central cavity.

But while it appears that in Trionyx the fourth pouch derivative is characterized by the tendency to cyst formation from its early stages and upward, a similar condition, and one which was not foreshadowed in the last-described stage (9.5-mm. c. l.) of this genus, occurs in the Chelydra embryos of 15-mm. and 16-mm. carapace length (figs. 20, 23). The greatest development of the vesicular portion is found in the smaller of the two embryos, where it not only exceeds any of the corresponding vesicles in Trionyx, but approaches closely the size of the larger ultimobranchial vesicle in the same embryo. It will be observed from the figures that only a part of the fourth pouch derivative is involved in the cyst, the whole being, as in Trionyx, composed of a glandular and a vesicular part. In the younger embryo the glandular part lies upon the ventrolateral wall of the bladder portion, while in the older specimen it lies upon the ventromedial and the dorsolateral surface, of right and left sides, respectively. At some points the cyst wall has reached a thinness bordering on the breaking-point, where the cells form a single layer and assume a mesothelial appearance. In all cases the cyst portion has cellular continuity with the glandular body, although, as in figure 20, the connection may at times be reduced to a very slender stalk.

The significance of the vesicular portion of the parathyreoid IV is not clear. As to its origin, however, it seems quite certain, from the conditions observed in Trionyx, that it is a part of the original cavity and wall of the fourth visceral pouch. The question will suggest itself whether it may represent a portion of the ultimobranchial vesicle which has separated, along with the

fourth pouch, and later manifests itself in the tendency to cyst formation that is so characteristic of that body. Again, it might conceivably be interpreted as a vestige of some other derivative of the fourth pouch, such as a thymus. Opposed to the first of these views, if not entirely to the second, is the fact that in both embryos of Chelydra (15-mm. and 16-mm. c. l.), although on the right side only, an exactly similar vesicle occurs in connection with the parathyreoid III (fig. 21). In the younger specimen the cyst is largely surrounded by thymous tissue. In the later series of Chrysemys the parathyreoid IV gives no evidence of cyst formation, but in an embryo of 15-mm. carapace length parathyreoid III contains an excentric cavity of moderate size whose wall is a single layer of cells, sharply differentiated from the surrounding tissue. In an embryo at hatching there is what appears to be a trace of such a cavity in the corresponding gland; in the parathyreoid IV evidence of such condition is doubtful.

The ultimobranchial body in all of the later stages mentioned, except that of hatching, shows merely a continuation of the process of reduction of the vesicle, begun in some of the younger embryos. In the present older specimens the vesicle is either completely broken down into a mass of diminutive vesicles and solid cell masses more or less spherical or cord-like in form, as in Chelydra of 16-mm. carapace length (fig. 23); or the main cyst is studded with sprouts and is extensively broken up and reduced in size, as in an embryo Chrysemys of 11-mm. carapace length. The process of reduction and transformation of the original vesicle apparently takes place, chiefly, by two methods: by the formation through evagination and separation (and perhaps also simply by constriction) from the main body, of smaller cysts of varying sizes and forms, and by the outgrowth and detachment from its wall of solid cellular sprouts. In the sprouts the cells at first have a radial or epithelial arrangement in section, and while in some of them an actual lumen may appear, in others such is seemingly not the case. The secondary vesicles undergo further reduction in the same manner as the parent structure. In the older specimen of Chelydra (16-mm. c. l.)

and in Trionyx of 13-mm. carapace length the ultimobranchial body assumes a structure resembling that of the thyreoid in the same specimens, but, nevertheless, distinct and readily distinguishable from it by the complete absence of colloid within the vesicles, by the comparatively small number of such vesicles or tubules, as well as by their irregular form, thicker walls, and often ill-defined lumina. A rudimentary right ultmobranchial body is present in all of the later stages described and it undergoes parallel differentiation with that of its much larger fellow.

In Chrysemys at the time of hatching the ultimobranchial body has assumed an appearance very much like that of the parathyreoid of the same embryo, namely, a rather lightly staining lymphoid structure in which traces of the earlier arrangement and grouping of the cells are clearly recognizable only in a few places. The position of the body remains the same. Deeply imbedded within the left ultimobranchial body lies the parathyreoid IV, which, however, is surrounded by a thin connective-tissue capsule of its own. The ultimobranchial body of the right side consists of a small mass of tissue on the medial side of and partly investing the parathyreoid IV; in structural differentiation it is like its fellow of the opposite side.

SUMMARY

1. The development of the branchial derivatives was studied in turtles of the genera Chelydra, Chrysemys, and Trionyx.

2. The persisting thymus arises from the dorsal portion of the third visceral pouch. In the corresponding portion of the second visceral pouch there is a cellular bud which is interpreted as a rudimentary, transitory thymus.

3. The ventral portion of the third visceral pouch gives origin to a persisting parathyreoid.

4. The fourth visceral pouch gives rise to a persisting parathyreoid, but so far as available material indicates there is no indisputable evidence that a persisting thymus arises from this pouch. A rudimentary thymus which is transitory probably occurs.

5. The fifth visceral pouch seems to disappear soon after it attains its greatest development, which in Chelydra was found to be in embryos of 7.5 mm. to 9 mm. greatest length.

6. A conspicuous ultimobranchial vesicle is usually present on each side in the early stages, but the one on the right, as a rule, soon reaches limitations in growth and becomes greatly exceeded in size by its fellow of the opposite side. The body on the right may apparently at times be wholly lacking. Where both are present, they appear to undergo parallel differentiation, at least up to the time of hatching. The relatively huge dimensions sometimes attained by the dominant ultimobranchial vesicle is a striking feature.

7. In turtles the fourth and fifth visceral pouches and the ultimobranchial diverticulum originate in a single conspicuous evagination from the lateral pharyngeal wall. In this evagination the fourth pouch is the first to be differentiated; next appears the ultimobranchial diverticulum, and lastly the fifth pouch may be distinguished, which is closely associated with the ultimobranchial diverticulum and is very small.

8. The fourth pouch and the ultimobranchial diverticulum become separated as a unit from the pharynx, but remain connected with each other until a comparatively late stage in their development.

9. The fourth pouch in subsequent development exhibits more or less of a tendency toward cyst formation. This seems to be manifested earlier in Trionyx than in the other two genera studied; but in later stages very large cysts, relatively speaking, were found in connection with the fourth pouch in Chelydra. In Chelydra such tendency was observed also in connection with the parathyreoid III. The significance of the cysts is not clear.

10. At the time of hatching the thymus is a rather voluminous body of oblong shape, and parathyreoid III is a relatively small rounded body which is more or less deeply imbedded in the caudal portion of the former gland.

11. Parathyreoid IV, similar in size and shape to the parathyreoid III, is usually found in close association with or partly

or wholly imbedded in the ultimobranchial body (on the left side); or (on the right side, where the ultimobranchial body is rudimentary) it may be adjacent to parathyreoid III, and with it becomes partly surrounded by thymous tissue; or it may lie further caudad in association with the ultimobranchial body.

12. The ultimobranchial body, by the time of hatching, has been transformed almost completely into a lymphoid organ, resembling the parathyreoids at this stage. It is in no way associated with the thyreoid.

BIBLIOGRAPHY

Bemmelen, J. F. van 1893 Ueber die Entwicklung der Kiementaschen und der Aortenbogen bei den Seeschildkröten, untersucht an Embryonen von Chelonia viridis. Anat. Anz., Bd. 8.

1886 Die Visceraltaschen und Aortenbogen bei Reptilien und Vögeln. Zool. Anz., Bd. 9.

Johnson, C. E. 1918 The origin of the ultimobranchial body and its relation to the fifth pouch in birds. Jour. Morph., v. 31.

Liessner, E. 1888 Ein Beitrag zur Kenntnis der Kiemenspalten und ihrer Anlagen bei amnioten Wirbelthieren. Morph. Jahrb., 13.

Maurer, F. 1899 Die Schildrüse, Thymus und andere Schlundspaltenderivate bei der Eidechse. Morph. Jahrb., 27.

Meuron, P. de 1886 Recherches sur le développement du thymus et de la glande thyreoïde. Dessertation. Geneve.

Peter, K. 1900–01 Mittheilungen zur Entwicklungsgeschichte der Eidechse. II. Die Schlundspalten und ihrer Anlage, Ausbildung und Bedeutung. Arch. f. mikr. Anat., Bd. 57.

Saint-Remy et Prenant 1903–04 Recherches sur le développement de dérivés branchiaux chez les Sauriens et les Ophidiens. Arch. de Biol., T. 20.

Verdun, P. 1898 Dérivés branchiaux chez les vertebrés supérieurs. Thése, Toulouse.

ABBREVIATIONS

A.a., aortic arches
Ar.car., carotid artery
Ao.r., aortic root
Br., bronchus
D.ao., dorsal aorta
Oes., oesophagus
Par. III, IV, parathyreoids, derived from the third and fourth pouches, respectively
Ph., pharynx
Ph.div., pharyngeal diverticulum
S.a., systemic arch
Thy., thymus
Thr., thyreoid
Tr., trachea
U.b., ultimobranchial vesicle or body
Vag., vagus nerve
V.a.5, fifth visceral arch
Ves., vesicular portion of parathyreoids
V.p. 2, 3, 4, 5, visceral pouches, second to fifth

PLATE 1

EXPLANATION OF FIGURES

1 Frontal section through the posterior pharyngeal region of an embryo Chelydra serpentina 5 mm. long. × 80.

2 Frontal section through the corresponding region of an embryo Chrysemys marginata 6.5 mm. long, showing developing fifth visceral arch and the differentiated fourth and fifth visceral pouches. × 80.

3 Same embryo as figure 2, section taken farther ventrally, showing relation of fifth pouch to ultimobranchial vesicle. × 80.

4 Frontal section from an embryo C. serpentina 7.5 mm. long, passing through the main body of the ultimobranchial vesicle and showing also progressive development of the fifth visceral pouch. × 80.

5 Frontal section from an embryo C. serpentina 9 mm. long, showing connection between fourth visceral pouch and ultimobranchial vesicle and also a further step in development of the fifth visceral pouch and corresponding visceral arch. × 80.

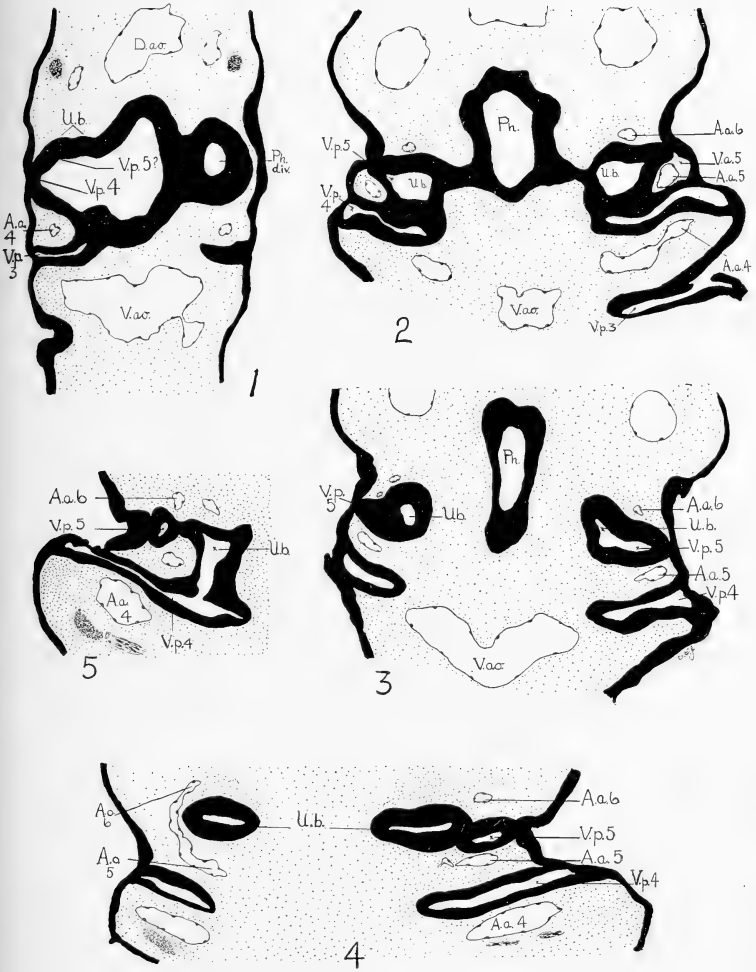

D.ao.
U.b.
Vp.5?
Vp.4
Ph. div.
A.a. 4
Vp. 3
V.ao.
1
Ph.
Vp.5
Vp. 4
U.b.
A.a.6
V.a.5
A.a.5
A.a.4
V.ao.
Vp.3
2
A.a.6
Vp.5
U.b.
A.a. 4
Vp.4
5
Ph.
Vp. 5
U.b.
A.a.6
U.b.
Vp.5
A.a.5
Vp.4
V.ao.
3
A.a. 6
U.b.
A.a. 5
A.a.6
Vp.5
A.a. 5
Vp.4
A.a. 4
4

PLATE 2

EXPLANATION OF FIGURES

6 Transverse section through the ectodermal duct of the second visceral pouch in an embryo Chelydra 9.5 mm. long. × 300.

7 Section through the third visceral pouch of the same embryo as figure 6, showing early step in the development of the thymus. × 230.

8 Transverse section through the fourth pouch derivative of an embryo Chelydra with carapace 9.5 mm. long. × 300.

9 Transverse section through the left ultimobranchial vesicle of an embryo Chelydra 10.5 mm. long. × 230.

10 Transverse section through the developing thymus of the right side of an embryo Chelydra 10.5 mm. long. × 230.

11 Transverse section through the fourth pouch derivative and the ultimobranchial body of the right side of an embryo Chelydra 10.5 mm. long. × 300.

12 Transverse section through a part of the left lateral wall of the left ultimobranchial vesicle and attached fourth pouch derivative, from an embryo Chelydra with carapace 9.5 mm. long. Same embryo as figure 8. × 300.

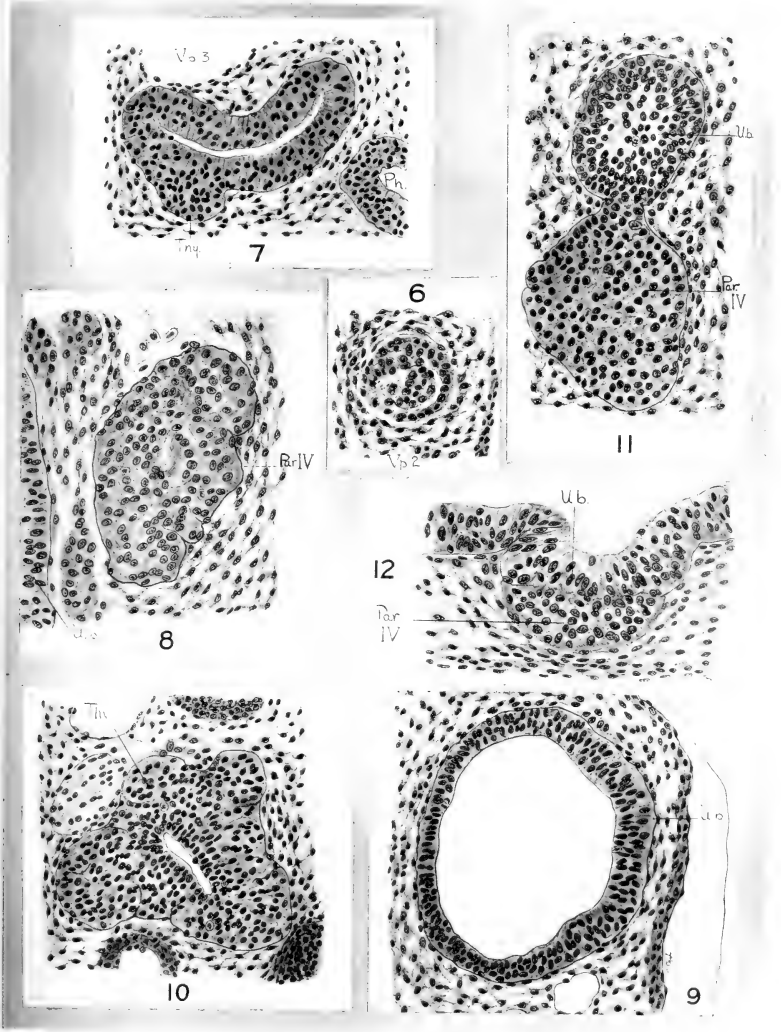

Vo 3
Pn
Tng
7
6
Vb 2
Ub
Par IV
11
Par IV
8
Ub
12
Par IV
Thy
10
9

PLATE 3

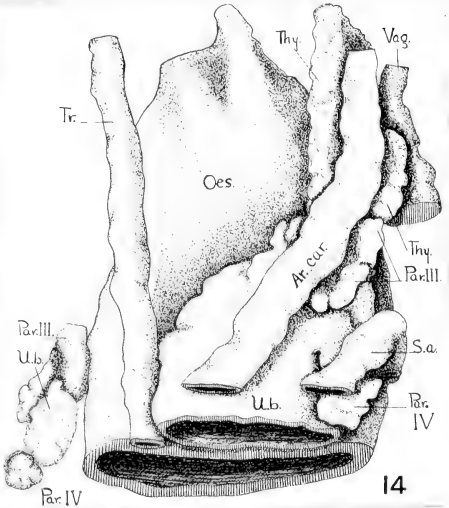
EXPLANATION OF FIGURES

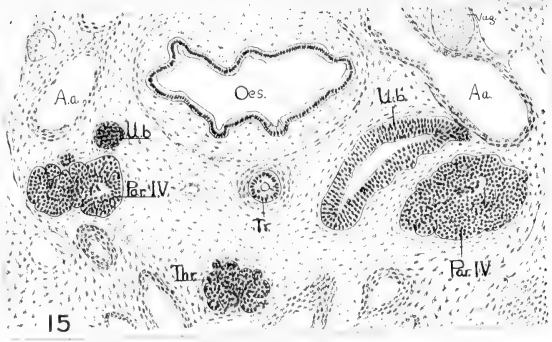
13 Wax-plate reconstruction of the posterior pharyngeal region of an embryo Chrysemys 6.5 mm. long. Ventrolateral view, left side. × 100.

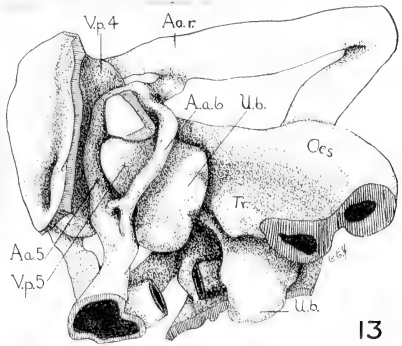
14 Wax-plate reconstruction of the visceral-pouch derivatives of the left side (and *par. III*, *par. IV* and the *U.b.* of the right side) of an embryo Chelydra with carapace 9.5 mm. long. Ventrolateral view. × 66.6.

15 Transverse section through the region of the ultimobranchiai vesicles and the fourth pouch derivatives of an embryo Chelydra 10 mm. long. × 75.

16 Section from the same specimen as figure 15, taken nearer the anterior end of the left ultimobranchial vesicle. × 75.


Thy.
Vag.
Tr.
Oes.
Ar. cur.
Thy.
Par III.
Par III.
U.b.
S.a.
U.b.
Par IV
Par IV
14


Vag.
A.a.
Oes.
U.b.
Aa.
U.b.
Par IV
Tr.
Thr.
Par IV
15


Vp.4
Ao.r.
A.a.6
U.b.
Oes
Tr.
A.a.5
Vp.5
U.b.
13

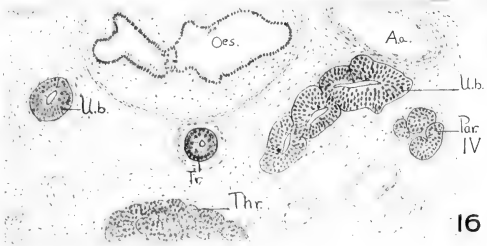

Oes.
Aa.
U.b.
U.b.
Par IV
Tr.
Thr.
16

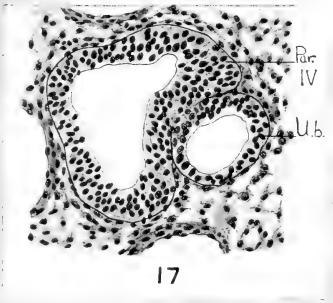
PLATE 4

EXPLANATION OF FIGURES

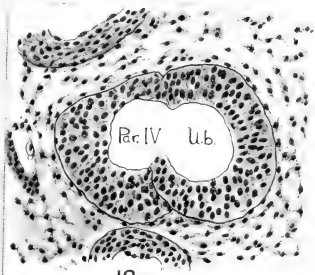
17 Transverse section through the fourth pouch derivative and the ultimobranchial vesicle of the right side of an embryo Trionyx sp. with carapace 9 mm. long. × 230.

18 Transverse section through the same structures of the right side of an embryo Chrysemys 6 mm. long. × 230.

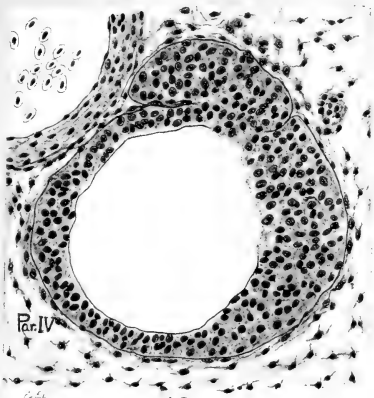
19 Transverse section through the fourth pouch derivative of the right side of an embryo Trionyx with carapace 13 mm. long. × 230.



17



18



19

PLATE 5

EXPLANATION OF FIGURES

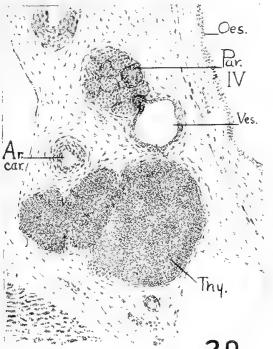
20 Transverse section through the parathyreoid IV and the caudal portion of the thymus of the right side of an embryo Chelydra with carapace 16 mm. long. × 50.

21 Same embryo. Section through parathyreoid III and the thymus of the right side. × 50.

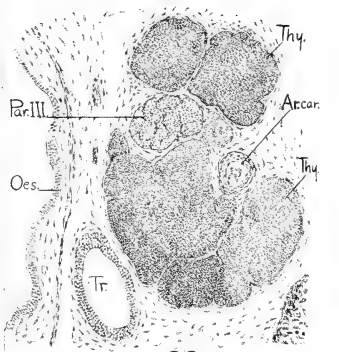
22 Same embryo. Section taken farther anteriorly than that of figure 21, showing thymus and parathyreoid III of left side. × 50.

23 Transverse section through the ultimobranchial body and the parathyreoid IV of the left side; from same embryo as figures 20 to 22. × 75.

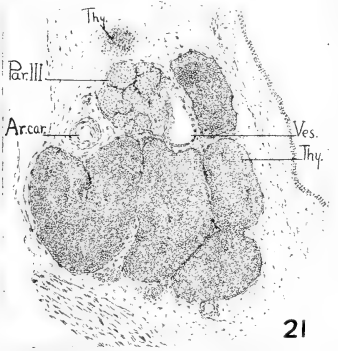
24 Transverse section through the left ultimobranchial body and the parathyreoid IV of an embryo Chrysemys at hatching. × 50.


Oes.
Par. IV
Ves.
Ar. car.
Thy.

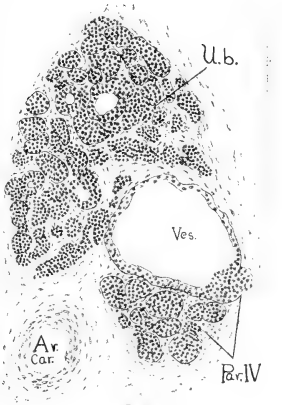
20


Thy.
Par.III
Ar.car.
Thy.
Oes.
Tr.

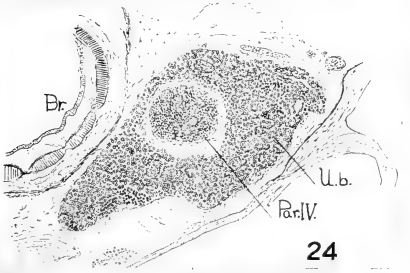
22


Thy.
Par.III
Ar.car.
Ves.
Thy.

21


U.b.
Ves.
Ar. Car.
Par.IV

23


Br.
U.b.
Par.IV.

24

Resumen por el autor, Horace W. Stunkard.

Los neurómeros primarios y la segmentación de la cabeza.

La literatura sobre la segmentación de la cabeza de los vertebrados presenta grandes diferencias, tanto de observación como de interpretación. Después de publicada una serie de trabajos antiguos sobre los neurómeros, Locy ('95) y Hill ('00) han descrito la segmentación primaria del sistema nervioso de Amblystoma, Squalus, el pollo y otras formas. Los investigadores han dudado a menudo de la exactitud de las observaciones de Locy y Hill, y una repetición de su trabajo, usando medios de exámen tan semejantes a los suyos como es posible, demuestra que los "neurómeros primarios" no pueden considerarse como metaméricos. Las divisiones mediales observadas en la placa neural de Amblystoma y consideradas por Griggs ('10) como neurómeros verdaderos se deben en gran parte, si no totalmente, a la segmentación del mesodermo, y por consiguiente deben considerarse tan solo como rasgos de importancia secundaria. Los neurómeros primarios de Locy y Hill, así como los de Griggs y otros autores que han estudiado el neuromerismo, son de tamaño irregular, en número inconstante, de posición asimétrica y no pueden servir como criterio bien establecido de la metamería de la cabeza de los vertebrados.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 16

PRIMARY NEUROMERES AND HEAD SEGMENTATION

HORACE W. STUNKARD
New York University

TWENTY FIGURES

The problem of the segmentation of the vertebrate head, old as Oken and Goethe, has possibly attracted as much interest and incited as much investigation as any other one question in vertebrate morphology. The first investigators advanced a theory based upon superficial external features the sutures of the skull. Subsequent workers have investigated every structure enclosed within the skull, with the hope that light may be thrown upon the obscurity and uncertainty enveloping the evolution of the head. This complex, intricate structure manifests evidences of past ages; remnants and vestiges of the long period of developmental history still persist, but the character of the evidence, the complications, omissions, and reversals have baffled all attempts at solution.

Huxley ('58) overthrew the vertebral theory of the skull, Balfour ('78) introduced mesodermal head cavities as criteria of segmentation and clues to the number and relationship of the cephalic somites, and Gegenbaur ('87) added cranial nerves and visceral arches as segmental criteria. Van Wijhe ('86), ('89) considered the dorsal ganglia of importance and formulated criteria to determine the true segmental nerves. He regarded the olfactory and optic nerves as parts of the brain and not of segmental value.

Von Baer ('28) noticed symmetrical folds in the hindbrain of the chick; Dohrn ('75) related them to the mesodermal somites, and Beraneck ('84) to the cranial nerves. Balfour reported that the first gives rise to the cerebellum, and considered it doubtful whether the other constrictions have any morphological

importance. Von Kupffer ('85) observed a 'primary metamerism' in the neural tube of Salamandra atra embryos which appeared before the segmentation of the mesoderm, and Orr ('87), studying the embryology of the lizard Anolis, noticed a number of symmetrical constrictions in the lateral walls of the hindbrain, "giving the walls in horizontal section an undulated appearance." Kupffer called these 'medularfalten' and Orr adopted for them the name neuromeres. This author formulated the first criteria for determining the identity of the neuromeres. He described two in the primitive forebrain, one in the midbrain, and six in the hindbrain. McClure ('90), working on embryos of Amblystoma punctatum, Anolis sagroei, and the chick, found "a continuous and symmetrical series of neuromeres increasing in size anteriorly, which extend from the lateral walls of the embryonic brain, throughout the entire length of the neuron." He believed that the primary forebrain contained two neuromeres, that the midbrain consisted of two neuromeres, and that the third and fourth nerves were the nerves of these somites. Froriep ('91) found neuromeres prior to the segmentation of the mesoderm, but did not attach any segmental importance to them, and later ('92) decided they were the results of underlying mesoblastic somites. He found the constrictions in the median part of the cephalic plate, while the neural tube is still open, four in Salamandra maculosa and five in Triton cristatus. Waters ('92) confirmed the observations of McClure, and found three segments in the forebrain. Eycleshymer ('95) observed certain markings in the neural folds which might be interpreted as neuromeres, yet he noted that their arrangement was decidedly irregular and the structures were probably due to the action of killing reagents. The transverse markings in the neural plate he regarded as due to the formation of the myomeres.

The tendency to regard the neuromeres as segmental structures reached a definitive stage with the work of Locy ('95). This author reviewed the work on neuromeres exhaustively. He made observations on Squalus acanthias, Amblystoma, Diemyctylus, Rana palustris, Torpedo ocellata, and the chick. In all these forms he described neuromeres in very early stages,

as soon as the neural folds are established, and before there is any division of the mesoderm into protovertebrae. In the open neural groove, the neuromeres of the hindbrain are, he stated, merely the more apparent constrictions of a neuromerism that involves the entire neural plate. He traced the neuromeres to the anterior end of the medullary groove and those earliest formed without a break into the later stages, identifying them with the neuromeres of the closed neural tube. In the chick he described neuromeres visible in the blastoderm of the twelfth hour of incubation, and stated that this segmentation extends into the primitive streak. In Amblystoma, at the stage with a broadly expanded neural plate and widely open neural groove, he found "the neural folds divided throughout their length into a series of segments with no especial distinguishing features between those of the head and those of the body region. The median plate included between the neural ridges is smooth at this stage; at a slightly later period, however, while the groove is still widely open, the median plate exhibits very faint transverse markings." He pointed out that these median divisions do not correspond with those in the neural ridges, and he attached no morphological significance to them. He claimed that in all the forms studied "The cells in the neural segments are characteristically arranged, even in the earliest stages, and their arrangement and structure would indicate that they are definite differentiations of cell areas, not merely mechanical undulations." Locy summarized his work on neuromeres by stating that they cannot be artifacts, that they arise before there is any segmental division of the mesoderm, and so cannot be dependent upon the latter. He concluded that neuromeric segmentation is more primitive than mesodermic segmentation, and for this reason may well serve as a basis for the study of the segmentation of the head.

Neal ('98) was unable to verify Locy's statements in Squalus. He found the edges of the plate slightly and irregularly lobed, but the lobes on the opposite margins of the plate did not correspond either in number or position, nor did they show any definite relation to the mesodermal somites. Regarding these 'segments'

as the results of unequal growth along the margin of the neural plate, he contended that "it is obviously not necessary to regard such irregularities of the edge of a rapidly expanding plate of tissue as of morphological importance. A disassociation of cells or rapid proliferation of cells, which certainly does occur in this region, would lead to such phenomena." Neal found it impossible to trace definite segments into the later stages, for in these stages, before the closure of the neural tube, in the majority of specimens little or no evidence of segmentation along the cephalic plate could be seen. In Squalus acanthias he found the posterior boundary of the cephalic plate coincides with the posterior boundary of encephalomere VI, opposite which the auditory invagination takes place. Showing discrepancies in Locy's statements regarding the position of the auditory vesicle and the posterior limit of the cephalic plate, Neal says, "I can see no escape from the conclusion that he (Locy) has not traced neural segments accurately up to the time they form neuromeres." Furthermore, Neal warned against formulating conclusions from observation of a single organ system and applying them to the phylogenesis of the vertebrate head. He contended that primitively there existed a correspondence between neuromerism, mesomerism and branchiomerism, and the problem of phyletic cephalogenesis is to explain the present lack of correspondence. In Squalus he found five mesomeres alternating with six neuromeres in the otic and preotic region.

Hill (1900), working on Salmo and chick embryos, confirmed the statements of Locy. He reported complete agreement as regards the number and position of the neural segments in the trout and chick embryos. The forebrain has three and the midbrain two segments which, in the earliest stages, do not differ in any essential features from those of the medulla. They antedate the historic divisions, forebrain and midbrain, and precede the optic evaginations. The primary neuromeres were constantly and normally present in the early stages of all the embryos examined by him. Speaking of the external and corresponding internal constrictions which separate the segments, he says that in the early stages these grooves encircle the encephalon,

but in later stages the primary segmentation is confined to its base and lateral walls, owing to the neural expansion and the appearance in the dorsal region of a thin roof. He pointed out that in the position occupied by the third, fifth, and sixth segmental grooves, deep internal constrictions appear that form the posterior limits respectively of the forebrain, midbrain, and cerebellum; that all the primitive grooves disappear during embryonic growth, those of the forebrain first, those of the midbrain second, and lastly those of the hindbrain. In the chick, when the neural folds close to form the neural tube, the walls of the latter expand, not uniformly, but intrasegmentally, and the position of the internal grooves is thus passively elevated upon crests. Contrary to the statement of Locy, he found that in younger embryonic stages of the chick and also of the trout, the histology is very simple, the radial arrangement of cells is absent, the nuclei do not recede intrasegmentally from the inner surface of the brain but are uniformly distributed. In these stages he reports that the only criteria by which he has counted neural segments were external and corresponding internal grooves. Concerning the value of various segmental criteria, Hill stated that mesomeres are found only in elasmobranchs, amphibians, and reptiles, and added that in elasmobranchs, the only group in which their development has been traced, their study has led to a greater divergence of opinion and more conflicting views than is generally supposed. He dismissed branchiomeres with a quotation from Minot that the gill clefts are not segmental and concluded therefore that the branchial nerves are not in segmental order. He argued that Neal's ('98) conclusions were based on negative evidence and that he had observed the segments where Neal failed to find them.

Johnston ('05) accepted the number of neuromeres described by Locy as necessary to account for all the nerves and sense organs connected with the brain, and stated that observations, then incomplete, on embryos of Amblystoma punctatum seemed to confirm Locy's work. He contended that the nervous system, acting in the rôle of a connecting and coördinating system, might well act as a key for the interpretation of the facts secured by a study of the other structures.

Von Kupffer ('06) reviewed the work of Locy and Hill and maintained that the question is still unanswered. Concerning Hill's work on the neuromeres of the chick, he says, "Mit einiger Ueberraschung werden wohl allgemein die Abbildungen aufgenommen worden sein, mit denen Hill seine Beobachtungen über die Primären Neuromeren bein Hühnchen belegt. Es macht den Eindruck, als wenn das subjective Moment die Führung des Zeichenstiftes doch wohl etwas zu stark beeinflusst hätte"; and (p. 248) "Ich kann diese Angaben, mangels gleich ausdehnter Beobachtungen, zwar nicht bestätigen aber ich will sie nicht beanstanden." Neal ('14) translated 'mangels' in this last sentence to mean 'in spite of,' which somewhat alters the original meaning.

Filatoff ('07) argued that neuromeres are mechanical results, due to growth in a restricted space. He rejected Hill's contention that neuromeres are the chief and only certain criteria upon which to build a judgment concerning the primitive metamerism of the head, and agreed with Neal ('98) and Koltzoff ('01) that the proper method by which to attack the problem is to establish an agreement between the neural segments and the somites, nerves, and gill clefts.

Wilson and Hill ('07) could not accept the conclusions of Locy and Hill, and maintained that Hill had not adequately met the contention of Neal ('98).

Belogolowy ('10) maintained that the neuromeres are only form changes of uncertain nature and irregular appearance, possibly the results of mechanical factors, and that they are of most uncertain value as criteria of the segmentation of the head.

Griggs ('10) sought again to establish neuromerism as a basis for determining the segmentation of the head. He described four neuromeres in the procephalic part of the open neural plate of Amblystoma embryos, and in a few specimens of later stages noted neuromeres which appear posterior to the four procephalic lobes, but the history of these posterior neuromeres could not be traced nor their number or arrangement determined. He agreed with Locy that in the early stages the plate and neural crests are not segmented in the same way; he found occasional slight

beadings or lobes on the neural crests, but did not regard them as of morphological importance. These lateral lobulations he found vary both in number and arrangement and as the neural crests close over the plate all signs of segmentation behind the procephalic lobes disappear. He described three distinct grooves, the anterior and posterior germinal depressions and the 'blastogroove' which appear in the location later occupied by the neural groove. Griggs stated that the primary neuromeres described by Locy were not apparent in any of the embryos which he examined. He concluded that the median transverse grooves separate the true neuromeres, that the first contributes to the formation of the forebrain, the second and third to the formation of the midbrain, and the fourth to the formation of the anterior part of the cerebellum.

Smith ('12) described grooves which appeared very early in the neural place of Cryptobranchus embryos, one regularly antedated the others, and this I believe corresponds to the transverse cephalic groove of Griggs. Anterior to this groove he noted six transitory furrows and posterior to it an undetermined number, but expressed the suspicion that these grooves are connected with the formation of the mesodermal somites. In early stages of the formation of the neural folds he observed occasional transverse grooves, but stated that they are often irregular and bear no definite relation to the segments of the neural plate. He argued that the true segments are to be found between the transverse grooves of the neural plate, and pointed out that in the region of the mesodermal somites the transverse grooves of the plate are in line with the intersomitic grooves and the neuromeres are in line with the somites. He was unable to follow the various structures of the neural plate into the definitive divisions of the embryonic and adult brain.

Graper ('13) reviewed the literature on neuromeres extensively; he criticised Hill's "überraschenden und von niemand bestätigten Zeichnungen" and was unable to confirm his observations in the chick.

Neal ('14) argued that the hindbrain neuromeres manifest a segmentation that cannot be explained upon purely mechanical

grounds, but contended that the differences in observation and the divergent conclusions of investigators who have examined neuromeres militate against the confidence of Locy, Hill, Johnston, Griggs, and others who hold that through the study of neuromerism the primitive segmentation of the head will be ascertained. Quoting Dr. Bashford Dean, he stated that in the forebrain of Bdellostoma there appear two, three, or four neuromeres on one side or the other, never paired; in the midbrain there is any number from one to eight; while in the hindbrain the number varies from three to twenty-four, differing in number on different sides, a difference of ten having been noted in the right and left sides of the same individual. He added that he had examined hundreds of Squalus embryos in an attempt to confirm Locy's results, and only two or three showed symmetry or regularity in the segmentation of the edges of the neural plate, while the beaded thickenings were not only asymmetrical, but quite variable in different specimens. He maintained that the primary brain vesicles and not their secondary subdivisions are homologous with the hindbrain neuromeres and that the correspondence in number of primary brain vesicles, myotomes, and cranial nerves argues strongly for the metameric value of these structures.

Smith ('14) observed transverse markings in the procephalic plate of Desmognathus fusca embryos which were very transient, varied in position in different individuals, and which he was unable to trace through from one stage to another in living specimens. In the posterior part of the plate the markings were more uniform, persisted longer, and were subject to but slight variation. In some specimens they corresponded closely to the outpocketings in the medullary folds, but not in other individuals. He also described plications in the medullary folds which appeared early and persisted until fused and absorbed in the expanding prosencephalon. These lateral irregularities did not correspond to the median grooves and he ascribed their formation to mechanical factors. He pointed out that it would be easily possible to select from the material a series which would show a uniform development and fate of these foldings, but

after the examination of a large number of specimens decided that they had no definite significance or fate. He regarded the folds in the medullary plate as normal, but not constant, and no evidence was found in the cephalic portion of the plate of divisions to which a segmental value should be assigned.

Neal ('18) has admirably summarized the evidence for and against the metameric importance of neuromeres. Adducing evidence from a thorough study of the problem of head development, he contends that the neuromeres of the spinal cord are passive results of the mechanical pressure of the adjacent mesodermic somites; that the rhombomeres have arisen in correlation with the visceral arches with which they are functionally connected; and that the only structures anterior to the medulla which may be considered as segmental are the primary forebrain and midbrain segments.

A number of other investigators have worked on different phases of the head-segmentation problem and a more extended review of the literature may be found in the bibliographies of the papers cited here.

It is apparent from the disagreement in the results of former investigators that the nature, number, and significance of the neuromeres are far from determined. While neuromeres have been frequently observed in many animals, and widely discussed, the conception of their value as segmental criteria has been largely developed by Locy, Hill, Johnston, and Griggs. It is extremely difficult, if not impossible, to correlate the observations and interpretations of the various authors, but the repeated observation as to some kind of division in the neural crests and open neural plate is sufficient to warrant further investigation. At the suggestion of Prof. J. S. Kingsley, the writer has studied the early stages in Amblystoma and the chick. The work was begun in 1914 and carried on for two years in the zoological laboratory of the University of Illinois. It was interrupted for two years because of military service, but was continued and completed in the biological laboratory of New York University. The writer wishes here to express to Professor Kingsley his appreciation for the many helpful suggestions

received in the course of the study. An attempt was made to determine whether in Amblystoma a segmentation of the neural crests is regularly and uniformly present, and to compare this division with that of the neural plate. Further, to determine, if possible, which, if either, is of metameric significance. The persistent doubt regarding the accuracy of the observations of Locy and Hill on chick embryos makes a reinvestigation and confirmation of their work very desirable.

The study of Amblystoma was made upon several hundred embryos, collected near Champaign-Urbana, Illinois. The entire series of changes involved in the formation and closure of the neural tube was repeatedly observed under the binocular. To make more careful observation, parts of the neural crests and medullary plate were dissected and observed from all angles and with various means of illumination. For material to supplement the study of living specimens, embryos at all stages of development from the wide-open to closed neural tube were killed in various fluids and sections were cut in transverse, frontal, and sagittal planes.

In Amblystoma, as the blastopore narrows to a small oval structure, a distinct longitudinal groove forms anterior to it. In a few specimens the groove appears to extend to the lip of the blastopore, but in the large majority of embryos when the groove is first formed a short distance separates it from the blastopore. In some embryos the groove extends anteriorly and posteriorly in a continuous manner, so that with the closing of the blastopore, it forms the definitive neural groove. In other specimens, however, another faint groove, usually shorter than the first, may appear anterior to it. This observation agrees with that of Griggs ('10), although the appearance of the grooves does not show the regularity or constancy reported by him. The first of these grooves he termed the posterior germinal depression and that anterior to it the anterior germinal depression. The groove formed by the concresence of the lateral lips of the blastopore he called the blastogroove. There is considerable variation in the uniformity and regularity with which these grooves appear, often separate germinal grooves are entirely absent and the

neural groove forms without the previous appearance of separate depressions. Griggs stated that it is sometimes impossible to distinguish between the anterior depression, posterior depression, and neural groove. The depressions described by Griggs appear in the same position as the neural groove, and I see no good reason for considering them as anything other than stages in the formation of the neural groove itself. The anterior end of the neural groove is marked by a depression, the anterior pit of Griggs, which becomes deeper, extends anteriorly, and, with only slight variations in the process of development, becomes the infundibulum. In these early stages it is sometimes possible to distinguish in the neural groove faint alternating lighter and darker areas, which in a few specimens suggest a segmental condition, but observation of a large number of embryos shows such variation and irregularity as to preclude such an interpretation.

Lateral longitudinal depressions at the sides of the neural plate could not be distinguished before the appearance of the neural crests. With the thickening of the ectoderm to form the crests, these structures are slightly elevated and a lateral linear depression is visible, not only on the median, but often also on the lateral side of the neural crest. The neural ridges increase in size and length, growing anteriorly, posteriorly, and dorsally until they become continuous in front of and behind the neural plate. As the neural crests grow dorsally, the anterior part of the neural fold rises prominently, and the embryo has the appearance shown in figure 1. At this stage the blastopore has closed to a narrow slit and the neural groove extends from the blastopore almost to the anterior part of the neural fold. On either side, just caudal to and within this anterior part of the fold, there is visible occasionally a small depressed circular or oval deeply pigmented area. These depressions were described by Eycleshymer, and, according to him, are the initial stages of the paired eyes.

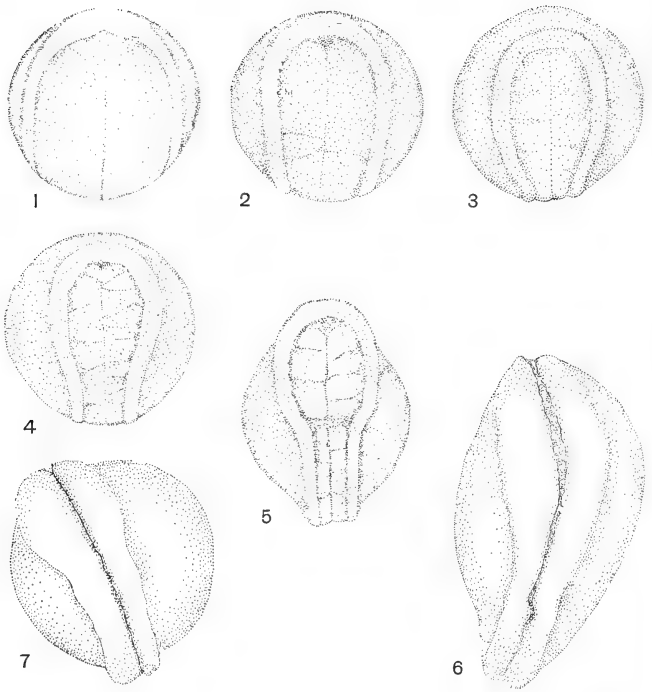
In many of the embryos a few (usually three to five) faint transverse grooves appear in the anterior part of the medullary plate, but they are not constant in number or regular in position.

In some specimens other similar divisions appear posterior to these, but they are less distinct and gradually fade out posteriorly so that their number could not be determined. Normally, the transverse grooves first appear at the lateral edges of the plate and extend toward the neural groove. Frequently one appears on either side before the others and since these first ones are at a corresponding level, their fusion forms a furrow which I regard as the transverse cephalic groove of Griggs. There is considerable irregularity in the formation of the grooves, however; often those of the two sides are formed at different levels and do not meet at the median line. The areas between the grooves are then irregular in size and shape; frequently they are almost triangular as the transverse grooves converge or meet, either at the neural groove or at the lateral edge of the plate. The transverse grooves in the two sides of the neural plate of the same embryo do not regularly correspond, and this lack of correspondence is manifest in the figures of Griggs and other authors. Only in the occasional and unusual specimen is there present the regular arrangement described by Griggs. The neural folds close rapidly and it is possible to observe the changes that take place during the process. It is a significant fact that the grooves do not always retain precisely their original aspect during the closing process. Some of the grooves shift slightly or fade out entirely and other grooves appear in different positions.

Divisions of the neural plate caused by the transverse grooves could not be clearly or satisfactorily demonstrated in sections, but such study shows that, with the appearance of the transverse grooves, the mesoderm has developed to a stage where it is assuming a segmented condition, and I regard the formation of the transverse grooves as due to the formation of the mesodermal somites. I am convinced that certain of the transverse grooves coincide with the divisions between somites, and I am inclined to believe that it is true of most if not all of the transverse grooves. It is possible, however, that grooves are also due to associated mechanical factors, pressure produced by the multiplying cells and the infolding of the neural crests.

In the lateral ridges a beaded appearance is sometimes present, but in no case did it show the regularity described by Locy. The lobulations along the neural crests are often entirely absent, and, when present they do not show definite regularity, either in size or arrangement. The number of lobes varies from two or three to as many as fifteen on one side, and little if any correspondence could be detected between the lobulations of opposite sides of the same embryo. Sections of the crests show the cells to be distributed uniformly with occasional slight irregular groupings, but there is no evidence of a segmental arrangement. These aggregates or clusters of embryonic cells are not differentiated into regular areas, but appear to be centers of rapid cell proliferation. Before the neural folds close the forebrain and midbrain are clearly outlined by thickened enlargements, and by the time of complete fusion, the three primary brain vesicles are distinctly defined. The crests close rapidly and in essential respects these observations confirm the description given by Eycleshymer ('95). The median divisions disappear with the closure of the neural folds, and no definite relation between them and the brain vesicles could be determined. Sections of many embryos seem to indicate that their fate is not uniform, but differs in different individuals.

For the study of chick embryos, several hundred eggs were incubated, and over one hundred embryos were obtained for study, giving a series of stages from the formation of the primitive streak to the formation of the brain and spinal cord. Most of the embryos were removed at the stage when the neural groove is open, as this, according to Locy and Hill, is the most favorable period at which to observe the primary neuromerism of the nervous system. In the study of the living embryo most of the work was done with a binocular although both dissecting and compound microscopes were used. For illumination, transmitted light, as well as reflected light from an electric arc, a gas light, and also direct sunlight were used. Following Locy's suggestion that "a dead black background is of course the best surface for observing anything of this kind by reflected light," a circle of dead black paper was placed under the specimen in the



Figs. 1 to 7 and 12 to 14, camera-lucida drawings of Amblystoma embryos, showing successive stages of development, the so-called primary neuromeres, and the neuromeres of later stages.

Fig. 1 Early stage in the formation of the neural folds, showing the anterior thickening.

Fig. 2 Embryo showing neural groove, the lobulations along the neural folds, and transverse grooves of the neural plate.

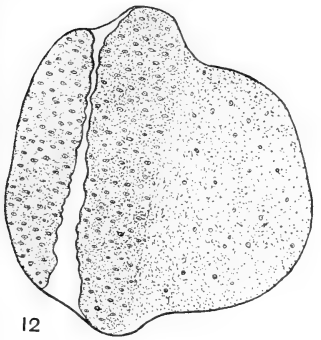
Fig. 3 Embryo with no evidence of segmentation in the neural folds and regular transverse grooves of the neural plate.

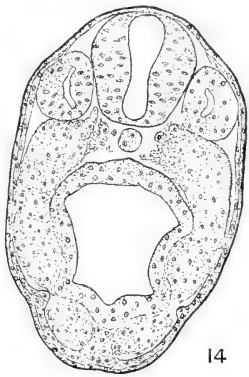
Fig. 4 Embryo showing irregular character of the divisions of the neural folds and neural plate.

Fig. 5 Embryo showing expanded anterior part of the neural plate, with irregular divisions of the neural folds and neural plate.

Fig. 6 Embryo elongated, with partial fusion of the neural crests.

Fig. 7 Complete fusion of neural crests and appearance of the brain vesicles.





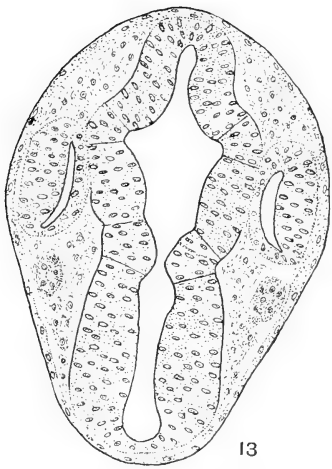


Fig. 12 Frontal section through the open neural crests of an embryo, showing irregular lobulations of crests and indefinite cell arrangement.

Fig. 13 Frontal section through the developing brain vesicles of embryo, showing the later neuromeres.

Fig. 14 Cross-section of embryo at same state of development as figure 13, showing mesodermal segmentation.

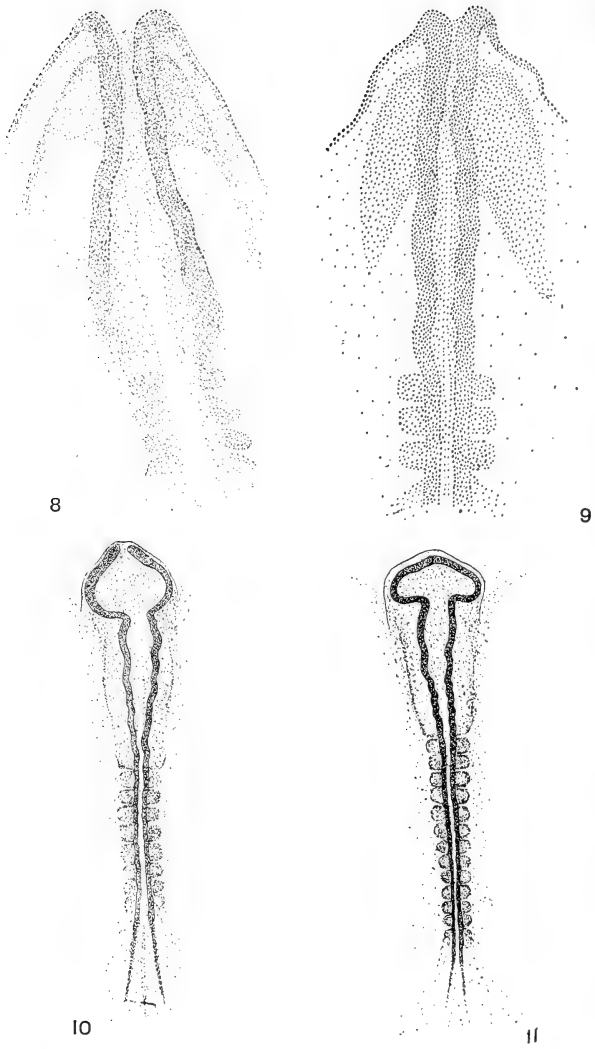
bottom of the watch-glass. The neural tube was swept clean of all surrounding tissues by the use of dissecting needles and fine brushes, the sides of the neural tube were dissected and also sections were cut of the roof and floor. In order to get shadows, specimens were tilted and rotated to secure all angles and degrees of illumination. Kleinenberg's, Bouin's, and Gilson's killing fluids were used, and some of the embryos were examined after faintly staining them with borax carmine and Conklin's picro-haematoxylin. To supplement the study of whole and dissected specimens, sections were cut in transverse, frontal, and sagittal planes and stained with Ehrlich's acid haematoxylin and Heidenhain's iron haematoxylin.

No indication of anything that could be interpreted as segmentation could be observed in the primitive streak, or before the neural folds were clearly outlined as elevated ridges. At this stage along the elevated margins of the medullary plate certain lobulated irregularities are formed, giving a beaded appearance to the crest, and these structures are present with more or less uniformity in most embryos up to the closure of the neural tube. They are, however, irregular in number in different embryos and do not correspond in the two sides of the same individual; their limits are often so obscure that they cannot be determined with certainty, and the wide variation in their relative position makes it impossible to correlate them, either in the two sides of the same embryo or in different embryos. They differ greatly in size; often in the same embryo there are two or three on one side while the corresponding region of the opposite side will consist of a single lobe, or perhaps the edge will be smooth, showing no lobulation. The variation in size, together with the uncertain position and desultory arrangement suggest strongly that this marginal lobulation is due entirely to differences in rate of cell proliferation at different points along the rapidly expanding wall of tissue.

As the neural crests increase in size, they rise rapidly and begin to fold over toward the median plane. At this stage especial care was exercised to detect any indication of segmentation in the medullary folds or in the plate between the folds.

Occasional faint lines could be distinguished, but their position and appearance were so irregular and variable that no segmental importance could be attached to them.

Segmentation of the neural folds was reported by Hill ('00), who described the marginal segments as separated by constrictions that "in early stages completely encircle the encephalon, and in later stages are confined to its base and lateral walls." In the examination of large numbers of embryos, I have found on the external surface of the neural folds faint constrictions that appear as lines when the best shadow effects are obtained, but I fail to find the regularity described and figured by Hill. On the contrary, the grooves are irregular in number and position, and often a single groove will divide to form two. The grooves do not regularly encircle the encephalon, but a groove will often fade out at some point and slightly anterior or posterior to it another groove will appear, so that the number of constrictions is different for the two sides. These constrictions are so faint that they can be traced only with difficulty, and in no specimen approximate the condition shown in Hill's figures. Furthermore, dissection shows that internal grooves do not regularly correspond with external constrictions. I find external grooves are present at these stages with no corresponding internal constrictions and internal grooves with no corresponding external constrictions. Later in ontogeny Hill says the internal grooves are elevated upon the apices of internal ridges, but the groove at the apex of the internal ridge is not present with sufficient constancy to be of value in determining the constrictions that are of segmental importance. That there are grooves in addition to those considered by Hill to be segmental, he admits when he says, page 423, "secondary divisions that frequently are present would eventually be confused with the primary ones." But he gives no criteria by which to distinguish between primary and secondary constrictions, and in his figures certain ones are exaggerated as 'primary,' while others are suppressed as 'secondary.' He states that in these early stages the only criteria by which he determined segments are the external and corresponding internal grooves, and that he considers the internal ridge as a secondary



Figs. 8 to 11 and 15 to 20, camera-lucida drawings of chick embryos, showing successive stages of development, the so-called primary neuromeres and the neuromeres of later stages.

Figs. 8 and 9 Embryos of three somites, dorsal view, drawn from living specimens, showing the early beaded appearance of the neural crests.

Figs. 10 and 11 Embryos of eight and thirteen somites, respectively, dorsal view, drawn from living specimens, showing the brain vesicles and later neuromeres.

modification. Observation of a large number of embryos affords no evidence to support the statement of Hill that "eleven constrictions are present on both inner and outer surfaces of the open neural groove, that they are constant in number and nearly equal in size and that they appear earlier in ontogeny than the historic encephalic divisions, forebrain, midbrain, and

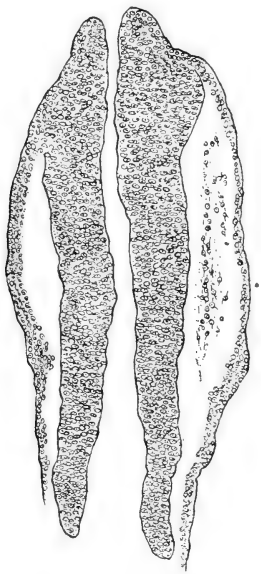


Fig. 15 Frontal section through the neural crests of an embryo of three somites, showing the irregular lobulations of the crests, absence of corresponding external and internal grooves, and indefinite cell arrangement.

hindbrain." Hill reported that the third and fifth grooves are deeper than the others and mark the posterior limits of the forebrain and midbrain. While it is true that with the appearance of the so-called secondary division of the neural tube into forebrain, midbrain, and hindbrain, the limits that separate them are clearly marked, the present study fails to confirm his statement

"that an earlier segmentation is incorporated into this division as follows: in the forebrain three primary somites; in the midbrain two; and in the hindbrain, six or six and one half if the portion of segment twelve that lies in front of the first somite is added to the latter."

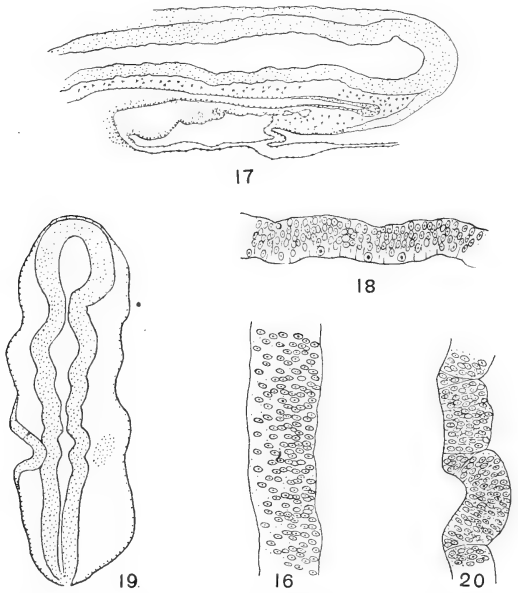


Fig. 16 Frontal section through the right neural crest of an embryo of five somites, showing same features as figure 15.

Fig. 17 Sagittal section through the closing neural tube of an embryo of ten somites, showing same features as figure 15.

Fig. 18 Sagittal section of the floor of medullary tube of an embryo of five somites, showing same features as figure 15.

Fig. 19 Frontal section through the developing brain vesicles, showing the later neuromeres.

Fig. 20 Same section as figure 19, showing cell arrangement in the right wall of the hind brain just anterior to the otic invagination.

As the neural crests approach each other, the primary brain vesicles and neuromeres of the hindbrain are well defined, and fusion of the folds first occurs in the region of the midbrain vesicle or slightly posterior to it. After the closure of the neural tube there are clearly six segments anterior to the auditory invagination. These brain vesicles and neuromeres of the hindbrain are so well-known that further description is unnecessary. In these divisions there is present the definite, characteristic cell arrangement designated by Orr as distinguishing true neuromeres. In the open neural groove there is no arrangement of cells in the medullary ridges or floor that even suggests a segmental condition. The cells are evenly distributed and do not manifest any tendency toward groupings that would give morphological significance to the grooves which serve as the basis of the primary neuromerism of Locy and Hill.

DISCUSSION

A survey of the literature on the subject of head segmentation shows most unusual differences, both in observation and interpretation. These observations are based on the study of different morphological features, and it has been impossible satisfactorily to explain the discrepancies and differences reported. In the study of neuromerism in urodeles, Kupffer, Froriep, Eycleshymer, Neal, Locy, Griggs, Smith, and others have described as many as eleven and as few as three segments in the cephalic region. Locy considered the divisions of the neural crests as segmental, and did not regard the divisions of the cephalic plate as of metameric importance. Kupffer, Froriep, Eycleshymer, Griggs, Smith, and others have agreed that in any consideration of neuromeric segmentation, the divisions of the medullary plate are of primary importance, but these authors have not agreed as to their number or metameric significance, and most regard them as due to the segmentation of the mesoderm. Locy pointed out that the median divisions do not correspond with those in the neural ridges; he reports four or five divisions in the median plate and ten or eleven segments in the neural ridges of the same region. He says, page 530,

"Whether we find the median plate smooth in Amblystoma or faintly segmented depends on the stage at which the examination is made, and we recognize that the appearances in any one egg are not constant throughout the open groove stage; further, that eggs of closely related animals are by no means necessarily similar at corresponding stages." According to Griggs, the beaded appearance of the neural crests was not apparent in any of the embryos of this stage examined by him, and he argues for the metameric significance of the median divisions. Locy and Griggs both attempt to establish neuromerism as a basis for determining the segmentation of the head, but their results are mutually exclusive and contradictory.

Locy's statement that primary neuromeres are visible in the blastoderm of the chick at the twelfth hour of incubation, just as the head fold is first outlined, and that they extend into the primitive streak, finds absolutely no support in any of the material of the present investigation. His further statement that "the cells in these segments are characteristically arranged, even in the earliest stages, and their arrangement and structure would indicate that they are definite differentiations of cell areas" was denied by Hill, and in the present study evidence to support this statement of Locy is also entirely wanting. Neal ('98) called attention to the fact that "none of the reproductions of Locy's photographs, with two possible exceptions, show a segmentation of the neural folds in either the trunk or embryonic rim." He might well have added that none show neuromeres of both sides and that the same embryo was not photographed twice, in two different positions (which probably would be necessary) that the neuromeres of the two sides might be compared. In fact, Locy's photographs, in my opinion, deny rather than confirm his statement. He admits that his drawings "are a little too distinct" and "the exactness has been exaggerated." Hill's figures of the chick have called forth exclamations of surprise and astonishment on all sides. He figures constrictions of his primary neuromerism persisting in embryos with a closed neural groove, but I have been unable to observe such a condition. It is a significant fact that the cell arrangement of the

forebrain and midbrain at this stage does not show such segmentation. In the primary neuromeres, he admitted that the segmental arrangement of cells is absent, and argued that the radial cytological condition which later appears is due to the intrasegmental expansion of the walls. Thus the definite structure of the later neuromeres he attempted to explain on purely mechanical grounds. If, however, these definite and constant structural features be merely mechanical effects, one wonders how he can hope to substantiate the transitory, indefinite, and irregular beadings of the early stages as a primary neuromerism of phylogenetic importance.

Study of the neuromeres of the later stages has also led to great diversity of opinion. The neuromeres of the medulla are definite structures with characteristic morphological features. It is an open question whether or not they are homologous with the divisions of the neural tube anterior and posterior to them. The divisions of the spinal cord are undoubtedly formed by the pressure of the adjacent mesodermic somites, and anterior to the otic invagination the number and character of the somites are far from established. It is in the anterior part of the neural canal that the evidence is most scanty and indefinite. Neal ('18) argues that the primary brain vesicles are the true neuromeres of the region, and in the opinion of the writer his argument is clear and comprehensive. If the central nervous system of the primitive vertebrate were segmented, with the enlargement of the brain there would be an enlargement of the segments. The brain segments enlarge laterally and dorsoventrally, and it seems only natural that they should enlarge anteroposteriorly. It certainly is as reasonable to suppose that individual segments would expand anteroposteriorly as to account for the elongation of the brain by fusion of segments and the backward migration of the cephalic region with the concomitant incorporation of additional segments in the brain. While gill clefts, visceral arches, and epibranchial organs are cenogenetic, still they may be segmental, being predetermined in position by nerves, blood vessels, septa, and other segmental structures of the invertebrate. The later brain is highly developed, with great specialization of parts,

and ontogeny affords such fragmentary and inconclusive evidence of phylogeny that neuromerism alone can hardly explain the development of the head. The study of highly specialized forms like the chick must appear of less importance than that of more primitive forms, or at least of forms in which primitive conditions persist. In this connection, Neal ('18) has pointed out that neuromeres are more conspicuous in the embryos of higher, than they are in embryos of lower chordates, and this would hardly be expected if they are vestiges of a primitive neuromerism.

In ontogeny, segmentation regularly appears first in the mesoderm and the segmentation of the mesoderm is more constant and regular than segmentation in other tissue. Segmentation of other tissue normally results from and is in correspondence with segmentation of the mesoderm. Mesomeres are uniformly present in the lower chordates, and to disregard mesodermal segmentation is therefore to overlook an item of paramount importance in any study of head segmentation. In the ancestral vertebrate there was undoubtedly a correspondence of mesomeres, neuromeres, cranial nerves, and branchial organs, and all of these structures must be considered in an explanation of the present lack of correspondence.

The present study has shown that in Amblystoma and the chick at least, the structures described by Locy and Hill as primary segments cannot be regarded as metameric. Investigators have repeatedly questioned the accuracy of the observations of Locy and Hill, and a repetition of their work, using as far as possible identical means of examination, has in the present case not only failed to verify their observations, but disclosed a quite different condition. The three morphological features upon which neuromerism can be based, marginal beadings, external and internal grooves, and cell arrangement, all fail to give evidence to confirm the primary neuromerism of Locy and Hill. Neal could not confirm Locy's statements concerning Selachian embryos and I have been unable to confirm Locy's observations on Amblystoma or Hill's on chick embryos. In my opinion, the so-called 'primary metamerism' is based upon incorrect observation and

cannot be accepted. The median divisions observed in the neural plate of Amblystoma are largely if not entirely due to segmentation of the mesoderm, and so can be regarded only as features of secondary importance. The primary neuromeres of Locy and Hill, as well as those of Griggs and other students of neuromerism, are irregular in size, inconstant in number, asymmetrical in position, and cannot serve as trustworthy criteria of the metamerism of the vertebrate head.

BIBLIOGRAPHY

Von Baer, K. E. 1828 Ueber Entwicklungsgeschichte der Tiere. Königsberg.

Balfour, F. M. 1878 A monograph on the development of elasmobranch fishes. London.

Belogolowy, J. 1908 Zur Entwickelung der Kopfnerven der Vögel. Ein Beitrag zur Morphologie des Nervensystems der Wirbeltiere. Bull. Soc. Imp. Nat. Moscow, Hft. 3 und 4.

1910 German reprint, Moscow.

Beraneck, E. 1884 Recherches sur le développement des nerfs craniens chez les lizards. Rec. Zool. Suisse, T. 1.

Dohrn, A. 1875 Der Ursprung der Wirbelthiere und das Princip des Functionswechsels. Genealogische Skizzen. Leipzig.

Eycleshymer, A. C. 1895 The early development of Amblystoma, with observations on some other vertebrates. Jour. Morph., vol. 10.

Filatoff, D. 1907 Die Metamerie des Kopfes von Emys lutaria. Zur Frage über die korrelative Entwicklung. Morph. Jahrb., Bd. 38.

Froriep, A. 1891 Zur Entwickelungsgeschichte der Kopfnerven. Verh. Anat. Gesell. München.

1892 Zur Frage der sogenannten Neuromerie. Verh. Anat. Gesell. Wien.

Gegenbaur, C. 1887 Die Metamerie des Kopfes und die Wirbeltheorie des Kopfskelettes. Morph. Jahrb., Bd. 13.

Graper, L. 1913 Die Rhombomeren und ihre Nervenberichtungen. Arch. mikr. Anat., Bd. 83.

Griggs, L. 1910 Early stages in the development of the central nervous system of Amblystoma punctatum. Jour. Morph., vol. 21.

Hill, C. 1900 Developmental history of the primary segments of the vertebrate head. Zool. Jahrb., Abt. f. Anat. u. Ont., Bd. 13.

Huxley, T. H. 1858 The Croonian lecture: On the theory of the vertebrate skull. Proc. Roy. Soc. London., vol. 9.

Johnston, J. B. 1905 The morphology of the vertebrate head from the viewpoint of the functional divisions of the central nervous system. Jour. Comp. Neur., vol. 15.

von Kupffer, C. 1885 Primäre Metamerie des Neuralrohrs der Vertebraten. Sitzungsber. math. physik. Kl. München.

1906 Die Morphogenie des Centralnervensystems. Handbuch vergl. u. exp. Entwick. Wirbeltiere. Jena.

Locy, W. A. 1895 Contribution to the structure and development of the vertebrate head. Jour. Morph., vol. 11.

McClure, C. F. W. 1890 The segmentation of the primitive vertebrate brain. Jour. Morph., vol. 4.

Neal, H. V. 1898 The segmentation of the nervous system in Squalus acanthias. A contribution to the morphology of the vertebrate head. Bull. Mus. Comp. Zool. Harvard, vol. 31.

1914 The morphology of the eye muscle nerves. Jour. Morph., vol. 25.

1918 Neuromeres and metameres. Jour. Morph., vol. 31.

Orr, H. B. 1887 Contribution to the embryology of the lizard. Jour. Morph., vol. 1.

Smith, B. G. 1912 The embryology of Cryptobranchus. Jour. Morph., vol. 23.

Smith, P. E. 1914 Some features in the development of the central nervous system of Desmognathus. Jour. Morph., vol. 25.

Van Wijhe, J. W. 1882 Uber die Mesodermsegmente und die Entwickelung der Nerven der Selachierkopfes. Naturk. Verh. K. Akad. Wiss., Amsterdam.

1886 Ueber Somiten und Nerven im Kopfe von Vögel und Reptilienembryonen. Zool. Anz., Bd. 9.

1889 Die Kopfregion der Kranioten beim Amphioxus, nebst Bemerkungen über die Wirbeltheorie des Schädels. Anat. Anz., Bd. 4.

Waters, B. H. 1892 Primitive segmentation of the vertebrate brain. Quart. Jour. Micr. Sci., vol. 33.

Wilson, J. T., and Hill, J. P. 1907 On the development of Ornithorhynchus. Phil. Trans. Roy. Soc., London, vol. 199.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MARCH 27

THE ORIGIN OF BILATERAL SYMMETRY IN THE EMBRYO OF CRYPTOBRANCHUS ALLEGHENIENSIS

BERTRAM G. SMITH

Department of Anatomy, New York University and Bellevue Hospital Medical College

THIRTY-THREE FIGURES

CONTENTS

ANALYSIS OF THE PROBLEM

In the higher animals, the most obvious features of organization are those expressed by the terms bilateral symmetry, anteroposterior differentiation, and dorsoventral differentiation. Since bilateral symmetry is always accompanied by the other features mentioned, the expression bilaterality is alone sufficient to designate the type of structure under consideration.

Bilaterality is a feature of such fundamental importance in the organization of the embryo that we should expect it to appear very early in the ontogeny, and the problem of tracing its origin is one of considerable interest in relation to theories of heredity and development. In particular one desires to know the earliest manner of expression of the definitive bilateral symmetry of the embryo, and whether the hereditary factors that are undoubtedly at work can be modified by external influences.

In a previous communication (Smith, '12, II) it has been shown that the polarity of the late ovarian egg of Cryptobranchus allegheniensis, as expressed by its telolecithal character, establishes approximately the principal axis of the embryo: the anterior end forms about 40° from the animal pole, the posterior end quite accurately at the vegetal pole. But a careful study of the ovogenesis recorded in another paper (Smith, '12, I) has not revealed any feature of the ovarian egg that enables us to distinguish right and left, dorsal and ventral surfaces; in the mature but unfertilized egg the condition is one of radial symmetry and axial differentiation, with no trace of bilateral symmetry. Subsequent observations, made by examining in toto preparations of the ovarian eggs cleared in various oils, have not altered this conclusion. Even the polarity of the egg is not visibly expressed

in its organization from the beginning, but arises during ovogenesis.

In the gastrula stage, the bilateral symmetry of the embryo is expressed in a perfectly obvious manner. Our problem then requires us to look for the beginnings of bilateral symmetry in the cleavage stages, or possibly in the fertilization stage, and leads us to consider every deviation from strict radial symmetry that suggests the beginning of definitive bilateral symmetry. The following features must be considered in their relation to the position of the dorsal lip of the future blastopore, and as a check on the results it is desirable that these same features should be considered in their relation to each other:

1. The direction of the entrance-path of the spermatozoon.
2. The direction of the first cleavage furrow, which defines an axis of biradial symmetry in the cleavage pattern of the third and later cleavage stages.
3. The excentric development of the micromeres shown by the superficial cleavage pattern of the early blastula.
4. The excentric development manifested by the internal structure of the early blastula.
5. The bilateral symmetry of the superficial cleavage pattern in the lower hemisphere of the late blastula.
6. The bilateral symmetry manifested by the internal structure of the late blastula.

One desires to know the nature of the factors at work in producing bilateral symmetry or determining the direction of the median plane, when the egg is developing in its natural environment. The egg of Cryptobranchus is fertilized immediately after spawning. Under the influences of light and gravity, streaming movements may be induced in the cytoplasm of the fertilized but unsegmented egg, which modify the results due to the operation of internal factors; in the egg of the frog such complications have been observed by various investigators. Another circumstance that must be taken into account is the well-known fact that in the amphibian egg the direction of the early cleavage furrows may be changed by mechanical pressure.

In nature, the developing egg of Cryptobranchus is shielded from the light; the factor of pressure also is negligible. In experimental procedure these factors are best dealt with by eliminating them so far as possible from the conditions of the experiment. The only external influence that appears to be normally related to the life of the egg, in such a way that it might affect bilateral symmetry, is gravity. So long as the eggs are retained in the body of the female their orientation is variable and inconstant, but since there is comparatively little axial differentiation during this period, it is not likely that even under the most favorable conditions of orientation could gravity exert any appreciable influence on the organization of the egg. If the egg is at all susceptible to the influence of gravity in determining the direction of the median plane, the most favorable conditions are presented immediately after spawning and fertilization. At this time the cytoplasm accumulates rapidly about the animal pole. The newly laid egg is closely invested by the gelatinous envelope, and does not freely orient itself with the animal pole uppermost until after sufficient water has been absorbed to cause the capsule to become turgid and spring away from the egg—a process requiring from one to two hours. During this period, in which the polar axis may make any angle with the vertical, gravity might possibly rearrange the contents of the egg in such a way as to affect the direction of the median plane of the future embryo.

EXPERIMENTS AND OBSERVATIONS

All the experiments with living material were carried out in a cellar, where the temperature was favorable for the development of the eggs and the light could be controlled. So far as possible the eggs were shielded from the light; they were handled as gently as possible to avoid the disturbing effects of mechanical manipulation.

Some of the experiments about to be described involve placing the egg in a definite position and keeping it there for a considerable period of time. Trials of various methods showed that it is sufficient and most expedient to orient each egg in water in a separate watch-glass and leave it in a situation where it will not

be disturbed. In the stages under consideration the egg or embryo is devoid of cilia; observation of landmarks furnished by artificial markings show that during cleavage the eggs do not undergo any perceptible rotation on a vertical axis. The inertia of the heavy egg and contact of its lower surface with the substratum make it easy to guard against disturbances sufficient to affect the position of the egg. In the first two or three seasons' work, the Syracuse watch-glasses used for these experiments were placed on a massive walnut table resting on a gravel foundation and against a stone wall; in later experiments, comprising the greater part of the work, they were placed on the level top of a concrete wall. To make up for the loss by evaporation, water was each day added gently by means of a pipette. The probability of error increases, of course, with the length of time involved in the experiments. Wherever possible, the results were checked by other methods.

In certain experiments the eggs were removed from their gelatinous envelopes immediately after being taken from the uterus, thereby allowing them to orient themselves, in water, at once with the animal pole uppermost. This procedure eliminates, during the fertilization period, the possible influence of gravity in determining the direction of the median plane. The eggs used in most of the experiments were taken from nests, where spawning and fertilization took place in a natural manner; hence it was necessary to test the possible influence of gravity under conditions that sometimes occur in nature.

A. The possible influence of gravity upon the direction of the median plane

For this experiment eggs taken from the uterus of a ripe female were artificially fertilized without removal from their envelopes. Twenty-one eggs were placed each in a separate watch-glass without water and oriented with their polar axes in a horizontal position and parallel to each other; the animal pole was directed away from the observer. After allowing time for the capsule to adhere to the glass, a little water was added gently by means of a pipette. In the course of one or two hours the capsules absorbed

sufficient water to allow the eggs to rotate slowly until the animal pole was uppermost. Eighteen eggs survived to the gastrula stage. Figure 1 shows the direction of the principal axis of each embryo in the advanced gastrula stage; it is evident that there is no preponderance of any particular direction. So far as it goes, this experiment indicates that gravity acting on the egg

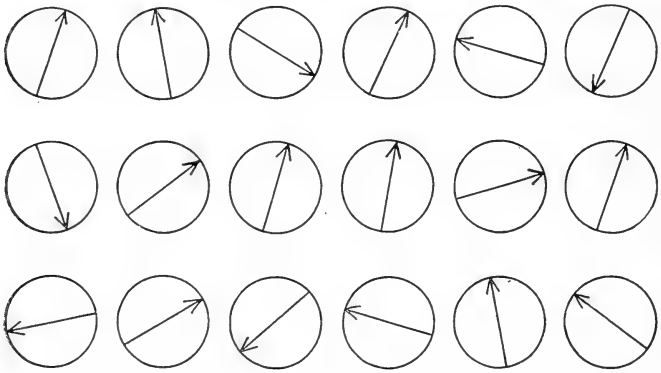


Fig. 1 Digrams showing the results of an experiment to test the possible influence of gravity in determining the direction of the median plane of the embryo of Cryptobranchus allegheniensis. The newly fertilized eggs were placed with their polar axes in a horizontal position and parallel to each other; the animal pole was directed away from the observer (i.e., toward the top of the page in the figure). After the absorption of water by the envelopes, each egg rotated slowly, in response to gravity, through 90° in such a manner as to bring the animal pole uppermost. The diagrams show the eggs in polar view at the time of gastrulation; the arrow, pointing anteriorly, indicates the direction of the median plane of the gastrula.

immediately after fertilization is not a factor of any importance in determining the direction of the median plane of the embryo.

B. The entrance-path of the spermatozoon

Various observations indicate that penetration by the spermatozoon induces profound cytological changes in the egg. In a previous paper (Smith, '12, I, figs. 7 to 12) I have described certain external phenomena consequent upon the entrance of

the spermatozoon; these changes were very clear and striking in one spawning of eggs, though less obvious in others. The internal phenomena alone are sufficient to show that the spermatozoon immediately exerts a very decided influence: a wave of condensation of the cytoplasm and finer yolk globules always marks the progress of the spermatozoon (Smith, 12, I, figs. 41 to 46). These features suggest that forces are at work comparable to those that produce the gray crescent of the frog's egg.

In order to investigate the relation of the entrance-path of the spermatozoon to other features of the developing egg, an attempt was made to control the direction of entrance of the spermatozoon, as follows: Unfertilized eggs were taken from the uterus of a ripe female, the accessory envelopes were removed and each egg immersed in water in a separate Syracuse watch-glass. Under these conditions the egg at once orients itself with the animal pole uppermost. Each egg was then fertilized with milt applied by means of a fine pipette to the right hand edge of the germinal disc or blastodisc. Great care was exercised to guard against any subsequent change in the position of the egg. The seminal fluid of Cryptobranchus is decidedly viscous and difficult to handle in small quantities, consequently the control of the direction of entrance of the spermatozoon cannot be assumed to be very accurate.

A number of circumstances combined to make the task of carrying out of this experiment decidedly troublesome and laborious. It was necessary to capture a large number of adult specimens in order to get a few females whose eggs were in precisely the right condition for fertilization. Less difficulty was experienced in obtaining males in condition for breeding, nevertheless some failures were due to the unripe or spent condition of the males. The operation of removing the egg envelopes and applying the seminal fluid requires some time, and unless the eggs are fertilized within a few minutes after their removal from the uterus, they fail to develop. Of the eggs experimented upon about 84 per cent either failed to develop or segmented in an irregular and probably abnormal manner; very few reached the gastrula stage. It is probable that this heavy loss was due in

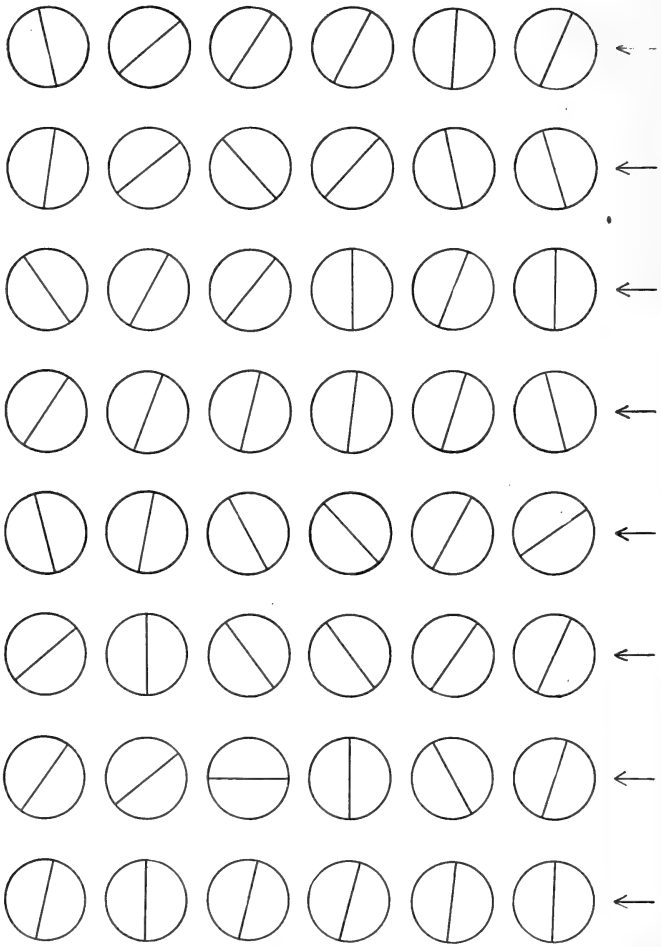


Fig. 2 Diagrams showing the direction of first cleavage in forty-eight eggs of Cryptobranchus allegheniensis in which an attempt was made to control the direction of entrance of the spermatozoon. The eggs are shown in polar view; the seminal fluid was applied to the edge of the germinal disc on the right-hand side of the egg.

large part to the removal of the envelopes, for it is comparatively easy to secure artificial fertilization and normal development when the envelopes are not removed. Excessive polyspermy, to which the eggs are peculiarly exposed after the removal of their envelopes, may account for many cases of abnormal development or failure to develop. In order to control the direction of fertilization it is necessary to apply the seminal fluid at some distance from the center of the blastodisc; no doubt it often happened that the spermatozoon entered the egg too far from the animal pole, and was unable to penetrate the yolk in order to reach the egg-nucleus. In order to obtain results from a sufficient number of eggs, the work was carried on each breeding season for five years. Over three hundred eggs were subjected to this experiment; the number does not include eggs that were rejected at once because of obvious inaccuracy in the control of the direction of application of the spermatozoa.

1. Relation to the plane of first cleavage. Forty-eight eggs segmented in a normal manner; in these eggs the direction of first cleavage with reference to the probable direction of entrance of the effective spermatozoon is shown in figure 2. It is evident that there is a decided tendency for the first cleavage furrow to come in approximately at right angles to the entrance-path of the spermatozoon.

2. Relation to the median plane of the gastrula. Sixteen eggs survived to form normal gastrulae. In these eggs the relation of the median plane of the gastrula to the probable direction of the entrance-path of the spermatozoon is shown in figure 3. The results indicate that there is no uniformity in the relation between the entrance-point of the spermatozoon and the plane of bilateral symmetry of the gastrula.

In interpreting the data, a slight complication arises from the fact that these eggs which survived to the gastrula stage included nearly all those exceptional cases in which the first cleavage furrow departed from the general rule of forming approximately at right angles to the direction of application of the seminal fluid. As an aid in studying this aspect of the situation, the direction of first cleavage in each egg is indicated in the diagram. If

we assume that the control of the direction of entrance of the spermatozoon was faulty and that the direction of first cleavage affords an index to the real direction of fertilization, we still find

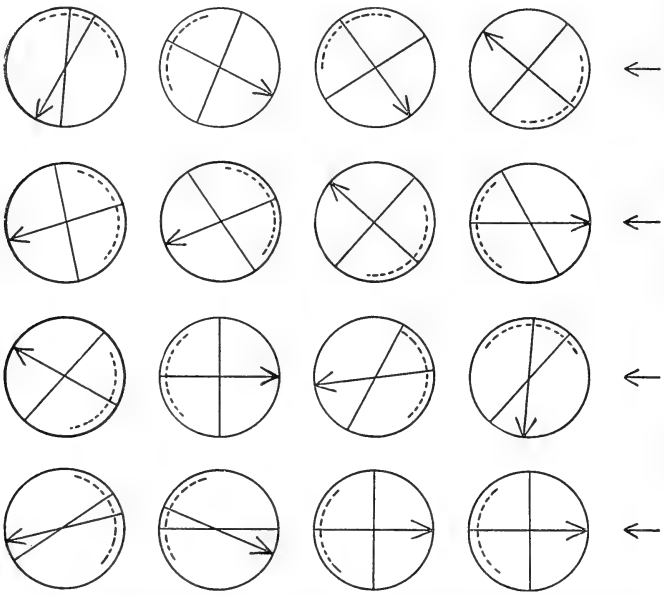


Fig. 3 Diagrams showing the direction of the median plane of the gastrula in sixteen eggs of Cryptobranchus allegheniensis in which an attempt was made to control the direction of entrance of the spermatozoon; the direction of first cleavage, also, is indicated. The eggs are shown in polar view; the seminal fluid was applied to the edge of the germinal disc on the right-hand side of the egg. In each egg an arrow, pointing anteriorly, shows the direction of the median plane of the gastrula; the position of the blastopore is indicated by a broken curved line.

that there is no uniform relation between the entrance-path of the spermatozoon and the median plane of the gastrula.

Indirect evidence in support of this conclusion is furnished by the results set forth in the next section. For it will be shown that

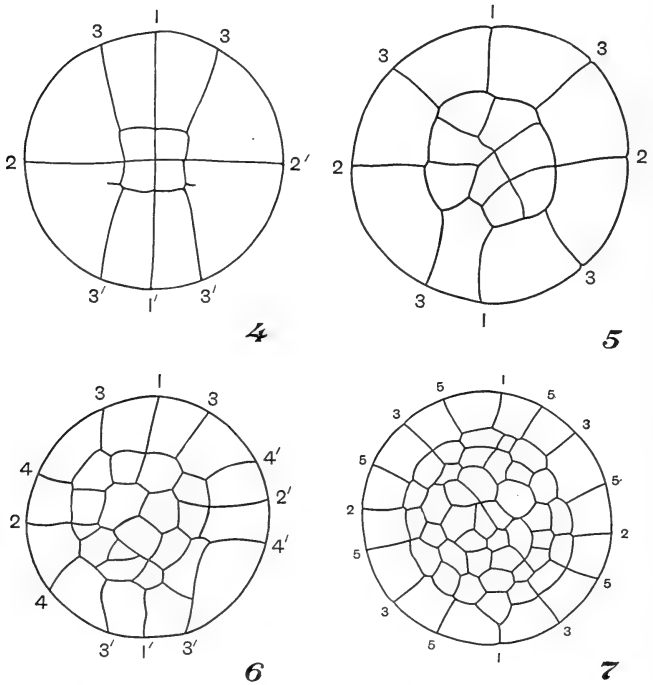
there is no fixed relation between the direction of the first cleavage furrow and the median plane of the gastrula. Since a fairly definite relation has been established between the direction of first cleavage and the direction in which the spermatozoon enters the egg, it follows that there is no fixed relation between the entrance-path of the spermatozoon and the median plane of the gastrula.

C. The relation of the first cleavage furrow to the median plane of the gastrula

Does the first cleavage furrow separate the material for right and left halves, or for anterior and posterior ends of the embryo? Beginning with the third cleavage stage, biradial symmetry is a conspicuous feature of the superficial cleavage pattern, and in the lower hemisphere serves as a ready means for identifying the early cleavage furrows up to the gastrula stage (figs. 4 to 13, 26 and 27). Is the cleavage determinate, and is this biradial symmetry a mode of expression of the definitive bilateral symmetry of the embryo? Since the axis of biradiality is marked by the first cleavage furrow, in answering this question it is necessary to consider only the relation of this furrow to the median plane of the gastrula.

1. Orientation experiments. The question was first investigated by the simple device of orienting, without removing the envelopes, a large number of eggs in the first cleavage stage in such a manner that the planes of first cleavage were parallel, and leaving them in this position to develop to the gastrula stage. At the time of first cleavage the egg naturally and freely orients itself within the envelope in such fashion that the animal pole is kept uppermost. This experiment was performed on several different occasions, using in all more than a hundred eggs; nearly all survived to the gastrula stage. The results, which were recorded by means of diagrams, fail to show any tendency for either the first or the second cleavage furrow to coincide with the median plane of the gastrula. It seems unnecessary to publish the data, since the point is conclusively established by the more exact experiments that follow.

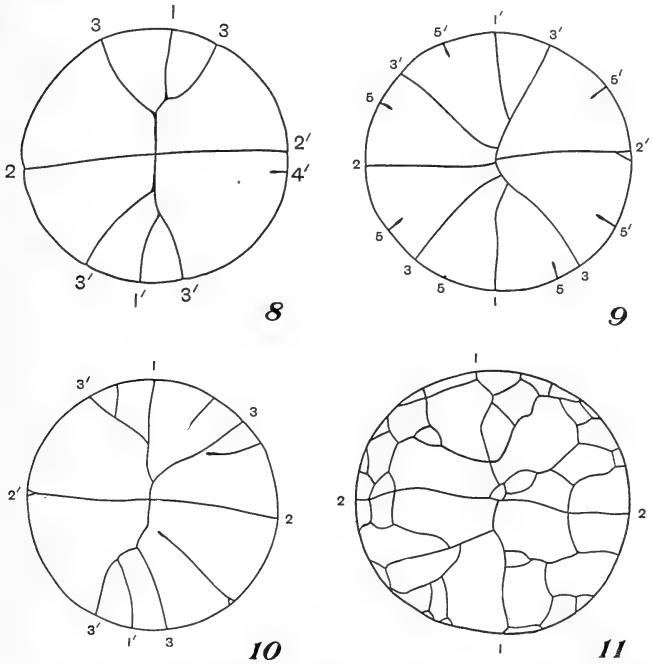
2. Constriction with a silk thread. Shortly after the appearance of the first cleavage furrow, the accessory egg envelope was removed and a silk cord tied around the egg in such a manner as



Figs. 4 to 7 Surface views of the upper hemispheres of four eggs of Cryptobranchus allegheniensis in early cleavage stages, showing the biradial character of the cleavage pattern. Figs. 4 and 5, stage 4; fig. 6, stage 5; fig. 7, stage 6. The figures were drawn with the aid of a camera lucida. ×7.

to constrict it slightly in the direction of the first cleavage furrow. The blastomeres were by no means entirely separated, and the egg developed as a whole; the device served merely to mark the direction of the first cleavage furrow. The operation is a delicate

one, and even after it had been accomplished with every appearance of success, the egg usually collapsed in a later cleavage stage. Out of a considerable number of eggs treated in this way,

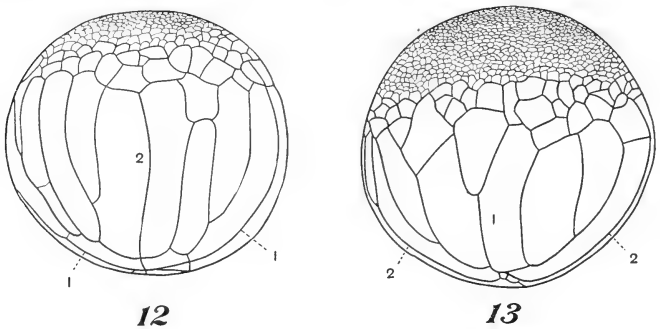


Figs 8 to 11 Surface views of the lower hemispheres of four eggs of Cryptobranchus allegheniensis, showing the biradial character of the cleavage pattern Fig. 8, stage 5; fig. 9, stage 6; fig. 10, stage 7; fig. 11, stage 9. The figures were drawn with the aid of a camera lucida. $\times 7$.

only nine lived to form gastrulae; the appearance of the lower hemispheres of these eggs in the gastrula stage is shown in figure 14. It will be observed that in two of these eggs the median plane of the gastrula coincides approximately with the plane of

first cleavage, in two others the median plane is at right angles to the plane of first cleavage, while in the five remaining eggs the median plane is oblique to the plane of first cleavage: a result that is about what we might expect if the relation between these two planes is held to be purely a matter of chance.

3. Staining experiments. A method of making permanent marks with Nile-blue sulphate on the living egg of Cryptobranchus has been described in a previous paper (Smith, '14). Since the stain is slightly toxic, certain precautions must be



Figs. 12 and 13 Equatorial views of two eggs of Cryptobranchus allegheniensis in advanced segmentation stages, showing the biradial character of the cleavage pattern in the lower hemispheres. Fig. 12, stage 8; fig 13, stage 9. The figures were drawn with the aid of a camera lucida. × 7.

observed. The egg is removed from its accessory egg envelope, but retains the chorion (the structural equivalent of the vitelline membrane of the frog's egg). The egg is then immersed in water in a Syracuse watch-glass, and a rather strong aqueous solution of the stain applied with a capillary pipette in such a manner as to make the smallest possible distinct spot. After about thirty seconds the excess of stain is removed with a pipette of larger caliber and the dish flooded with fresh water. Not more than one egg is placed in each watch-glass, and the water is changed several times during the first day, and once a day thereafter. With few exceptions, eggs so treated develop normally.

A microscopical examination of the stained substance of the egg, twenty-four hours after the application of the stain, showed that the cytoplasm, and not the yolk granules, takes the stain.

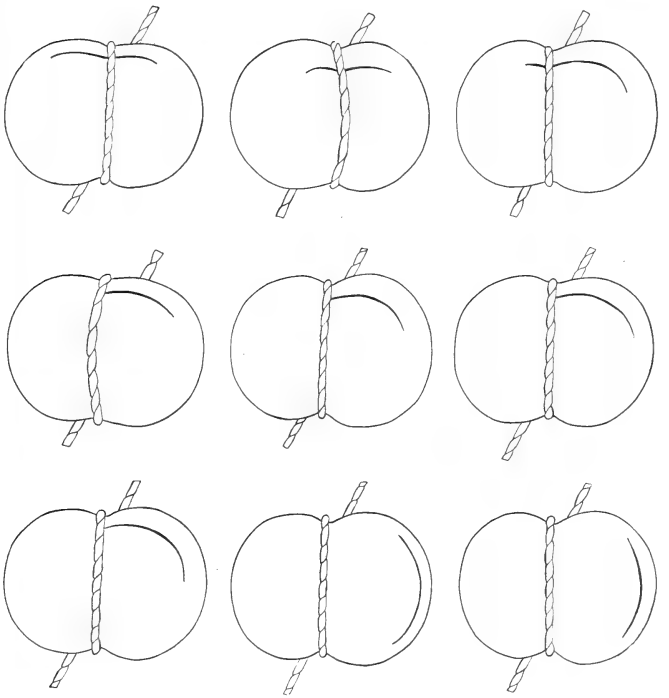


Fig. 14 Nine eggs of Cryptobranchus allegheniensis in the early gastrula stage, inverted to show the vegetal hemisphere; the plane of first cleavage has been marked by tying a silk thread around the egg. The figures were drawn with the aid of a camera lucida.

In order to mark the direction of first cleavage, eggs were taken shortly before the appearance of the second cleavage furrow; at this time the first cleavage furrow had not quite reached the equator. Two small spots were made on opposite sides of the

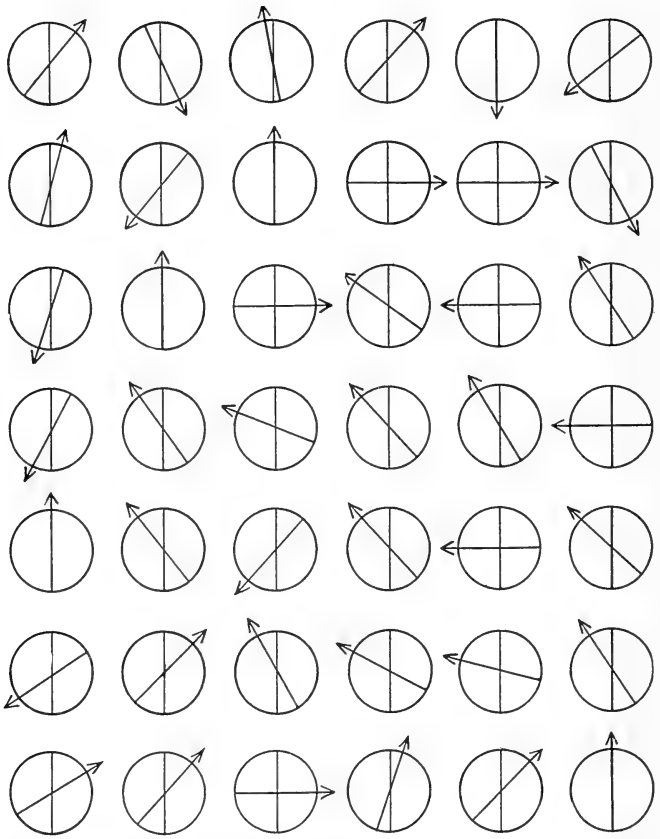


Fig. 15 Diagrams showing the relation between the direction of the first cleavage furrow and the median plane of the gastrula in forty-two eggs of Cryptobranchus allegheniensis. The vertical line indicates the direction of the first cleavage furrow, which was marked with Nile-blue sulphate; the arrow, pointing anteriorly, indicates the direction of the median plane of the gastrula.

egg where the plane of first cleavage intersects the equator; these spots remained perfectly distinct in the early gastrula stage. To determine accurately the relation between the first cleavage furrow and the median plane of the gastrula, it is necessary that the egg be examined immediately after the beginning of gastrulation; for during the progress of gastrulation one of the spots may, in certain cases, become involved more than the other in the shifting of material toward the median line, which has been described as a phenomenon of concrescence (Smith, '14). By taking observations promptly after the first appearance of the dorsal lip of the blastopore, this source of error may be avoided entirely.

Fifty-five eggs were thus treated; forty-two survived to the gastrula stage. In these forty-two eggs (fig. 15) there was no uniformity in the direction of first cleavage with respect to the median plane of the gastrula. Experience with the method here employed inspires one with so much confidence in the accuracy of the data obtained that the results of this experiment alone might be taken as a conclusive answer to the question under consideration.

4. Direct comparison. As already stated, the biradial symmetry of the cleavage pattern in the lower hemisphere enables one to identify first and second cleavage furrows in the late cleavage stages (figs. 26 and 27). It is sometimes possible, with the aid of a good dissecting microscope, to identify the first and second cleavage furrows in the region of the vegetal pole even after the appearance of the blastopore (figs. 28 and 29); this enables one to make a direct comparison between the direction of the first cleavage furrow and the median plane of the gastrula.

The first cleavage furrow was identified in the early gastrula stage in twenty-seven eggs. In six eggs the first cleavage furrow extended approximately in the median plane of the embryo, in eleven eggs it was oblique to the median plane, and in ten eggs it crossed the median plane approximately at right angles. The validity of these results depends of course on a correct identification of the first cleavage furrow. All cases that seemed doubtful were discarded, but there remains the possibility that one might

occasionally be deceived in distinguishing the first from the second cleavage furrow. One can hardly attribute to this method the degree of accuracy inherent in the two preceding methods. However, the results tend to confirm the conclusion that there is no constant relation between the direction of first cleavage and the median plane of the embryo.

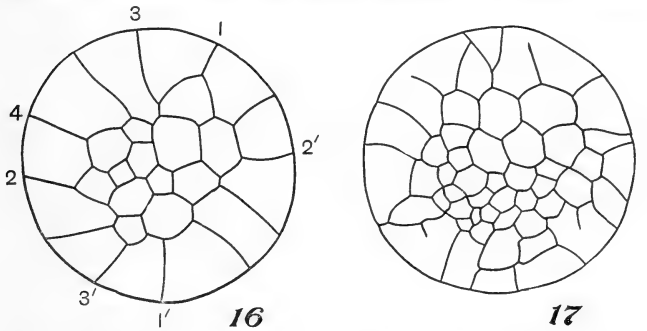
5. Instability of the micromeres. In studying the problem of orientation of the early cleavage furrows it is necessary to bear in mind the extensive shifting of the micromeres and consequent torsion of cleavage furrows that takes place from the beginning of second cleavage throughout the remaining early cleavage stages (Smith, '12, II). The portions of the early cleavage furrows that traverse the region of macromeres are relatively stable, but, on account of the instability of the micromeres during the early segmentation period, upper and lower portions of a meridional cleavage furrow may come to lie in different directions.

After keeping a living egg under constant observation during the early cleavage stages and sketching the cleavage pattern at frequent intervals, it is possible upon comparing these sketches to trace the first two cleavage furrows through the region of micromeres up to the sixth and sometimes the seventh generation of blastomeres. This has been done in a number of instances, and it has been found in every case that the path of a given cleavage furrow becomes very irregular. The most extensive shifting occurs during the early stages, beginning with second cleavage; later, as the micromeres become smaller, the distances involved in these movements are not so great.

D. The excentricity in the superficial cleavage pattern of the early blastula

Since the study of conditions arising during cleavage necessitates a rather precise designation of stages, we shall have occasion to refer to these stages by the serial numbers adopted in an earlier paper (Smith, '12, II) devoted to the external development; the entire segmentation period is divided into ten stages.

At first, cell division proceeds most actively at the animal pole; consequently we may regard this pole as the center of a primary area of cellular activity. In stages 5 and 6 (illustrated by figs. 16 and 17 of the present paper), also in the stages that immediately follow, the frequent occurrence of a secondary area of accelerated cell division has been noted (Smith, '12, II). On opposite sides of the circular area occupied by the micromeres, these cells are unequal in size and number; the smaller micromeres give evidence of more recent division, since the cleavage



Figs. 16 and 17 Surface views of the upper hemispheres of two eggs of Cryptobranchus allegheniensis in early blastula stages (stages 5 and 6, respectively), showing excentric development of the micromeres. The figures were drawn with the aid of a camera lucida. × 7.

furrows that bound them are superficially deeper and more open, as is always the case with newly formed furrows. In other words, the region of most active cell division is no longer confined to the vicinity of the animal pole, but extends from that pole a short distance along a meridian. A line drawn from the center of the secondary area of accelerated cell division through the animal pole defines an axis of excentricity in the superficial cleavage pattern of the micromeres.

The condition is really one of bilateral symmetry, but to avoid unwarranted implications I have tried to describe it without using this term. The question naturally arises whether this excen-

tricity in the superficial cleavage pattern of the early blastula is an expression of the definitive bilateral symmetry of the embryo. As we shall see, a somewhat similar condition exists in the lower hemisphere of the late blastula (figs. 26 and 27), and this is undoubtedly an expression of the definitive bilateral symmetry; but between these two stages, early and late, respectively, there intervenes a period in which it is more often impossible to detect any deviation from strict radial symmetry in the superficial cleavage pattern. Consequently, we should not assume that there is genetic continuity between these two similar phases that occur at widely separated stages; they require separate investigation. In this section we shall consider only the problematical bilaterality of the early blastula.

1. Relation to the median plane of the gastrula. *a.* Orientation experiments. Eighty-three eggs showing excentricity in the cleavage pattern of the early blastula (stages 5 to 7, inclusive) were oriented, each in a separate watch-glass, without removal from their envelopes. Seventy-five eggs lived to the gastrula stage. The results, which were recorded by means of diagrams, indicate that there is no constant relation between the axis of excentricity in the superficial cleavage pattern of the early blastula and the median plane of the gastrula. This result was wholly unexpected and difficult to reconcile with the impressions gained through the study of the internal development; consequently, the subject was investigated again by another method.

b. Staining with Nile-blue sulphate. A method more accurate than the preceding is to remove the envelopes and mark the axis of excentricity by means of a vital stain. The marking was readily accomplished by applying a small drop of Nile-blue sulphate to the side of the egg on which the larger micromeres occurred. The axis of excentricity is thus defined by an imaginary line drawn from the point marked, through the animal pole. To insure accuracy in the identification of this axis, each egg was examined with a dissecting lens, and those failing to show marked excentricity were rejected. The results were recorded promptly at the very beginning of gastrulation, to avoid possible errors due to concrescence.

Seventy-seven eggs in stages 5 and 6 were marked as above described; only twenty-three survived to the gastrula stage. This heavy loss was due to the fact that the staining fluid was inadvertently made too strong. The results for the twenty-three eggs that survived are shown by the first twenty-three diagrams, occupying the upper part of the page, in figure 18.

Forty eggs in stage 7 were marked in the same manner. In this stage the roof of the blastocoele is thin and translucent in a region which, as a rule, is slightly excentric with respect to the polar axis of the egg, and lies toward the side possessing the larger micromeres. The smaller micromeres now extend further from the animal pole than do the larger micromeres. Of the eggs marked, twenty-five survived to the gastrula stage. The results are shown by the last twenty-five diagrams, occupying the lower part of the page, in figure 18.

These experiments confirm the conclusion reached by the method of orientation. Whatever the origin and significance of the excentricity in the cleavage pattern of the early blastula, it is clearly of no value in foreshadowing the direction of the future median plane of the embryo. If it is indeed causally related to the development of the definitive bilateral symmetry of the embryo, then in its incipient condition this bilaterality must be an unstable thing, subject to a shifting of one of its axes of differential cellular activity.

It would be interesting to apply the same tests to stages 8 and 9, but in these stages the excentricity in the superficial cleavage pattern is not so easily recognizable, particularly in living material.

2. Relation to the first cleavage furrow. This comparison was made by identifying the first cleavage furrow in eggs that showed marked excentricity in the superficial cleavage pattern of stages 5 to 7, inclusive, using preserved material. In these stages the first cleavage furrow in the region of macromeres can be identified with certainty. The results for twenty-three eggs may be ummarized as follows: in nine eggs the axis of excentricity coincides approximately with the plane of first cleavage; in seven eggs the axis of excentricity is oblique to the plane of first cleavage

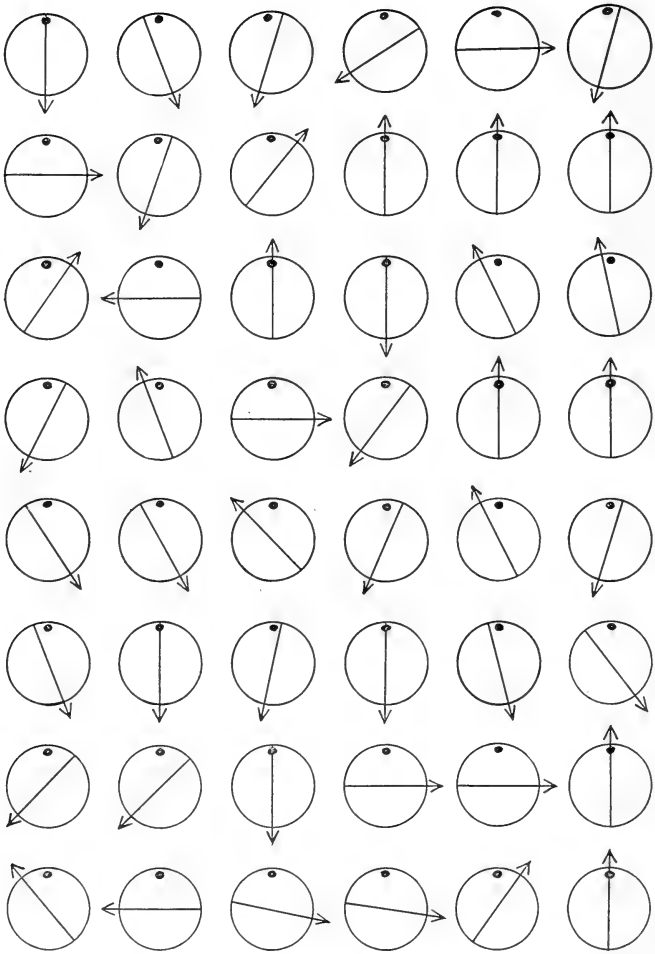


Fig. 18 Diagrams showing the relation between, *a*) the axis of excentricity in the superficial cleavage pattern of the micromeres of the early blastula and, *b*) the median plane of the gastrula in forty-eight eggs of Cryptobranchus allegheniensis. Each diagram represents the upper hemisphere of an egg; the small round spot on the side toward the top of the page indicates a mark made with Nile-blue sulphate on the side of the egg possessing the larger micromeres; the arrow, pointing anteriorly, indicates the direction of the median plane of the gastrula.

and in the seven remaining eggs the axis of excentricity is approximately at right angles to the first cleavage furrow. Evidently there is no constant relation between the two features considered.

3. Relation to the entrance-path of the spermatozoon. In the experiments already described in which an attempt was made to control the direction of entrance of the spermatozoon, excentricity in the cleavage pattern of the early blastula was observed in fifteen eggs. No doubt a much larger number of cases of excentricity would have been found had all the eggs been kept under continuous observation. In these fifteen eggs (fig. 19) there was an evident lack of uniformity in the direction of the axis of excentricity with respect to the probable direction of the entrance-path of the spermatozoon.

E. The excentricity manifested by the internal structure of the early blastula

In order to study the internal development of Cryptobranchus during the cleavage stages, entire eggs were embedded in paraffin and cut into vertical serial sections (i.e., sections taken in planes parallel to the polar axis of the egg). Of these sections the ones passing approximately through the center of the egg are designated as meridional. In a large number of cases the eggs were not oriented with reference to any structural features other than polarity. In examining sections of such eggs in stages 5 to 9, inclusive (early blastula to very late blastula), the writer was quickly impressed with the fact that the internal development of the micromeres is excentric with respect to the animal pole. On one side of the axis of polarity the micromeres composing the roof, and especially the lateral wall, of the blastocoele are smaller and more numerous, richer in cytoplasm, poorer in yolk, and in the later stages usually extend a little farther from the animal pole than on the opposite side. In favorable cases this inequality is revealed by a single meridional section (figs. 20 to 25); in other cases it is less readily recognizable through a mental reconstruction of the entire series.

The impression gained through the study of sections is that this differentiation is of the same general character throughout the entire blastula stage, having its beginning in stages 5 and 6, progressing rapidly in stages 7, 8, and 9, and continuing in somewhat different form through stage 10 (very late blastula). The condition is one of bilateral symmetry, and its development ap-

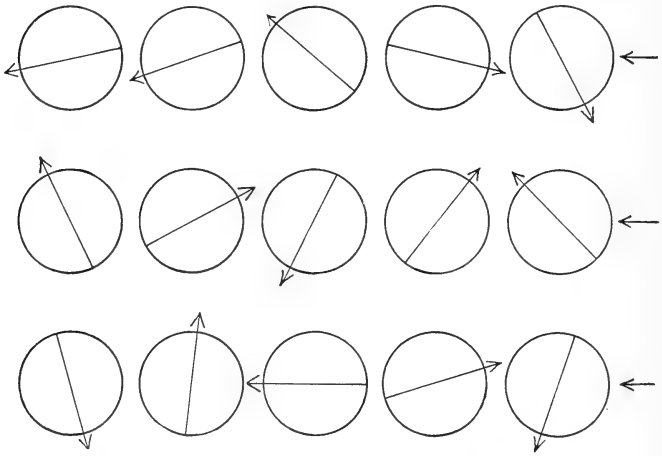
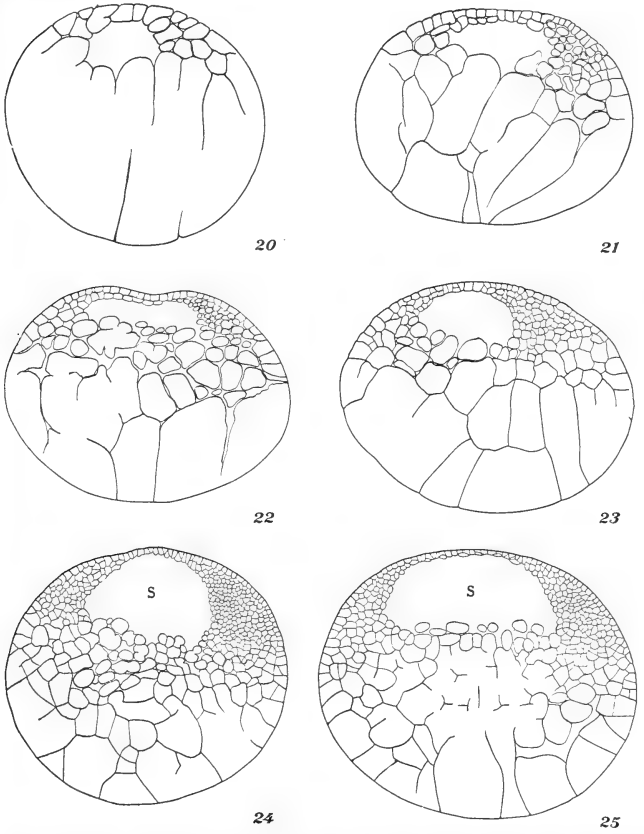


Fig. 19 Diagrams showing the direction of the axis of excentricity in the superficial cleavage pattern of the upper hemisphere of the early blastula in fifteen eggs of Cryptobranchus allegheniensis fertilized by seminal fluid applied to the edge of the germinal disc on the side indicated by the arrows on the right-hand margin of the figure. The arrow drawn through each circle indicates the axis of excentricity; the head of the arrow is placed on the side occupied by the larger and less numerous micromeres.

pears to be fundamentally a single continuous process. That the direction of differentiation of the micromeres, with reference to the positions of the more stable macromeres, is unchanged during this long period of development is of course not to be assumed without proof. In the early stages, before the excentric differentiation is well established, it seems likely to be subject to changes in direction; in the later stages it assumes an aspect of

greater stability. We shall here consider the relation of the excentric internal structure of the early blastula to some other features of organization.



Figs. 20 to 25 Meridional sections taken in the median plane of bilateral symmetry of eggs of Cryptobranchus allegheniensis in early to late blastula stages. *S*, segmentation cavity or blastocoele. Fig. 20, stage 6; fig. 21, stage 7; figs. 22 and 23, stage 8; figs. 24 and 25, stage 9. The figures were drawn with the aid of a camera lucida. × 8.

1. Relation to the excentricity in the superficial cleavage pattern. It is a very natural supposition that the excentricity in the superficial cleavage pattern is merely the outward expression of the excentric development of the micromeres revealed by the study of the internal structure. To test this hypothesis, sixteen preserved eggs in stages 5 to 8, inclusive, were split (with a razor blade) along the axis of excentricity in the superficial cleavage pattern and the internal structure examined with a lens. In every egg but one the roof and lateral walls of the blastocoele showed excentric development of the micromeres after the fashion previously observed in serial sections, and in twelve eggs the median plane of excentricity in the internal structure coincided with the plane of splitting.

The point was further investigated by means of specially prepared serial sections. Nineteen eggs in stages 6 to 8, inclusive, showing marked excentricity in the superficial cleavage pattern, were oriented in paraffin, and sectioned in planes parallel to the axis of excentricity. In seventeen eggs the meridional sections showed approximate coincidence in the direction of excentric development of the micromeres as manifested by external and internal features, respectively, while in the two remaining eggs the meridional sections gave no evidence of excentric development.

Therefore we conclude that the excentricity manifested by the internal structure of the early blastula is definitely correlated with the excentricity in the superficial cleavage pattern; these two features are different aspects of the same thing. The experimental evidence has shown that there is no constant relation between the axis of excentricity in the superficial cleavage pattern of the early blastula (stages 5 to 7, inclusive) and the median plane of the gastrula; we must now accept the same conclusion for the excentricity in the internal structure.

Incidently, the study of sections shows why, in stages 8 and 9, the excentric development of the micromeres is not so clearly expressed by the superficial cleavage pattern as it is by the deeper structure. In these stages there occurs a rather uniform flattening of the superficial layer of micromeres, which masks the

changes within; the outer layer of cells is becoming epithelial in character.

2. Relation to the plane of first cleavage. In the following experiments the first cleavage furrow was identified in the vegetal hemisphere only.

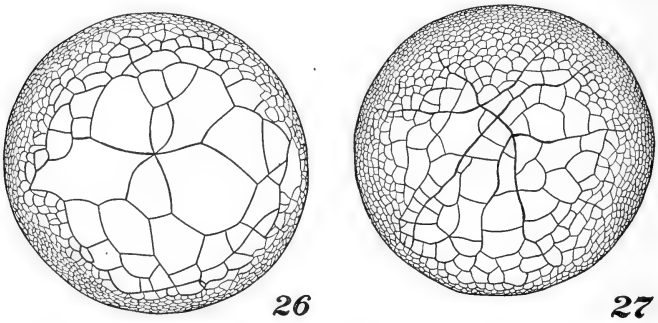
Thirteen preserved eggs in stages 5 to 8, inclusive, were split with a razor along the plane of first cleavage. In three eggs the first cleavage furrow coincided with the axis of excentricity as revealed by the internal structure, in seven eggs the first cleavage furrow was oblique to the axis of excentricity, and in the three remaining eggs the first cleavage furrow extended at right angles to it. So far as they go, these results indicate that there is no fixed relation between the plane of first cleavage and the axis of excentricity in the internal structure.

The matter was further investigated by means of serial sections. Eleven eggs in stages 5 to 8, inclusive, were cut into sections parallel to the first cleavage furrow; in nine of these eggs the meridional sections showed unequal development of the micromeres on opposite sides of the polar axis, while in the two remaining eggs the meridional sections gave no evidence of such differentiation. This result considered alone might be taken to indicate a tendency for the axis of excentricity to coincide with the plane of first cleavage; but this conclusion is nullified by the results of the preceding test, also by the one which follows: of eleven eggs in stages 5 to 8, inclusive, sectioned at right angles to the first cleavage furrow, six showed excentricity in the meridional sections, while in five the meridional sections were lacking in this feature.

F. The bilateral symmetry of the superficial cleavage pattern in the lower hemisphere of the late blastula

In the late blastula (stages 9 and 10) bilateral symmetry is usually recognizable in the cleavage pattern of the lower hemisphere. The vegetal pole, marked by the point of intersection of the first and second cleavage furrows, is excentrically situated within the area occupied by the macromeres: a more rapid multiplication of cells has occurred on one side of the egg, where

micromeres and transitional cells approach nearer the vegetal pole (figs. 26 and 27). In most eggs the transition from small to large cells is more gradual on the side where the more rapid multiplication of cells occurs; on the opposite side it is characterized by a rather abrupt line of demarcation. These features impose a phase of excentricity and bilateral symmetry upon the previously existing biradial symmetry of the cleavage pattern of the lower hemisphere. A meridian drawn through the vegetal

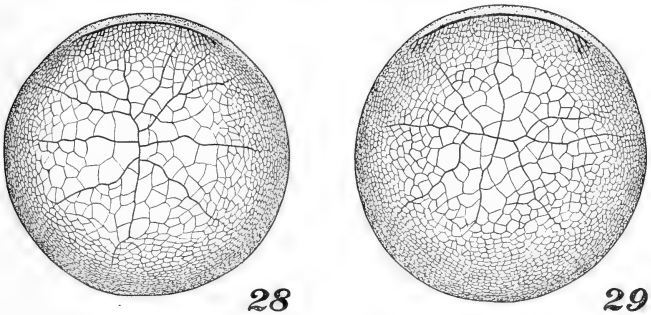


Figs. 26 and 27 Surface views of the lower hemispheres of two eggs of Cryptobranchus allegheniensis in late blastula stages (stage 10, early and late phases, respectively). In each egg the lower pole as determined by gravity lies at the center of the figure; the vegetal pole, at the intersection of the first two cleavage furrows, is slightly above this point and excentrically situated within the macromeres. The upper part of each figure represents the side on which the blastopore is to appear. The figures were drawn with the aid of a camera lucida. × 7.

pole and the center of the area occupied by the macromeres defines the axis of excentricity and bilateral symmetry in the cleavage pattern.

1. Relation to the median plane of the gastrula. In many eggs preserved in the early gastrula stage the cleavage pattern of the lower hemisphere is well-defined; it is sometimes possible to identify first and second cleavage furrows and thus to locate the vegetal pole at their intersection (figs. 28 and 29). The excentric position of the vegetal pole within the region of macromeres and the bilateral phase of the cleavage persist into the

gastrula stage. These features are usually better expressed than in the eggs shown in the figures, which were chosen because the distinctness of their early cleavage furrows enabled them to be drawn with a camera lucida. The dorsal lip of the blastopore serves as a landmark for determining the true median plane of bilateral symmetry of the gastrula, and in every case studied the axis of excentricity and bilaterality of the superficial cleavage pattern lies in this median plane; this was clearly made out in



Figs. 28 and 29 Surface views of the lower hemispheres of two eggs of Cryptobranchus allegheniensis in the early gastrula stage, showing the biradial character of the cleavage pattern which enables one to distinguish first and second cleavage furrows. In figure 28 the first cleavage furrow lies approximately in the median plane of the gastrula; in figure 29 it lies nearly at right angles to this plane. The drawings were made with the aid of a camera lucida. $\times 7$.

about a dozen eggs. We conclude, therefore, that the definitive bilateral symmetry of the embryo is indicated by the cleavage pattern of the late blastula; the earliest stage in which bilateral symmetry thus becomes unequivocally expressed is the one designated stage 9.

2. Relation to the first cleavage furrow. On account of the biradial character of the cleavage pattern of the lower hemisphere, it is often possible to distinguish the first from the second cleavage furrow in the vicinity of the vegetal pole of the late blastula. Observation of a considerable number of eggs makes it certain that the direction of the first cleavage furrow bears no constant relation to the axis of bilaterality in the cleavage pattern.

G. The bilateral symmetry manifested by the internal structure of the late blastula

When a living egg is examined in a very late blastula stage, the roof and lateral walls of the blastocoele are found to be slightly translucent. When such an egg is immersed in water and held between the observer and the source of light, it can usually be seen to possess a very definite bilateral symmetry. On one side

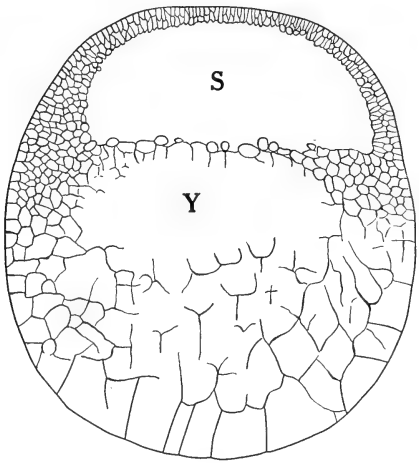


Fig. 30 Meridional section taken in the median plane of bilateral symmetry of an egg of Cryptobranchus allegheniensis in a very late blastula stage (late phase of stage 10). *S*, segmentation cavity or blastocoele; *Y*, yolk. The drawing was made with the aid of a camera lucida. × 15.

of the large blastocoele the roof is more translucent than on the opposite side and contrasts more abruptly with the opaque yolk. The condition is more clearly shown in meridional sections, but in order to observe the exact condition described, one must be careful to obtain an egg killed when it is just ready to begin gastrulation. When the plane of the section is sagittal, the contrast between the two sides is usually obvious enough (figs. 30 and 31). Not only has the roof of the blastocoele

become slightly thinner on one side, which we shall see becomes the dorsal side of the embryo, but on this side the floor of the blastocoele dips abruptly downward to form a small crevice, while on the opposite side it curves gradually upward. On the thinner side of the roof of the blastocoele, the cells are more columnar in form; on this side of the egg, below the level of the blastocoele, the micromeres usually, but not always, extend a

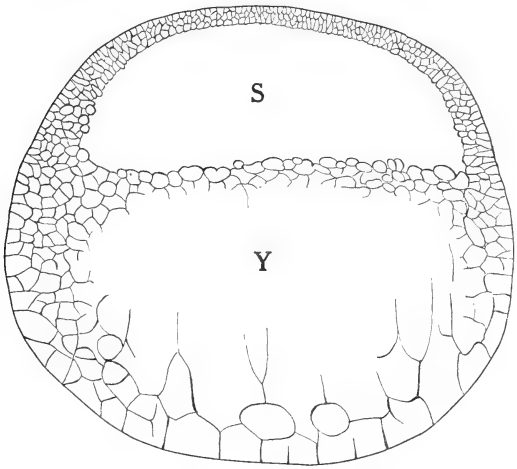


Fig. 31 Meridional section taken in the median plane of bilateral symmetry of an egg of Cryptobranchus allegheniensis in a very late blastula stage (late phase of stage 10). *S*, segmentation cavity or blastocoele; *Y*, yolk. The drawing was made with the aid of a camera lucida. × 15.

little further toward the vegetal pole. When the plane of the section is transverse, the structure revealed is symmetrical.

So far as one can judge from the study of sections, this differentiation of the very late blastula is genetically continuous with the excentric development of the micromeres in earlier stages, certainly as early as stages 8 and 9 (figs. 22 to 25). In these earlier stages the roof or lateral wall of the blastocoele is decidedly thicker on the side where the more rapid multiplication of cells

occurs; nevertheless, there are reasons for believing that this is the side that eventually becomes the thinner (dorsal) side. For in the very late blastula stage (figs. 30 and 31), the side with the thinner wall is in the more advanced stage of differentiation, as evidenced by the columnar character of its cells and the greater progress made in the extension of the cap of micromeres over the yolk cells. In the earlier stages mentioned, the depression or crevice in the floor of the blastocoele, if present at all, is located on the side of greater thickness and more advanced differentiation. Another evidence of a reversal in the relative thickness of opposite sides of the roof of the blastocoele is that in an intermediate stage (early phase of stage 10) they are equal in thickness, and can then be distinguished only by a careful study of the character of the cells and by the position of the crevice, which in this stage is a fairly pronounced and constant feature. The thinning-out of the originally thicker portion of the roof of the blastocoele is apparently accomplished in part by a migration of cells from its inner surface, away from the median plane, in part by a process of circumcrescence. Since the plates were prepared, I have sectioned additional material in which the gap between figure 25 and figure 30 is more satisfactorily bridged and the above conclusions confirmed.

1. Relation to the bilateral symmetry of the superficial cleavage pattern. When an egg in a late blastula stage is sectioned along the axis of bilaterality indicated by the superficial cleavage pattern of the lower hemisphere, this axis is found to coincide in direction with the median plane of symmetry of the internal structure. Thus the bilateral symmetry of the cleavage pattern is but the external expression of the more fundamental symmetry of the internal organization of the egg.

2. Relation to the bilateral symmetry of the early gastrula. In the living egg, it may be observed that the blastopore begins to form just below the equator, on the side where the lateral wall of the blastocoele is more translucent, but at a lower level than the floor of the blastocoele. The study of sections of the beginning gastrula shows that the bilaterality of the late blastula is carried over into the gastrula stage (figs. 32 and 33). Internally,

the first evidence that gastrulation is about to begin is the fact that the cells lying just below the crevice on the dorsal side of the egg, where the segmentation cavity dips downward, become rounded or oval in outline, preparatory to immigration or invagination.

The morphological evidence seems sufficient to connect the bilateral symmetry of the late blastula (stages 8 to 10) with the definitive bilateral symmetry of the gastrula, since it is very improbable that sudden changes in the direction of the bilateral organization of the blastula should occur after this condition is well established.

The meridian that bisects the beginning blastopore defines the median plane of bilateral symmetry of the gastrula, which ultimately becomes the sagittal plane of the embryo. As more fully described in previous contributions (Smith, '12, II; and '14), the anterior end of the embryo forms in this meridian about 40° from the animal pole, while the posterior end forms in the region where the blastopore closes, at the vegetal pole. Consequently, the dorsal side of the embryo forms mainly from materials which in the early gastrula stage lie between the beginning blastopore and a point situated some distance above it, in the thinner portion of the roof of the blastocoele. In the late blastula and early gastrula, this dorsal region coincides with the region of greatest cell activity, which is not confined to the thin portion of the roof of the blastocoele, but extends below the level of the blastocoele into the equatorial region on the side of the egg where the blastopore begins to form. This extension or shifting of the original area of excentric cellular activity is an expression of the circumcrescent or epibolic phase of gastrulation. The opposite and less active side of the egg, where the now thickest portion of the roof of the blastocoele joins the yolk cells, is ventral. Hence the differentiation which first establishes the definitive bilateral symmetry of the blastula is dorsoventral in direction, and constitutes a new embryonic axis secondary to the principal or polar axis of the egg. In other words, radial symmetry with axial differentiation gives place to bilateral symmetry as soon as a new axis of differentiation is established approximately at right angles to the first.

DISCUSSION

A. Bilateral organization previous to fertilization. In the egg of Cryptobranchus allegheniensis we find polarity arising during ovogenesis and bilaterality established only after cleavage has reached an advanced stage. The writer's observations give no support to the idea sometimes advanced that "bi-

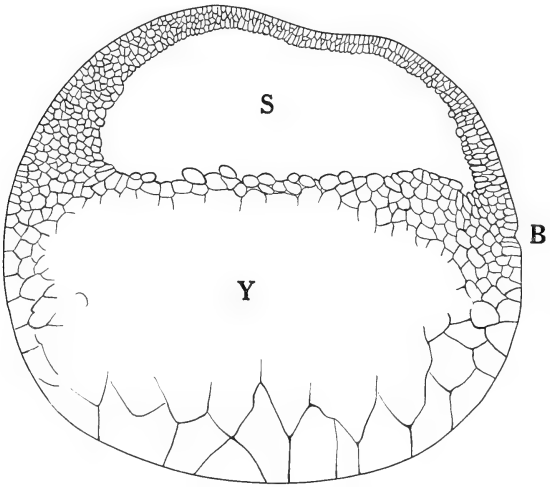


Fig. 32 Sagittal section of a beginning gastrula of Cryptobranchus allegheniensis. *B*, blastopore; *S*, segmentation cavity or blastocoele; *Y*, yolk. The drawing was made with the aid of a camera lucida. × 15.

laterality as well as polarity are inherent characters of the protoplasm and persist from generation to generation" (Bartelmez, '12). In many species of animals the egg is bilaterally organized before fertilization, but it has never been shown that this bilateral organization is present as such from the beginning of ovogenesis. The available evidence favors the theory of nuclear determination, as opposed to the hypothesis of cytoplasmic inheritance.

B. Influence of the spermatozoon and of environmental factors. Various observers (Roux, '83, '85; Schultze, '00; Morgan and Boring, '03) have shown that following fertilization and previous to cleavage the egg of the frog is bilaterally organized, and that this bilateral symmetry foreshadows the definitive bilateral symmetry of the embryo. In the fertilized egg the bilateral

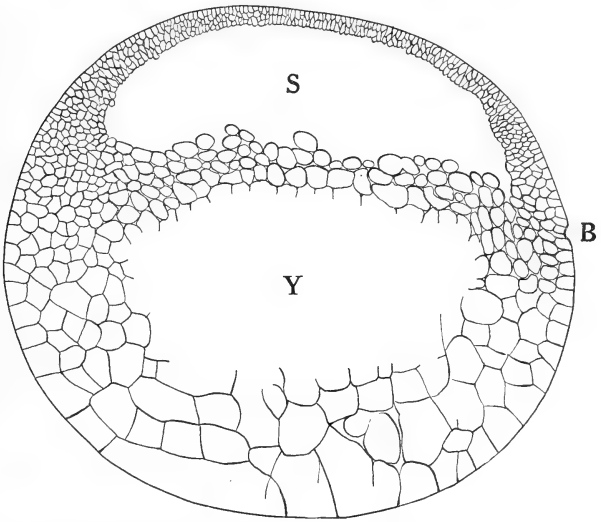


Fig. 33 Sagittal section of a beginning gastrula of Cryptobranchus allegheniensis. *B*, blastopore; *S*, segmentation cavity or blastocoele; *Y*, yolk. The drawing was made with the aid of a camera lucida. × 15.

organization expresses itself superficially in the formation of a gray crescent on the site of the future blastopore. Since the crescent is formed on the side opposite the point of entrance of the spermatozoon (Roux, '83, '85, '87, '03; Schultze, '00), Roux maintained that the point of entrance of the spermatozoon determines the direction of the median plane of the embryo.

That the egg is not dependent upon the spermatozoon for the establishment of its bilateral symmetry is shown by the

fact that in many species of animals the egg develops bilaterality in advance of fertilization, and many eggs are capable of developing parthenogenetically into organisms possessing the bilateral symmetry characteristic of the species. Consequently, there is no necessary relation between the fertilization meridian and the plane of symmetry.

The relationship which has been shown to exist in certain cases must therefore depend upon a certain time relationship in the course of the two processes. The influences radiating from the spermatozoon establish a gradient from its original excentric position, which may influence the direction of the plane of symmetry in which there is also a gradient, if its determination is synchronous, as in the frog (Lillie, '19).

In the egg of the frog, external influences such as gravity and light acting at the time of fertilization may exert an additional modifying influence in determining the direction of the median plane (Jenkinson, '09, Appendix A). In the egg of Cryptobranchus, neither the direction of sperm entrance nor the influence of gravity are factors of any appreciable importance in the determination of bilaterality.

In the frog's egg, the first cleavage furrow usually passes through the entrance-point of the spermatozoon (Newport, '54; Roux, '85, '87; Schultze, '00). According to Roux ('87), it is the entrance-path of the spermatozoon that determines the position of the gray crescent, while it is the latter part of the sperm-path, the 'copulation-path,' that determines the direction of first cleavage. Since the entrance-path and the copulation-path do not always lie in the same direction, we have here an explanation of the fact that, in the egg of the frog, the first cleavage furrow sometimes fails to coincide with the plane of symmetry (Jenkinson, '09, pp. 248, 307 and 308). In the egg of Cryptobranchus the first cleavage furrow tends to form at right angles to the fertilization meridian. I have not been able to follow satisfactorily the latter part of the sperm-path in Cryptobranchus, but it seems likely that the condition is the same as in the egg of the axolotl (Fick, '93), where the copulation-path forms at right angles to the entrance-path.

C. Relation of the first cleavage furrow to the median plane of the embryo. Recent observers agree that the cleavage of the am-

phibian egg is indeterminate in the sense that there is no causal connection between the direction of early cleavage furrows and the median plane of the embryo. This view has gained ground in spite of the fact that in particular species there is an approximate coincidence between either the first or the second cleavage furrow and the median plane.

In the frog's egg the plane of first cleavage tends to coincide with the median plane of the future animal, though the relation is far from exact (Newport, '54; Roux, '85, '87; Morgan and Boring, '03; Jenkinson, '09, pp. 165–168 and Appendix A). Brachet ('03, '05) demonstrated experimentally that each of the first two blastomeres of the segmenting egg of the frog is capable of producing an entire embryo only when the plane of first cleavage coincides with the previously determined plane of bilateral symmetry; right and left halves, dorsal and ventral sides, anterior and posterior ends, are predetermined in the undivided egg, and in normal development it makes no difference how the egg is cut up by the early cleavage furrows. McClendon ('09, '10) was able to remove completely one of the first two blastomeres of the egg of the tree-frog Chorophilus triseriatus. A large number of eggs were thus operated upon; in a considerable number of cases the remaining isolated blastomere gave rise to a complete normal embryo, and some of these lived to the larval stage. These results, considered in connection with the findings of other investigators, led this author to conclude that each of the first two blastomeres is totipotent only when the first cleavage furrow bisects the gray crescent.

In the newt Diemyctylus viridescens, Jordan ('93) found that in the majority of cases the first cleavage furrow forms at right angles to the direction of the future median plane. Jordan does not interpret this relation to mean that there is any causal nexus between the two. Spemann ('01–'03) found that in the newt Triton cristatus, the first furrow is usually (two-thirds to three-fourths of all cases) at right angles to the sagittal plane, and separates the material for the dorsal and ventral halves of the embryo; only occasionally (one-fourth to one-third of all cases) do sagittal plane and first furrow coincide. Herlitzka

('96, '97) had previously shown that by constricting these eggs in the two-cell stage by means of a noose of fine hair tied around the egg in the plane of the first furrow, it was sometimes possible to obtain two complete embryos of rather more than half size. Spemann showed that this result could be obtained only in those occasional cases where the first cleavage furrow coincides with the median plane of the embryo.

In the living egg of Necturus, Eycleshymer ('04) was able to keep the first cleavage furrow in the lower hemisphere under observation until the blastopore appeared. In the twenty-two eggs studied there was no fixed relation between the median plane of the embryo and the early cleavage furrows. The absence of a constant relation between the first cleavage furrow and the median plane of the gastrula has been demonstrated for the egg of Cryptobranchus by a variety of methods as recorded in the present paper.

In an extensive series of observations on the living, segmenting eggs of Ambylstoma, Diemyctylus, Rana, and Bufo, Jordan and Eycleshymer ('94) found that the first and second cleavage furrows undergo extensive torsion. This phenomenon seemed to the authors sufficient basis for the conclusion that the early cleavage planes and the embryonic axes have no vital connection, and that the coincidence, where it exists, is of no fundamental significance. If we assume that material for right and left halves of the body is segregated on opposite sides of either the first or the second cleavage furrow, then as a consequence of the shifting of micromeres we shall later find some of this material crossing the median line. Eycleshymer's ('04) later study of cleavage in the living egg of Necturus revealed a similar irregularity. Extensive shifting of micromeres and torsion of cleavage furrows occurs in the early stages of segmentation of the egg of Cryptobranchus.

D. The bilateral symmetry of the blastula. While the direction of the early cleavage furrows in the amphibian egg is thus shown to be without causal relation to the median plane of the embryo, bilaterality is indeed sooner or later made manifest in the cleavage pattern as a consequence of more rapid cell division on one side

of the region of micromeres. In the frog's egg, Morgan and Boring ('03) have noted that the pigmented cells on the gray crescent side of the egg are slightly smaller from the beginning than the other pigmented cells. Eycleshymer ('98, '02, '04, '15) has shown that in the blastula stages of Amblystoma, Necturus, Rana, Acris, and Bufo there is a secondary or excentric area of smaller cells, which, roughly speaking, lies within a sector of the circular area occupied by the micromeres, and that this area of accelerated cell division always lies on the side on which the dorsal lip of the blastopore is to appear.

> The primary area of cellular activity, at the upper pole of the amphibian egg, forms the basis of the cephalic end of the embryo. The secondary area of cell activity, on the blastoporic side of the egg, forms the basis of the greater portion of the posterior half of the embryo. These two areas constitute an embryonic tract, from which arise at least two-thirds of the embryo. The posterior end of the embryo is formed by a coalescence of the lateral portions of the blastoporic margins (Eycleshymer, '98).

In Eycleshymer's earlier writings emphasis is placed on the occurrence of this area of accelerated cell division in the late blastula stage, as illustrated by his figure of Amblystoma (Eycleshymer, '98, fig. 100); but in his later investigations he found that in several species excentric development is present in the early blastula, sometimes as early as the fourth or fifth cleavage stage. These two similar conditions, appearing respectively early and late in the amphibian blastula, he regarded as genetically continuous. I have been able to confirm Eycleshymer's observation concerning the early appearance of excentric development in the micromeres of Necturus; but in Cryptobranchus, experimental results make it necessary to distinguish between the problematical significance of the excentricity of the early blastula and the undoubted significance of the bilateral symmetry of the late blastula. According to Lillie ('08, pp. 42 and 47), the axis of excentricity in the early cleavage pattern of the pigeon's egg bears no constant relation to the median plane of the embryo. It is undoubtedly true that the excentric development of the blastoderm which truly marks the beginning of

dorsoventral differentiation appears at different stages in different species of animals.

Schultze ('00) was probably the first to describe the bilateral organization of the late blastula of the frog, and Ishikawa ('08, '09) has described a similar condition in the developing egg of the giant salamander of Japan.

In the segmenting egg of the frog, Bellamy ('19) has demonstrated a primary area of high susceptibility to the action of reagents, in a meridian that bisects the gray crescent and near the center of the pigmented hemisphere; also a secondary area of high susceptibility in the equatorial region immediately above the gray crescent, hence just above the site of the dorsal lip of the future blastopore.

E. Embryonic axes in relation to bilateral symmetry. In the ovarian egg of Cryptobranchus, the first visible differentiation having reference to the form of the adult is manifested in what we call polarity: an active pole, rich in cytoplasm, is differentiated from an opposite and relatively inactive pole concerned mainly with the storage of food materials. At this stage of development the structure in any plane taken at right angles to the polar axis is radially symmetrical. Eventually, this polar axis determines approximately the principal axis of the embryo, or the axis of anteroposterior differentiation.

The further step necessary for the establishment of bilateral symmetry is the appearance of a secondary axis, the dorsoventral axis, extending at right angles to the first. In the egg of Cryptobranchus this secondary axis becomes apparent through the unequal development of opposite sides of the roof of the blastocoele; the region of more active cell division becomes the dorsal side of the embryo.

It is perfectly obvious that these two axes supply all the differentiations necessary to establish a condition of bilateral symmetry, for the plane determined by the intersection of these two axes divides the egg into halves which were originally alike, and which are modified in a corresponding manner by the differentiation that proceeds along the secondary axis.

To explain the maintenance of bilateral symmetry throughout the subsequent development of the embryo, it is necessary to suppose that later differentiations are conditioned and controlled by the differentiations along the two axes already established; in particular, differentiation along the mediolateral axes of the body does not proceed independently, but only in subordination to the more potent and regulatory anteroposterior and dorsoventral differentiation.

It would seem simplest to assume that right and left halves of the body are not at all self-differentiated, but that they are conditioned by the other body axes. In this way the establishment of two axes each with two distinct poles (anterior and posterior; dorsal and ventral) would fully suffice to determine the bilaterality of the organism, because, if we presuppose a similar interaction of analogous anlagen, those lying in the third axis and giving rise to the right and left halves of the body should naturally arrange themselves so that they would represent mirror images of each other, when they occupy the same position in relation to the two differentiated axes (Przibram, '11).

SUMMARY

1. The polarity of the egg of Cryptobranchus allegheniensis arises during ovogenesis and establishes approximately the direction of the anteroposterior axis of the embryo.

2. The organization of the mature but unfertilized egg is characterized by radial symmetry with differentiation along the polar axis, but with no evidence of bilaterality.

3. Gravity acting at right angles to the polar axis of the egg during the fertilization period is without perceptible effect in determining the direction of the median plane of the embryo.

4. The direction of entrance of the spermatozoon is not a controlling factor in determining the direction of the median plane of the embryo.

5. The first cleavage furrow forms approximately at right angles to the direction of the entrance-path of the spermatozoon.

6. The direction of first cleavage bears no fixed relation to the direction of the median plane of the embryo.

7. In the early blastula stages, the direction of excentric development of the micromeres bears no constant relation to the direction of the median plane of the embryo.

8. In the late blastula, bilateral symmetry is manifested by both the superficial cleavage pattern and the internal structure, and this condition is undoubtedly an expression of the definitive bilateral symmetry of the embryo.

9. The bilateral symmetry of the late blastula is the consequence of dorsoventral differentiation imposed upon the preexisting radial symmetry and anteroposterior differentiation of the egg.

10. The subsequent development of the two lateral halves of the egg is conditioned and controlled by the differentiation which proceeds along the two axes (anteroposterior and dorsoventral) already established; these two axes suffice to determine and maintain the bilateral symmetry of the embryo.

BIBLIOGRAPHY

Bartelmez, Geo. W. 1912 The bilaterality of the pigeon's egg. Jour. Morph., vol. 23, no. 2.

1918 The relation of the embryo to the principal axis of symmetry in the bird's egg. Biol. Bull., vol. 35, no. 6, Dec.

Bellamy, Albert William 1919 Differential susceptibility as a basis for modification and control of early development in the frog. Biol. Bull., vol. 37, no. 5, Nov.

Brachet, A. 1903 Sur les relations qui existent chez le Grenouille entre le plan de penetration du spermatozoide dans l'oeuf, le premier plan de division et le plan de symetrie de la gastrula. Comptes Rendus de l'Assoc. des Anat., Sess. 5.

1905 Recherches experimentales sur l'oeuf de Rana fusca. Archives de Biologie, T. 21.

1906 Recherches experimentales sur l'oeuf non segmenté de Rana fusca. Arch. für Entwickelungsmech., B. 22.

Conklin, E. G. 1917 The share of egg and sperm in heredity. Proc. Nat. Acad. Sci., vol. 3.

Eycleshymer, A. C. 1898 The location of the basis of the amphibian embryo. Jour. Morph., vol. 14.

1902 The formation of the embryo of Necturus, with remarks on the theory of concrescence. Anat. Anz., B. 21.

1904 Bilateral symmetry in the egg of Necturus. Anat. Anz., B. 25.

1915 The origin of bilaterality in vertebrates. American Naturalist, vol. 49.

Fick, R. 1893 Ueber die Reifung und Befruchtung des Axolotleies. Zeitschr. für wiss. Zool., B. 56.

Herlitzka, A. 1896 Contributo allo studio della capacità evolutiva dei due primi blastomeri nell' uovo di tritone (Triton cristatus). Arch. für Entwickelungsmech., Bd. 2.

HERLITZKA, A. 1897 Sullo sviluppo di embrioni completi da blastomeri isolati di uova di tritone (Molge cristata). Arch. für Entwickelungsmech., Bd. 4.

ISHIKAWA, C. 1908 Ueber den Riesen-salamander Japans. Mitteilungen der Deutschen Gesellschaft für Natur- und Völkerkerkunde Ostasiens, Bd. 11, Teil 2.

1909 Note on the gastrulation of the giant salamander, Megalobatrachus sieboldii. Proc. Seventh Internat. Zool. Congress, Boston, Aug. 19–24, 1917.

JENKINSON, J. W. 1909 Experimental embryology. Clarendon Press, Oxford.

JORDAN, EDWIN O. 1893 The habits and development of the newt, Diemyctylus viridescens. Jour. Morph., vol. 8.

JORDAN, E. O., AND EYCLESHYMER, A. C. 1894 On the cleavage of the amphibian ovum. Jour. Morph., vol. 9.

LILLIE, FRANK R. 1906 The development of the chick. Henry Holt & Co.

1919 Problems of fertilization. The University of Chicago Press.

MCCLENDON, J. F. 1909 On the totipotence of the first two blastomeres of the frog's egg. American Naturalist, June.

1910 The development of isolated blastomeres of the frog's egg. Am. Jour. Anat., vol. 10, no. 3, July.

MORGAN, T. H., AND BORING, ALICE M. 1903 The relation of the first plane of cleavage and the gray crescent to the median plane of the embryo of the frog. Arch. für Entwickelungsmech., Bd. 16.

NEWPORT, G. 1854 On the impregnation of the ovum in the amphibia; and on the early stages of the development of the embryo. (Third series.) Philos. Trans. Royal Soc. London, vol. 144.

PRZIBRAM, HANS 1911 Experiments on asymmetrical forms as affording a clue to the problem of bilaterality. Jour. Exp. Zoöl., vol. 10.

ROUX, W. 1883 Ueber die Zeit der Bestimmung der Hauptrichtungen des Froschembryo. Leipzig.

1885 Ueber die Bestimmung der Hauptrichtungen des Froschembryo im Ei und über die erste Theilung des Froscheies. Breslauer ärtzl. Zeitschr.

1887 Die Bestimmung der Medianebene des Froschembryos durch die Kopulationsrichtung des Eikernes und des Spermakernes. Arch. für mikr. Anat., Bd. 29.

1893 Ueber die ersten Teilungen des Froschei und ihre Beziehungen zu der Organbildung des Embryos. Anat. Anz., Bd. 8.

1903 Ueber die Ursachen der Bestimmung der Hauptrichtungen des Embryos im Froschei. Anat. Anz., Bd. 23.

SCHULTZE, O. 1900 Ueber das erste Auftreten der Bilateralen Symmetrie im Verlauf der Entwicklung. Arch. für mikr. Anat., Bd. 15.

SMITH, BERTRAM G. 1912 The embryology of Cryptobranchus allegheniensis, including comparisons with some other vertebrates. Part I: Introduction; the history of the egg before cleavage. Jour. Morph., vol. 23, no. 1. Part II: General embryonic and larval development, with special reference to external features. Jour. Morph., vol. 23, no. 3.

1914 An experimental study of concrescence in the embryo of Cryptobranchus allegheniensis. Biol. Bull. vol. 26.

SPEMANN, H. 1901–1903 Entwickelungsphysiologische Studien am Triton-Ei. I, II, III. Arch. für Entwickelungsmech., Bde. 12, 15, 16.

Resumen por la autora, Edith Pinney.

La supresión inicial del desarrollo en los óvulos de fecundación cruzada.

I. Cruzamientos con el óvulo de Fundulus.

II. Cruzamientos recíprocos entre Ctenolabrus y Prionotus.

Las observaciones sobre las mitosis de la segmentación temprana en óvulos de híbridos peces indican la existencia de factores específicos que operan durante la anafase de segmentación. La división normal de los cromosomas no se lleva a cabo en muchos óvulos híbridos. Mientras que el trastorno posee características generales, tales como la aglutinación, retraso en el movimiento de las mitades de los cromosomas y su falta de separación, las cuales resultan en la distribución desigual de la cromatina en los blastómeros hijos, la distribución actual de los elementos cromáticos varía considerablemente. La autora interpreta dicho trastorno, por consiguiente, como la expresión de la falta de coordinación entre factores del desarrollo, tal vez cambios específicos de viscosidad, del citoplasma del óvulo y de la cromatina del espermatozoide. Las pruebas acumuladas no prestan apoyo a la hipótesis que supone que las cualidades hereditarias específicas de los cromosomas individuales juegan papel importante en la reacción. Este concepto de la naturaleza de los factores que producen comportamiento anormal de la cromatina en los óvulos híbridos de los peces está en armonía con los resultados del desarrollo obtenidos en varios cruzamientos que hasta el presente no han podido explicarse basándose en las relaciones taxonómicas. La comparación de los resultados de la hibridación en los peces demuestran que si dos especies se cruzan reciprocamente sin trastorno en la mitosis de segmentación, los óvulos de estas dos especies reaccionarán de un modo semejante cuando se fecunden con el mismo esperma extraño. Este resultado se aplica a todos los cruzamientos llevados a cabo con peces hasta el presente, así como los efectuados en los equinodermos. Además, verifica las expectaciones de nuestra hipótesis de que el comportamiento anormal de los cromosomas de los óvulos híbridos depende de la coordinación entre los sistemas que cambian físicamente en el citoplasma del óvulo y la cromatina del espermatozoide.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, APRIL 17

THE INITIAL BLOCK TO NORMAL DEVELOPMENT IN CROSS-FERTILIZED EGGS

I. CROSSES WITH THE EGG OF FUNDULUS

II. RECIPROCAL CROSSES BETWEEN CTENOLABRUS AND PRIONOTUS

EDITH PINNEY

Lake Erie College

SEVENTEEN FIGURES (TWO PLATES)

There is a marked absence of specific fertilization qualities among the germ cells of teleosts, such as has been demonstrated in the eggs of certain invertebrates. It is true that the per cent of fertilized eggs in fish hybridization varies widely, both in different crosses and in the same crosses at different times, but no quantitative study of this variation has been made. There is, however, a specificity factor present in fish eggs which has been met with in the eggs of echinoderms (9). It expresses itself in a disturbance of the mitotic process during the first cleavage anaphase and forms the first critical block to development. All of the nuclear phenomena following fertilization up to the time that the equatorial plate is formed in preparation for the first cleavage are normal in all of their visible morphological aspects. During the anaphase, however, abnormalities arise.

In an earlier paper (15) it was shown that the abnormalities in development in certain crosses could be traced to these abnormalities in the mitosis of early cleavage. Two general types of mitotic behavior during the first cleavage division were observed and described; one type being normal, division of the chromatin occurring with undisturbed mitotic precision; the other type being abnormal, showing a number of irregularities in the accurate division of the chromosomes, such as unequal distribution, fragmentation, and, possibly, elimination. That this early

mitotic disturbance was not the cause of all of the abnormalities appearing during the course of development in fish hybrids is quite certain, since hybrid eggs which undergo normal mitosis in early cleavage often fail to develop (12). The cause of the later-appearing derangements still remains to be determined. The two problems are not necessarily identical and the present paper will be limited to a consideration of the abnormal mitoses immediately following fertilization and their significance.

In the paper referred to above (15) I suggested that the success of the first cleavage mitosis depends upon certain specific physical conditions of the substratum, namely, the egg protoplasm. The new crosses described in this paper strengthen that interpretation and, by extending the field for comparison, throw light also, I believe, upon some hitherto rather obscure and puzzling results of fish hybridization. I refer to the absence of any correlation between developmental results and taxonomic relationships.

For a detailed account of the behavior of the chromatin in the Ctenolabrus crosses with Fundulus heteroclitus, Menidia menidia notata and Stenotomus chrysops, the reader is referred to my earlier paper (15). The methods used in the present investigation are also fully discussed there. I will confine the descriptive part of this paper to new crosses or new observations on previously described crosses.

I. DESCRIPTION OF CROSSES WITH FUNDULUS

1. Fundulus heteroclitus ♀ × *Ctenolabrus adspersus* ♂

This cross was discussed in my earlier paper. At that time I had only a few preparations of my own. These taken in conjunction with the figures published by Morris (11) and compared with my own preparations of normal Ctenolabrus eggs, formed the material basis of my conclusions in regard to this cross. In order to clear up any doubt to which the former limitations in material might give rise, I wish to report here upon a new lot of preparations which furnish abundant evidence that the Ctenolabrus chromatin in the Fundulus eggs is very unequally

distributed in the first anaphase, as well as in subsequent divisions. Figures 1a and 1b are from an egg in the first cleavage, as are also figures 2a and 2b. Figure 3, a and b, and figure 4 show the anaphase of the second cleavage. The conditions displayed here are so typical for all of the sections studied that it seemed superfluous to multiply the evidence by many drawings of practically the same thing. The only variation between first anaphase spindles in these eggs is in the amount of undivided chromatin which lags at or near the equator. This variation indicates that, whether chromatin is actually eliminated or not, and there is much reason to think that it is, the foreign chromatin which does remain in the egg is distributed in a variety of ways. Undivided chromosomes can pass to one pole only and, therefore, one of the first two blastomeres lacks some chromosomes which the other contains. Thus extreme variation between blastomeres arises and is increased during early cleavage. It is a significant fact that the behavior of the chromatin during the first anaphase is duplicated during the second cleavage.

Perhaps a word might be added here in description of the lagging masses of chromatin. Those which are nearer the daughter groups of chromosomes resemble in contour and size the smaller chromosomes of the foreign species (15). Other masses, usually lying on or near the equator of the spindle, cannot be reconciled with single chromosomes of either species. They resemble more nearly the undivided chromosomes of the early metaphase stages. Some of the masses show rod-like projections which suggest that perhaps two or even more chromosomes have adhered to each other during their partial journey to the pole. The appearance may be due, however, to nothing more than a collision such as frequently occurs in normal anaphases. Figure 3a shows what is plainly a split chromosome, the halves of which are still adherent, passing to one pole. I have yet to observe an anaphase figure in this cross at these early stages in which there is no lagging chromatin.

2. Fundulus heteroclitus ♀ × *Prionotus carolinus* ♂

In this combination there is the same abnormal behavior on the part of the sperm chromatin in the early mitoses that is seen in the cross just described. Figures 5 to 8, inclusive, show first and second cleavage anaphases. All of the chromosomes crowded at the ends of the spindle could not be included in the drawing without altering their spatial relations. The lagging chromatin, however, has been depicted as accurately as is possible with such minute objects. There can be no doubt that here again we would obtain a great range in variation in the chromatin content of early blastomeres. As before, the second cleavage anaphase repeats the abnormal behavior of the first.

Observations upon the prophase stages of the first cleavage were not made, but it seems reasonable to assume that they resemble the prophase stages of the second cleavage. After the first cleavage two normal-appearing nuclei are reformed, the centrosome divides, the asters which are to function in the second cleavage appear, and their growth proceeds normally. This process is beautifully clear in the Fundulus egg. That the rays of the aster are formed by the rearrangement of the cytoplasmic reticulum, as described by Wilson (17) for the sea-urchin egg, is quite obvious, and one feels convinced that, however much the structural appearances in fixed material differ from the actual state of the living egg, the relation between astral system and cytoplasm is the same in both. When the astral rays have extended well out into the cytoplasm, approximately half-way to the cell wall, division of the chromatin, which meanwhile has formed the equatorial plate, begins. It is during this ensuing anaphase stage that abnormalities arise. The point I wish to make is that if the second anaphase which is abnormal is preceded by perfectly normal processes as far back as the first anaphase which was likewise abnormal, then we may assume that it in turn is preceded by a normal prophase. Morris gives figures which show the early stages preceding the first cleavage anaphase in the cross Fundulus ♀ × Ctenolabrus ♂ to be normal (11). The same sort of observations were made by Godlewski

on a cross between members of two different classes of Echinoderms (5).

It must be remembered that in speaking of normal processes I refer only to the visible, morphological changes which follow each other in orderly succession in the course of cleavage. This cycle is uninterrupted and the changes themselves appear normal. If any deviations from the normal course of affairs are present, they are far too slight to be recognized even in a very careful study.

In order to meet the possible objection that this characteristic lagging and clumping is not typical of this and the foregoing cross, I should perhaps emphasize the fact that the peculiarities described appear in every egg observed at this stage. That it is not an artifact, due to poor fixation, is proved by the fact that preparations of other crosses with the same egg in which the same technique was used show normal anaphases as consistently as these exhibit abnormal anaphases. If these appearances were artifacts, we should not expect to find this regularity in their occurrence. It should also be mentioned in this connection that in all of the crosses with Fundulus eggs, the eggs are taken from several females and placed in the same dish. When the sperm is added the eggs are stirred about so that in taking out a pipette full of eggs to fix it is certain that the eggs are well mixed and that those fixed in any stage originate from several females. The character, then, which determines the behavior of the chromatin during the anaphase is not an individual character, but is common to the species.

3. *Fundulus heteroclitus* ♀ × *Menidia menidia notata* ♂

The behavior of the chromatin in this cross has been described by Moenkhaus (10), and the facts have become such a familiar part of our cytological knowledge that any further description of the details of cleavage are unnecessary. My purpose in repeating his observations was to determine whether the conditions he describes are common to all hybrid eggs of this cross. My preparations resemble his descriptions and figures so closely and so consistently that there is left no room for doubt that here

we have a cross in which this block to normal mitosis of the anaphase period is absent. Figures 9 and 10 are from my material and present first and third cleavage conditions during the critical period. Division is normal.

4. Fundulus heteroclitus ♀ × *Stenotomus chrysops* ♂

Of this cross I am able to give only second-cleavage figures (11, a and b, and 12, a and b). These show the usual conditions in straight-fertilized eggs. There is no lagging or clumping, and I feel that one may safely conclude that the same conditions prevail during the first cleavage. I base this inference upon observations made on other crosses reported here and elsewhere (15).

Figure 12 shows the two sections of one spindle. The chromosomes were so well separated that it was possible to count them. There are forty-six at either pole. In figure 11a the individual chromosomes have been drawn spread out laterally, so that all of the rods of each group are not reproduced in their actual positions. Such a drawing illustrates how much more can be gained from a study of the preparations than is indicated by the drawings themselves. In this figure the longer Fundulus chromosomes are easily identified, as are the smaller Stenotomus elements. The rods of medium length cannot with certainty be ascribed to either species. There is a definite grouping on this second-cleavage spindle. Evidently, the plane of the section has passed to one side of the Fundulus group, as all of the long rods are in one section. The smaller chromosomes in figure 12 b belong to the Stenotomus group.

This cross, therefore, shows the type of behavior that is characteristic of self-fertilized eggs. It falls in the same group as the cross with Menidia.

II. RECIPROCAL CROSSES BETWEEN CTENOLABRUS ADSPERSUS AND PRIONOTUS CAROLINUS

In all of the reciprocal crosses with the cunner which I had studied up to this time there was a marked difference in the chromatin behavior during the early anaphases. In the crosses

in which the egg of Ctenolabrus was used normal mitotic division was the rule. The eggs of Stenotomus, Fundulus, and Menidia, however, when fertilized with the sperm of Ctenolabrus showed the typical abnormality during the anaphase cleavage which is described above. It was, therefore, a matter of interest to find a cross with this species in which the reciprocals were alike in their early mitotic behavior, as is the case in the cross between Ctenolabrus and Prionotus. The behavior is normal in both eggs. Nothing earlier than second-anaphase figures of both of these crosses were observed. From much observation of other crosses I feel convinced that the second cleavage mitosis resembles the first very closely, and that therefore in these reciprocal crosses no elimination of chromatin or abnormalities in mitosis occur during the first cell division.

I thought that perhaps some evidence on this point might be gained from a study of polar groups of chromosomes after actual division had occurred. With this in mind, I attempted to estimate the chromosomes to be expected in polar groups and then determine whether second-anaphase groups fulfilled this expectation. The sources of error in such a study are numerous, and the estimates that I have made can only claim to be approximate. Counts of both polar and lateral views of anaphase groups place the number of chromosomes in the normal Prionotus egg near fifty. My earlier counts of the species Ctenolabrus give indications that the number there is about forty-four. We should then expect between forty-five and fifty in the hybrid eggs. Actual counts are as follows:

$$\text{Ctenolabrus } ♀ \times \text{Prionotus } ♂, \frac{52}{51'}\ \frac{53}{56'}\ \frac{56}{59'}\ \frac{49}{\text{not counted}}$$

$$\text{Prionotus } ♀ \times \text{Ctenolabrus } ♂, \frac{55}{58'}\ \frac{53}{50'}\ \frac{55}{50}.$$

The unexpectedly large number here is probably due to the sectioning of single chromosomes. No entire spindles or even single polar groups were found. Obviously, such material is not adapted to accurate counting. As evidence, while it may show that no great elimination of chromosomes has occurred, as regards irregular distribution of chromosomes it has no value.

The best evidence of regular division is obtained from early anaphases. Such a stage is drawn in figure 13, a and b. There is no abnormality. Mitosis here is wholly orthodox. Corresponding daughter chromosomes on their way to opposite poles can be identified.

As seen from this figure and figures 14 and 15, Prionotus chromosomes are indistinguishable in a Ctenolabrus egg. The only difference between the two species of elements lies in the probable presence of more hooked-shaped chromosomes in Ctenolabrus, but it has never been determined that shape is a constant feature in fish chromosomes. Figures 16 and 17 are from the cross Prionotus ♀ × Ctenolabrus ♂.

SUMMARY OF DATA

The following table presents, in a summarized form, the data for all of the crosses thus far studied. In case of the cross, Menidia ♀ × Fundulus ♂, the results stated here are those

	CTENOLABRUS ♀	FUNDULUS ♀	STENOTOMUS ♀	MENIDIA ♀	PRIONOTUS ♀
Ctenolabrus ♂	×	Early mitosis abnormal	Early mitosis abnormal	Early mitosis abnormal	Early mitosis normal
Fundulus ♂	Early mitosis prevailingly normal	×		Early mitosis normal	
Stenotomus ♂	Early mitosis normal	Early mitosis normal	×		
Menidia ♂	Early mitosis normal	Early mitosis normal		×	
Prionotus ♂	Early mitosis normal	Early mitosis abnormal			×

reported by Moenkhaus (10). I have not verified his results. Figure 23 of his paper was earlier interpreted by me as indicating lagging in this hybrid. The figure, however, shows only one pole of the spindle and there is nothing to indicate the position of the equatorial plane, which fact I overlooked in my diagnosis. His other figures are free from the undivided masses of chromatin which are characteristic of the irregular mitosis found in some crosses. This behavior justifies its inclusion in the group of normally reacting crosses.

DISCUSSION

1. The significance of the early mitotic disturbance in hybrid eggs

Teleost eggs clearly exhibit a factor which regulates the activity of the sperm chromatin in cleavage. The nature of the mitotic disturbances which have been described above would indicate that the immediate factor was a physical condition of the egg cytoplasm, probably the normal state of fluidity or viscosity characteristic of the division phase. The possibility that physical factors of the sperm take part in this reaction should not be excluded, but in the progress of cleavage the egg cytoplasm plays a more active rôle, while the elements contributed by the sperm are relatively passive (15). In this sense the egg determines the cleavage phenomena, the process of chromosome separation as well as the rate of the antecedent processes.

The results of heterogeneric hybridization in echinoderms are analogous to those in fishes. We have several accounts of the cytological events following cross-fertilization in these forms, and in all of them, with one or two exceptions, the description of the behavior of the chromatin resembles very closely that given for fishes (1, 4, 5, 7, 8, 16).

In order to be convinced that the phenomena are similar in both forms, the reader has only to compare the figures given here for the two Fundulus crosses in which the sperm of Ctenolabrus and Prionotus was used and the figures already published by the writer of the mitotic process in eggs of Stenotomus and

Menidia fertilized by the sperm of Ctenolabrus (15) with the figures given by Herbst (7) for Sphaerechinus ♀ × Strongylocentrotus ♂, by Baltzer (1) for the eggs of Strongylocentrotus, Echinus, and Arbacia fertilized by Sphaerechinus sperm, and by Tennent (16) for the reciprocal crosses between Arbacia and Toxopneustes.

The behavior of the chromatin in the cross between Echinus acutus ♀ × Echinus esculentus ♂, reported by Doncaster and Gray (4), shows slight differences from those described by the foregoing investigators. In the latter cross the chromosomes form vesicles and are eliminated during the anaphase. The vesicles make their appearance in the prophase, which may indicate that the origin is different from that causing the lagging occurring in other forms. It appears that lagging was also observed by these authors in connection with the cross Echinus acutus ♀ × Echinus milearis ♂, for of this cross they say, "In the hybrid eggs we found that some of the chromosomes developed vesicles, but no other elimination occurred except the possible non-division of certain chromosomes, about which we are uncertain."

Godlewski (5) reports chromatin elimination in his interclass cross between Echinus ♀ and Antedon ♂. The Antedon chromosomes take part in the first mitosis, but are eliminated later. Counts of polar views of anaphase groups are given as evidence. The figures accompanying this paper are unsatisfactory in that they give no evidence as to the manner in which elimination is accomplished.

In the cross studied by Kupelwieser (8) we have an extreme case of elimination, due probably to factors operating earlier in development.

The actual elimination of chromosomes from the mitotic mechanism rests on more conclusive evidence in the case of echinoderms than it does in that of fishes.

Unequal distribution of paternal chromatin unquestionably occurs in both, although aside from its casual mention by Doncaster and Gray (4), Herbst (7) is the only investigator to report this for echinoderms. Baltzer's figures, however, show unmis-

takable cases of undivided chromosomes passing to one pole (1). It is more difficult to decide in the case of the figures given by Tennent (16), since the matter is complicated by the presence of the large V-shaped chromosomes of Toxopneustes.

The evidence from fish crosses does not support the idea that the chromosomal behavior is highly specific. If only certain paternal chromosomes were affected, as Baltzer claims does occur in certain of his crosses (1), then we would expect to find in every anaphase of the first cleavage the same picture. This is not the case. The amount of lagging varies. It also occurs in the cleavage following the first and always at the anaphase. In Baltzer's crosses, as his figures show, mitotic disturbances appear in all of the early cleavages including the fourth. I have elsewhere (14) emphasized the difficulties in the way of identifying many of the extruded chromosomes in echinoderm crosses. If elimination or unequal distribution depends upon the specific nature of individual chromosomes, we would expect more variability in the character of the disturbance, more regularity in the extent of its occurrence during any one mitotic phase, as well as an expression of its specificity during other periods of the mitotic cycle. The fact that it appears only during the anaphase, that it is variable in extent, and that it occurs in the second, third, and fourth cleavages as well as in the first is in harmony with the suggestion that the behavior is the result of a general physical reaction between the egg cytoplasm and the sperm chromatin.

All of the figures referred to strengthen the impression that the disturbance is due to a general physical reaction involving the entire sperm chromatin and the egg cytoplasm and is not the result of a differential action of the egg components toward individual paternal chromosomes due to the specific differences existing in the hereditary quality of the latter. According to modern ideas of heredity, the chromosomes determine taxonomic characters. In so far they are specific. Their relation in cell division is the same for all. If some paternal chromosomes undergo abnormal distribution or elimination and others escape, it is a result of chance rather than of specific hereditary qualities.

The whole phenomenon is one that is concerned with developmental rather than with hereditary factors.

If, as was originally suggested by Bütschli (18, 2), protoplasmic currents are concerned in the movements of chromosomes, we might have in the specific character of such currents the physical factor necessary to regulate chromosome division in these cases. The results of Chambers (3) and Heilbrunn (6) are highly suggestive in this connection. Both of these workers have demonstrated changes in the viscosity of the cell protoplasm during mitosis. Specific viscosity differences in the eggs at the anaphase period may cause the abnormal division of chromosomes occurring in some heterogeneric hybrids. The conditions of viscosity that prevail during the cleavage of these hybrids are, I believe, the normal conditions always present in the egg and are not deviations from the normal caused by the foreign sperm. I infer this from the fact that the egg cytoplasm exerts a differential effect toward the two sorts of chromosomes which it contains. The egg chromosomes divide normally. The sperm elements show abnormal behavior.

There are two exceptions to this. Doncaster and Gray (4) consider that the abnormally behaving chromatin in the cross Echinus acutus ♀ × Echinus esculentus ♂ is of maternal origin. The phenomenon described by them for that cross, however, is of an apparently different nature and need not be considered in this category. The other exception occurred in the cross, Arbacia ♀ × Toxopneustes ♂, in which Tennent (16) observed the elimination of chromosomes of both species from the nucleus. These eggs were, however, given rather drastic treatment to cause penetration of the foreign sperm. The eggs stood in sea-water for four hours and were then treated with alkaline sea-water. If this treatment in any way changed the egg cytoplasm, the results are no longer inconsistent, but follow the expectations of the hypothesis expressed here. The point could be tested perhaps by self-fertilizing Arbacia eggs treated in the same manner.

2. Chromosome behavior and taxonomic relationships

Whatever the nature of the physical condition governing the mitotic processes at the critical anaphase stage, the condition itself is no doubt an expression of the specific chemical composition of the egg cytoplasm. This chemical composition may be more highly specific than the physical state which it conditions, that is, it is conceivable that the egg protoplasm of two species of fish may differ chemically and yet resemble each other so closely in their physical characters that they may react alike in crossing. In other words, protoplasmic relationships between species are not necessarily correlated with the physical conditions present in their germ cells at corresponding morphological stages. This, I believe, explains the fact that the results of heterogeneric hybridization show no correlation with taxonomic relationships.

While the presence or absence of abnormal mitosis in early cleavage is not correlated with taxonomic relationships, a comparison of the crosses made shows some indication of an underlying relationship based on the egg's behavior in this respect which is independent of species affinities. For instance, reference to the tabular summary above shows that when the germ cells of Menidia and Fundulus combine reciprocally, development is not hindered by this block to normal mitosis, although it may, and usually does, meet with some disturbing factor later on. Both of these eggs exhibit such a block to the sperm of Ctenolabrus; that is, both show the same behavior to the same foreign sperm. Further, the reciprocal crosses of Ctenolabrus and Prionotus show similar behavior in that both proceed normally. The egg of Fundulus produces the same reaction in the sperm of both of these species. I should like to have made further tests by crossing Menidia ♀ with both Prionotus ♂ and Stenotomus ♂, but unfortunately Menidia eggs were not obtained in 1921. One of course hesitates to draw conclusions from so little evidence, but the facts are certainly significant.

A review of the reactions of echinoderm eggs in hybridization reveals certain similarities of behavior which favors the

same interpretation. Lillie (9, p. 191) has tabulated the results of Baltzer's crosses. From his table it is seen that Echinus and Strongylocentrotus cross reciprocally with no elimination. The eggs of both species eliminate Sphaerechinus chromosomes at the first cleavage. The egg of Sphaerechinus, on the other hand, tolerates the male chromatin of either species. In addition the two sorts of eggs eliminate Arbacia chromosomes, but not until the blastula stage is reached.

In connection with these echinoderm crosses one should remember that cross-fertilization was only possible after treating the eggs with alkaline solutions. The primary effect of this treatment is to alter the normal cortical reaction of the egg to the foreign sperm. If it affects the physical character of the cytoplasm, it is more than probable that it does this in a uniform manner so that the same relative conditions obtain in the treated eggs as would exist in eggs that were not treated.

Norman (13) showed a difference between the eggs of Ctenolabrus and Fundulus, forms which behave differently in crossing, by subjecting them to the action of heat: 30°C. was sufficient to stop segmentation in Ctenolabrus, while it required a temperature of 38°C. to produce the same effect in Fundulus. This indicates some specific difference in the cytoplasm of these two eggs. It is not unreasonable to suppose that other fish eggs would show different points of susceptibility to heat in this regard. Whether such susceptibility points would follow the taxonomic affinities of species or show independent variation would be an interesting point to determine in this connection.

The evidence so far accumulated seems to me to point to the variation in the physical factors controlling mitosis as one basis upon which the lack of correlation between developmental success in fish hybrids and taxonomic relationships can be explained. If this view is correct, it should be possible to reproduce these phenomena experimentally in both straight-fertilized eggs and in crosses. In that direction lies the hope of further analysis.

LITERATURE CITED

1 Balzer, F. 1910 Ueber die Beziehung zwischen dem Chromatin und der Entwicklung und der Vererbungsrichtung bei Echinodermenbastarden. Archiv. f. Zellforschung, Bd. 5.

2 Bütschli, O. 1900 Bemerkungen über Plasmaströmungen bei der Zelltheilung. Archiv. f. Entwickelungsmechan., Bd. 10.

3 Chambers, R. 1917 Microdissection studies. II. The cell aster; a reversible gelation phenomenon. Jour. Exp. Zoöl., vol. 23.

4 Doncaster, L., and Gray, J. 1913 Cytological observations on the early stages of segmentation of Echinus hybrids. Quart. Jour. Mic. Sci., vol. 58.

5 Godlewski, E. 1906 Untersuchungen über die Bastardierung der Echiniden und Crinoidenfamilien. Archiv. f. Entw. Mech., Bd. 20.

6 Heilbrunn, L. V. 1920 An experimental study of cell-division. I. The physical conditions which determine the appearance of the spindle sea-urchin eggs. Jour. Exp. Zoöl., vol. 30.

7 Herbst, Curt 1909 Vererbungstudien VI. Archiv. f. Entw. Mech., Bd. 27.

8 Kupelwieser, Hans 1909 Entwicklungserregung bei Seeigeleiern durch Molluskensperma. Archiv. f. Entw. Mechan., Bd. 27.

9 Lillie, F. R. 1919 Problems of fertilization. Univ. of Chicago Press.

10 Moenkhaus, Wm. J. 1904 The development of the hybrids between Fundulus heteroclitus and Menidia notata, with especial reference to the behavior of the maternal and paternal chromatin. Am. Jour. Anat., vol. 3.

11 Morris, Margaret 1914 The behavior of the chromatin in hybrids between Fundulus and Ctenolabrus. Jour. Exp. Zoöl., vol. 16.

12 Newman, H. H. 1915 Development and heredity in teleost hybrids. Jour. Exp. Zoöl., vol. 18.

13 Norman, W. W. 1896 Segmentation of the nucleus without segmentation of the protoplasm. Archiv. f. Entw. Mechan., Bd. 3.

14 Pinney, Edith 1911 A study of the chromosomes of Hipponoe esculenta and Moira atropos. Biol. Bull., vol. 21.

15 1918 A study of the relation of the behavior of the chromatin to development and heredity in Teleost hybrids. Jour. Morph., vol. 31.

16 Tennent, D. H. 1912 Studies in cytology. II. The behavior of the chromosomes in Arbacia-Toxopneustes crosses. Jour. Exp. Zoöl., vol. 12.

17 Wilson, E. B. 1895 Archoplasm, centrosome and chromatin in the sea-urchin's egg. Jour. Morph., vol. 11.

18 1901 Experimental studies in cytology. II. Some phenomena of fertilization and cell-division in etherized eggs. Archiv. f. Entw. Mechan., Bd. 13.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida. The optical equipment consisted of a 1.8 oil-immersion objective with a no. 8 ocular. The drawings, made at table level, at a magnification of 1600 ×, are reproduced as drawn. It is not possible to produce a drawing in two planes which will show the objects as adequately and convincingly as they appear when studied in three planes. Added to this is the difficulty of drawing such minute objects as fish chromosomes accurately. The drawings are as faithful a representation of the facts as can perhaps be hoped for under these conditions.

PLATE 1

EXPLANATION OF FIGURES

1 to 4 Fundulus heteroclitus ♀ × Ctenolabrus adspersus ♂.

1, a and b An anaphase of first cleavage in two sections. Some chromosomes omitted for clearness. All of the lagging chromatin is included in the drawings. Chromosomes of Fundulus are more numerous in a. Ctenolabrus chromosomes appear in b.

2, a and b A late anaphase in two sections. Two types of chromosomes can be recognized. There is lagging, but the amount of chromatin involved is less than that in figure 1. Not all of the chromosomes were drawn.

3, a and b Two sections of one spindle. One split chromosome is seen passing to the lower pole of a. The hooked chromosomes in a are characteristic of Ctenolabrus.

4 One section of a fairly late anaphase of second cleavage, showing an acute case of irregular distribution of chromatin. This is typical for the rest of the two spindles present in this egg.

5 to 8 Fundulus heteroclitus ♀ × Prionotus carolinus ♂. The chromosomes of both species can be identified in these drawings. The Prionotus type are short rods. There are fewer hooks than were found in Ctenolabrus.

5 An early anaphase of second cleavage. Only one of the two sections of the spindle is given. Marked lagging.

6 The middle section of a first-cleavage anaphase that appeared in the preparations in three sections. Some chromosomes omitted.

7 An early anaphase of the second cleavage. Marked lagging.

8 An older spindle of the same stage. Only one section drawn. Both types of chromosomes are seen at the poles.

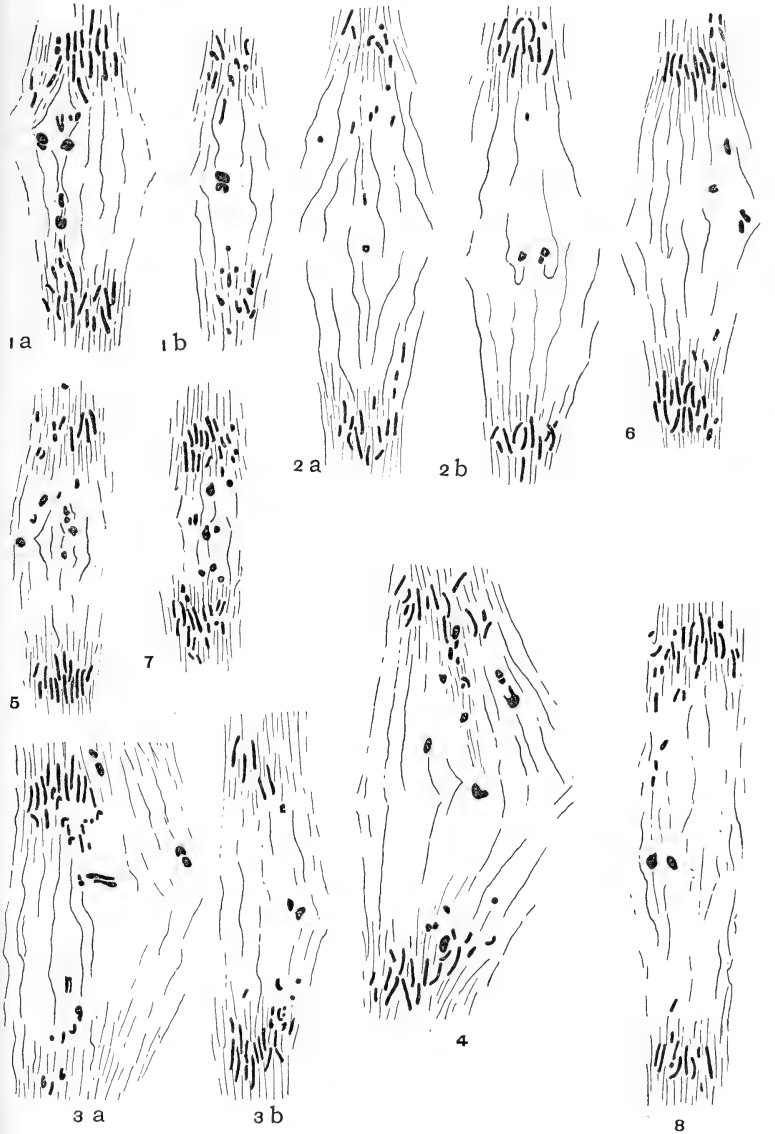

1a
1b
2a
2b
6
5
7
4
3a
3b
8

PLATE 2

EXPLANATION OF FIGURES

9 Fundulus heteroclitus ♀ × Menidia menidia notata ♂. First-cleavage anaphase. A few of the Fundulus rods have been displaced by the knife. Only one section drawn.

10 Third-cleavage anaphase of the same cross. Many chromosomes omitted. No lagging.

11, a and b Fundulus heteroclitus ♀ × Stenotomus chrysops ♂. Two sections of an anaphase of second cleavage. All of the chromosomes are drawn. There are forty-six at either pole. Those in a have been spread laterally in drawing.

12, a and b Second-cleavage spindle of the same cross. No lagging. Some chromosomes omitted in drawing.

13, a and b Ctenolabrus adspersus ♀ × Prionotus carolinus ♂. Two sections of an early anaphase of the third cleavage. The daughter halves of dividing chromosomes could be easily identified not only for those drawn, but in the case of those omitted from the drawing.

14 A later anaphase of the same cross. Third cleavage. No lagging.

15 Same as 14.

16 Prionotus carolinus ♀ × Ctenolabrus adspersus ♂. Second cleavage. No lagging.

17 Same cross. Later anaphase of second cleavage. Normal mitosis.


9


10

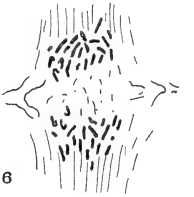

11a


11b


12a


12b


14


16


13a


13b


15


17

Resumen por el autor, Oliver P. Hay.

Sobre la filogenia del caparazón de los Testudinata y las relaciones de Dermochelys.

El presente trabajo renueva una discusión comenzada en 1899 sobre las relaciones de la tortuga con caparazón coriáceo, Dermochelys, con las otras tortugas. La primera posee en la parte dorsal del caparazon siete filas de grandes placas óseas; en la parte ventral cinco filas solamente. El estudio de otros miembros del mismo órden demuestra que estas filas están representadas por el mismo número de escudos córneos; algunas de las filas han sido halladas solamente en unas pocas especies. En casos raros existen elementos óseos debajo de estos escudos.

Las tortugas más antiguas poseían un caparazón externo (el de Dermochelys) y uno interno (el de las tortugas ordinarias). Dermochelys heredó el caparazón externo perdiendo la mayor parte del interno; las otras tortugas perdieron el externo quedando solamente vestigios. Varios autores se han opuesto a esta teoría, especialmente Verluys y sus discípulos. En el presente trabajo el autor intenta responder a sus críticas, llamando la atención acerca del caparazón del género Chelys, en el cual ha encontrado huesos distintos debajo de los escudos córneos de las cinco filas superiores (media, primera lateral y periférica) y debajo de dos de las filas del caparazón ventral (segunda fila a partir de la línea media). A consecuencia de esto el otro órden de los Testudinata consta de dos sub-órdenes, Athecae y Thecophora. La presencia de otros huesos dérmicos es de díficil explicación. Pueden ser equivalentes a los huesos encontrados en Dermochelys entre las filas de los huesos más grandes.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MAY 22

ON THE PHYLOGENY OF THE SHELL OF THE TESTUDINATA AND THE RELATIONSHIPS OF DERMOCHELYS

OLIVER P. HAY

Associate of the Carnegie Institution of Washington

ONE TEXT FIGURE AND TWO PLATES

Some years ago the testudinate genus Dermochelys was an object of interest to the writer, and he discussed the structure and origin of its peculiar shell and the systematic position of the animal (Amer. Naturalist, vol. 32, 1898, pp. 929–948. The Fossil Turtles of North America, 1908, p. 23). Since that time several important papers on the subject have been published, especially by Dr. J. Versluys and his students. The writer wishes to take up again briefly the subject. Inasmuch as Doctor Versluys' paper, "Über die Phylogenie des Panzers der Schildkröten und über die Verwandtschaft der Lederschildkröte Dermochelys coriacea" (Palaeont. Zeitschr, Bd. 1, 1914, S. 321–347), furnishes a résumé of the results obtained by himself and his coworkers, this paper only will be directly considered.

Doctor Versluys rightly emphasizes the importance of Dermochelys, recognizing that either it represents a very old lateral branch of the testudinate stem or that in its shell it presents a remarkable example of a rapidly divergent development. He concludes that the view has been confirmed which makes of Dermochelys a not very distant relative of the Cheloniidae.

Dermochelys is regarded by Doctor Versluys as belonging to the Cryptodira for two principal reasons. The first is that the neck is bent in a vertical plane, as in the Cryptodira, instead of a horizontal one, as in the Pleurodira; the second, that the individual vertebrae conform in the shapes of their articular ends to the arrangement in the Cryptodira. As to the first proposition

it may be said that the primitive testudinates had relatively undifferentiated cervicals and short necks which could be bent equally well in all directions. A retraction of the head for defense, first between the fore legs and later into the shell by bending the neck in a vertical plane, is the action that has been adopted by the great majority of turtles, not only the Cryptodira, but also the Trionychoidea. The method of protecting the head resorted to by the Pleurodira is a special one, and must have been the result of special conditions. Of living species of turtles about four-fifths bend the neck in a vertical plane, only one-fifth in a horizontal. Of known extinct species apparently many more than four-fifths belong to Cryptodira and Trionychoidea. Hence if Dermochelys was derived from an independent branch of the protestudinates, there are certainly more than four chances out of five that the species would have adopted the habit of bending the neck in a vertical plane.

To Versluys' second proposition one may reply that it is not true that the forms and the order of succession of the cervicals are as fixed in the Cryptodira, as might be supposed from his statement. The reader may consult Vaillant's paper on this subject (Ann. Sci. Nat., ser. 6, vol. 10, art. 7, pp. 1 to 106, pls. 25 to 31).

Variations in the form of the articular surfaces are found in the cervicals of other groups of turtles. In the pleurodires they are constructed so as to permit easy movement in a horizontal plane; but there exist deviations from the general plan. In the Trionychoidea (essentially Cryptodira) the typical arrangement is for all except the first and last to be convexoconcave.

Probably no one is able to say what advantages result to the cryptodires in having the fourth so generally biconvex, with those in front of it convexoconcave and those behind it concavoconvex. Versluys (p. 325) has suggested that it is in adaptation to the strong curvature of the neck during retractions of it; but in the trionychids the curvature is excessive, and here all the vertebra, except the first and the last, are convexoconcave. That it is a matter of indifference one can hardly believe. We seem to be justified in concluding that the forms of these cervi-

cals may be modified to suit the requirements of the creatures, and we need not suppose that these modifications required great periods of time. If indeed Pyxis has all of its cervicals procoelous, we can hardly conclude that its ancestors back to the primitive turtles had such cervicals.

However the ancestors of Dermochelys took their origin, the neck was, and has probably always been, short. If they formed one of the two divisions resulting from the first cleavage of the order they may have very early taken to a habitual aquatic existence. Leading such a life and possessing short necks, it is improbable that they would have developed side-bending necks. Having the same number of cervicals, each composed of the same primary elements, and experiencing the same needs in sustaining the head in swimming and in protecting it as did the Cheloniidae, there seems to be no reason why exactly the same kind of cervicals should not have been produced. If slight differences at first existed, we must suppose that these would have been eliminated in time, unless we believe that heredity prevailed over adaptability.

It will be impracticable to consider all of the seven structures which Doctor Versluys discusses as showing a probable close relationship between Dermochelys and the Cheloniidae. One may grant that his arguments possess force, without admitting that they subvert other considerations. Some of the structures, as the intertrabecula and the pouches in the nasal passages, are of obscure origin and purpose and in need of further investigation. As regards the intertrabecula, may it not have been possessed by the protestudinates and transmitted by them to the Thecophora and the Athecae alike? It may later have been lost by most members of the former group. Relatively few testudinates have been examined for this structure, and the discovery of it in any one species of Cryptodira outside the Cheloniidae, in any of the Pleurodira, or of the Trionychoidea would be fatal to the conclusion that has been drawn from its presence in the Cheloniidae and Dermochelyidae.

As to the structure of the roof of the mouth, the palatine bone, and the position of the choanae, one might easily admit all that

Doctor Versluys affirms, without admitting that his conclusion follows. A secondary palate is a possession of some turtles of all the higher divisions of the order, and there is hardly a possibility that these secondary structures have been derived in all cases from a common source. The early representatives of the Athecae were probably swamp- or coast-frequenting species and they may have subsisted on hard food; the mastication of this may well have developed a secondary palate. Having later taken more and more to life in the sea and to soft food, the palate may have gradually degenerated to its present state.

We are indebted to Doctor Versluys for the finding of a large parasphenoid bone in Dermochelys (Zool. Jahrb. Anat., Bd. 28, S. 283–294) and his discovery appears to be confirmed by two disarticulated skulls in the U. S. National Museum. Inasmuch as this bone has not been recognized in any of the other sea turtles, Versluys concluded that there was no close relationship between the Cheloniidae and Dermochelys. Certainly, if the latter genus had been derived from any of the Cheloniidae, we might expect that some of the Cretaceous members would possess a parasphenoid.

On the part of those who believe that Dermochelys and its allies have been derived from the chelonioid Cryptodira, much importance has been given to the fact that the eighth cervical in both the Cheloniidae and Dermochelys forms an articulation with the nuchal, and Doctor Versluys makes allusion to it. To the writer it appears that this articulation has lost its importance as a mark of kinship. From Versluys (p. 322, footnote) we learn that Menger has discovered that the nuchal is a composite bone, one layer of which may have been derived from the ribs of the hindermost cervical. This could hardly have come to pass without a close connection of the neural arch of that vertebra with the nuchal. Jaekel (Palaeont. Zeitschr., Bd. 2, S. 102) has found that in his Stegochelys (Triassochelys) the spinous process of the eighth cervical (Jaekel's first dorsal), as well as that of the succeeding vertebra, is attached without suture to the nuchal. In the great majority of these reptiles the connection has been dissolved; in the sea-inhabiting members of the group it has, for special reasons, been retained.

Versluys (p. 326) holds the view that, since the Cryptodira possess the thecophore shell inherited from the Amphichelydia, the primitive ancestor of Dermochelys must also have possessed such a shell, and by this there appears to be meant a practically complete shell such as that of the Cheloniidae. The present writer holds, however, that Dermochelys was not derived from the Amphichelydia and has therefore nothing to do with the cryptodires. The common progenitor of the Athecae and the Thecophora possessed the elements of the armor found now in Dermochelys; likewise, perhaps in a rudimentary form, the elements which constitute the carapace and the plastron of the other existing turtles. Proceeding from this common condition, the Thecophora lost the superficial skeleton, but developed the deeper-seated one, while in the Athecae the inner one became more and more reduced.

Versluys appears to be in doubt whether or not the epithecal armor of Dermochelys was secondarily developed. He is inclined to regard it as composed partly of new elements, partly of old. The median and costal rows of enlarged scutes of the leatherback may, he thinks, be new structures, and he refers to those epithecal bones found alternating with the neurals in Toxochelys and the more numerous ones of Archelon. He thinks it possible that new epithecal bones might arise under the horny scutes at their center of growth. This appears to be a reasonable proposition. It would provide for rows of four or five bones; but how would Doctor Versluys account for the approximately fifty bones in each of the seven rows of the carapace of Dermochelys? Where did all the little plates of bone originate that fill the spaces between the rows? If it be assumed that the species of Toxochelys were developing a new epithecal shell, two questions may be asked: 1) Why should they have been providing for themselves a new armor whilst the old one was yet in good order? 2) Those epithecal[1] neural bones had a tendency to coossify with the underlying neurals. How could a new shell be produced under such circumstances? As old useless elements

[1] These have been called by Wieland epineurals, but the term had long before been applied to very different bones in the fishes.

one can see why they might coossify with the neurals; otherwise, not.

The marginal rows of osseous elements in the armor of the leatherback are regarded by Versluys as being equivalent to the peripheral bones of the thecophores and both as belonging to the epithecal skeleton. In Archelon a supramarginal bone has been found to articulate with two of the peripherals. The supramarginal is an epithecal bone; therefore, argues Versluys, the peripherals are likewise epithecal bones. However, one might insist with equal right that these peripherals are thecal elements because in the great majority of turtles they articulate with the costal plates and with the nuchal. Horny scutes alternate in the same way with both the costal plates and the peripherals. Versluys recognizes that in the case of the other scutes they correspond with epithecal bones that have disappeared; but he appears to believe that the scutes overlying the peripherals form an exception. It would be very remarkable if the scutes once coincided with the epithecal elements and later came to alternate with them as they do with the thecal bones. We ought at least to have satisfactory evidence that such a change has been effected.

Inasmuch as the plastral bones, omitting the epiplastrals and the entoplastron, are derived from gastralia, the peripherals of each side may possibly have originated from an outer longitudinal row of gastralia.

Those investigators who have access to skeletons of the South American pleurodire Chelys are invited to make a study of its shell. In the United States National Museum there is a mounted skeleton which presents some features which appear to have a bearing on the relationships of the various groups of the Testudinata. This skeleton has the catalogue number 29545 and the record shows that the animal came from Caicara, Venezuela. As is well known, there is, on the lateral keels of the species of this genus, near the hinder border of each costal scute, an elevation, or boss. In the skeleton mentioned there is found on each of the bosses of the second scute areas, right and left, a cap of thin bone which is joined suturally to the underlying costal

bone. These plates of bone are thin, about 25 mm. long, and about half as broad. On the bosses of the other scute areas no such bones are found, but the summits of these bosses present adequate evidence that they were once capped by similar thin plates. It appears probable that some of these plates were lost in the preparation of the skeleton; others may have been absorbed during the life of the animal.

On the bosses situated on the neural bones and near the hinder end of the vertebral scutes no thin bones distinct from the neurals are found, but on each boss there is a rough and pitted surface which suggests that such a bone was once there. Coming now to the borders of the shell, we may examine the projecting points of the peripheral bones, those points which are situated at the rear of the various marginal scutes. No bones distinct from the peripherals are there found, but there are indications that such bones may have been present. On several of these points, or bosses, are found pitted surfaces, to each of which appears to have been joined by suture a bone of considerable size. On the plastron of the skeleton referred to are surfaces which suggest the former presence of thin superficial bones, and these are situated at the center of growth of each plastral scute. The one on each pectoral scute is very large and rough. If the bone was once there it may have been lost during the maceration of the shell.

From the American Museum of Natural History, New York, through the courtesy of its Department of Herpetology, the writer has received three shells of the genus Chelys. One of these, having the number 7167, is disarticulated. On this last-mentioned shell the following observations have been made.

On the fifth neural (fig. 2) there is a triangular patch of thin bone which is joined to the underlying neural by suture, but which in places around the edge appears to be coossified with the neural. The area occupied by it is about 16 mm. long and at the rear 15 mm. wide. The upper surface of this bone is rough and pitted. The thin plate has the appearance of being partially absorbed. The sulcus bounding the third vertebral scute lies behind the area described and on the sixth neural. The

presence of this bone confirms the conclusion that was reached regarding these bones on the neurals of the specimen in the United States National Museum.

On the third neural of this specimen, at the rear of the second vertebral scute, there is an area which is rough and pitted, but no overlying plate of bone is found. This has probably been completely absorbed. A smaller similar area is seen at the rear of the first vertebral scute, on the first neural. Near the rear of the fourth vertebral scute, on the peak of the high ridge there is found, lying also partly on the seventh neural and partly on the eighth, a patch which is very uneven and deeply pitted; but if there was ever an overlying plate of bone there it is now gone. On the hinder part of the narrow ridge of the surface occupied by the vertebral scute is a long rough tract, but no overlying bone is found.

Coming now to the costal bones, attention will be given first to the fourth of the right side, that costal into which is inserted the buttress of the right hypoplastron. Capping the summit of the boss forming a part of the lateral keel and near the rear of the second costal scute area is a plate of bone (fig. 3) distinctly sutured to the underlying costal. It is about 15 mm. long and nearly as wide. Where it comes to the suture between the third and fourth costal bones, it is nearly 4 mm. thick. On the corresponding elevation of the left fourth costal there is a pitted area similar in size and shape to that on the right side, but the cap of bone has either been absorbed or has fallen off during maceration. One cannot doubt that it was at some time present. Coming forward to the boss at the rear of the first costal scute area, on the second costal bone, we find a rough and deeply pitted area much like that found on the fourth costal, but no plate of bone caps it. The impression is again given that this plate has been lost in maceration. It appears to have extended forward on the first costal bone. On the corresponding boss on the right side is a surface in size and shape like that of the left side, but it is smoother. The bosses near the rear of the third and fourth costal scute areas indicate that they may once have been furnished with thin plates of bone, but of these there are now no traces.

Turning, now, our attention to the peripheral bones, we find, at the peaks of the tooth-like processes along the border, areas so similar to those found on the neurals and costals that we can hardly doubt that they were once covered each by a thin bone. These may have been lost during preparation of the skeleton. On the left fourth peripheral (fig. 4) there is a fragment of one of these bones sutured to the peripheral. It is only 10 mm. long and 4 mm. wide, but evidently it was once about 15 mm. long and 5 mm. wide. A part of it appears to have been absorbed. On the upper surface of the peripheral an impressed area extends 8 mm. from the edge, and the bone mentioned appears to have once covered this area. The latter does not show well in the figure; but on the lower face of the peripheral the impressed surface is larger and deeper. On no other peripheral is there found a separate bone, but the surfaces for receiving them are usually distinct, sometimes conspicuously so. Figure 5 of the plate presents a view of the border of the first and of a part of the second right peripherals of carapace 7167. The view is partly from below. The lines radiating from the letters, *a*, *a*, call attention to the rough surfaces which appear to have supported bony plates. Similar surfaces are present even on the projecting points of the pygal bone. These appear to have been spread out as thin laminae over the upper surface as far forward as the sulcus in front of the marginal scutes.

Another carapace (no. 6596) appears to have belonged to an old captive individual, and the borders of the shell are considerably worn, especially over the hind legs. No bones corresponding to the superficial ones above described are observable, but their former presence is in some places distinctly indicated. On the front of the nuchal scute area (fig. 6, *a*) there is, however, a bone 16 mm. long from side to side and 3.5 mm. wide. This is placed at the center of growth of the nuchal scute. The third carapace (no. 5911), apparently belonging to a species different from the others, appears to present no features that add to or subtract from what has been observed in the others.

Interesting results are secured in a study of the plastra. That of the specimen no. 7167 must first receive attention, and a

figure of it is presented (fig. 9). Beginning at the rear, there is found on the right xiphiplastral (*e*) a thin plate of bone now 30 mm. long and 10 mm. wide, but it was evidently once 6 mm. longer. The greatest thickness is 3 mm. On the left side this bone is missing, but the surface to which it was articulated is distinct. These bones, as in other cases, are situated at the center of growth of the corresponding scutes. Coming forward to the femoral scutes, it is found that nearly the whole of the outer border of each is occupied by two epithecal bones (*d*, *d*). One of these lies on the xiphiplastral, the other on the hypoplastral; but on the left side the hinder of the two bones has scaled off. The length of the two bones is 62 mm.; the breadth 11 mm.

Along the hinder border of the right abdominal scute, at the lower end of the bridge (*c*), there is a thin bone 22 mm. long, 10 mm. wide, and 4 mm. thick at the hinder end. On the left side there is no corresponding bone, but a small scar marks its position. On the hinder border of each pectoral scute at the upper end of the bridge (*b*, *b*) is a large plate of bone, the length being 45 mm., the width 20 mm., the greatest thickness 5 mm. At the outer hinder corner of the humeral scutes there is hardly any indication of the epithecal bones that might be looked for there. On the right side is a rough surface where the little plate was probably once seated. On the gular of each side is a rough surface where evidently a plate of bone was once attached. The scar on the right side is 20 mm. long and 11 mm. wide; the one on the left side is narrower (*a*, *a*).

One might expect to find some evidences of the presence of an epithecal bone within the area of the intergular scute, but none is certainly found. From the plastron of no. 6596 most of the epithecal bones have been lost. Those on the femoral scutes were not so large as in no. 1167. On the abdominal scute areas traces of them are mostly gone. On the pectorals the epithecal bones are large. On the right side the bone is missing, but there is a deeply pitted surface where it was lodged. On the left side the bone consists of two pieces, the intermediate part having probably been absorbed. The two pieces taken together measure 38 mm. in length; the rear piece is 23 mm. wide. The borders

of those scute areas and a part of that of the humerals appear to have been covered by epithecal bones; if so, the latter have disappeared. No bones or surfaces worthy of note appear at the centers of growth of the gulars and the intergular. On the plastron of no. 5911 no epithecal bones corresponding to those mentioned are found, but plain traces of most of them are present. They appear to have been thinner and usually to have been absorbed. Nearly the whole free edge of the epiplastra within the intergular scute area of the specimen in the National Museum is occupied by two or three rough surfaces to which were probably attached epithecal plates.

Some months after the preceding paragraph had been written, Dr. L. Stejneger found in his collection nearly all of the horny scutes which had been removed from the shell of the mounted specimen, no. 29545, above mentioned. These confirm the writer's conjecture that the bones interesting us had been lost from the skeleton in the course of preparation. Three vertebral scutes are preserved. On the inner surface of the first one, at the point where the bone is to be looked for, there is a patch of tissue 10 mm. long and 3 mm. wide; but, when it is thoroughly moistened and then treated with hydrochloric acid, no reaction is seen. The bone salts had probably been absorbed. The second and third vertebral scutes are not preserved. On the fourth there is a very distinct bone 14 mm. long and about 10 mm. wide. Above, it is partly exposed by abrasion of the horny scute. On the fifth scute there is distinct bone forming a patch 27 mm. long and 8 mm. wide. It is partly exposed on the upper surface. All of the costal scutes are preserved except the left second. Each of the first costal scutes bears on the under surface a large and thick patch of bone. That on the left side is 21 mm. long and 13 mm. wide. The bone of the right side is partially exposed above; that of the left side is not. As stated above, the plates of bone belonging under the second costal scutes remain on the mounted skeleton. The left third costal scute retains its plate of bone, 21 mm. long and 7 mm. wide. When a piece of it was removed and put in acid abundant gas was liberated. The scute of the right side also has its bone. Neither this nor that of the

left side has the horny scute eroded from the surface. The bone beneath each of the fifth costal scutes is small. When a fragment was dug out and treated with acid gas was liberated.

About fifteen of the marginal scutes are present. Of these nearly all retain patches of bone which correspond to the projections along the border of the carapace. These bones are partially exposed outwardly by the wearing away of the projections against objects during the movements of the animal. It has not been convenient to determine the position of all these scutes on the margin. One however, is the left eleventh; another apparently the right twelfth. One, probably the ninth left, seems to have a strip of bone 25 mm. long, which formed the edge of the carapace under that scute. At this point may be mentioned the nuchal scute. At the middle of its front border there is a fragment of bone which responds readily on the application of acid.

The scutes of the plastron are present and they bear on their inner surfaces those patches of bone which the writer judged from the marks on the mounted skeleton must have been present. As these are better displayed on specimens described below, nothing more will be said about them.

Now must be described another set of bones, the meaning of which is yet to be determined. These are small, thin, flat plates which are likely to be indicated anywhere on the surface that was covered by the horny scutes. Often the plates themselves are present and, after the bone is moistened, may be picked out of their resting places. In other cases they appear to have fallen out during maceration. Sometimes they have evidently become coossified with the surrounding bone; sometimes there is present only a scar which seems to show that long before the death of the animal the plate had been absorbed. Occasionally it is difficult to determine whether or not a depression in the bone represents one of these plates. The latter are usually more or less nearly circular or polygonal, but are sometimes irregular in form. A full-sized illustration of the lower face of the right fifth and sixth peripheral bones of no. 6596 of the American Museum of Natural History is here presented (fig. 7). A little above and to the

left of the center of the figure is a little bony plate marked by a conspicuous border. This was taken from its resting place and returned. Near it, on the right hand, is a larger patch, slightly lower than the general surface and in which there was once a little five- or six-sided plate. Near the upper left-hand corner is a pretty large irregular and rather indistinct surface which rises onto the scute area in front of it. The appearance indicates that the plate of bone which occupied it had long been absorbed. On its right again there is a little plate which has become pretty thoroughly coossified with the bone around it. At the lower end of the figure are two plates whose outlines are rather indistinct. A good many similar areas are found scattered here and there over the surface of the carapace of no. 6596. Also on the carapace 5911 a few such areas are found. On the disarticulated carapace 7167 many shallow pits are found which appear to have been filled by little plates of bone; but these may have come away with the horny scutes at the time of maceration. On this shell they appear to be clustered especially around the bosses of bone belonging to the various scute areas, but they are found also elsewhere. They do not appear to be due to any abnormal condition of the bone, and they were certainly buried under the horny scutes.

Many of these small plates which are distributed without order are found on the flat part of all of the three plastra from the American Museum. On no. 7167 (fig. 9) a number of these are seen fixed in their pits. In other cases they are gone, absorbed or lost in maceration. On the plastron of no. 6596 have been many such plates. A few remain, but of others only their impressions are left. An oval one is 10 mm. long; another apparently occupied by a single plate is still larger. On the plastron of no. 5911 are seen shallow depressions in which had rested bony plates, some of them of considerable size.

After the greater part of this paper had been written, still another specimen of Chelys was put into the writer's hands for examination. This had been in the Zoological Park for some months. It had never been known to take any food, and it probably died of starvation. Since a hole is found bored through

the hinder edge of the shell, it is judged that the animal had been kept in captivity before it was brought to this country. The length of the carapace is 400 mm. After maceration and cleaning, an examination has been made of the shell. On the carapace not as many of the scute areas have furnished epithecal bones at the centers of growth of the scutes as was hoped. Nevertheless, a thin cap of bone was found on the rear of the third vertebral scute and a small bone at the rear of the second right marginal scute and another on the left. Distinct evidence of similar bones occurs at other points where they might be expected to occur. On the plastron there is a scar on the right side of the front edge of the intergular where there may have been a plate of bone. On nearly the whole of the front of the right gular there is a surface (*a*) from which a bone was certainly lost during maceration. No plates of bones are found on the outer hinder angles of the humeral scute areas. On the outer hinder angle of the plastral portion of each of the pectoral (*b*) and the abdominal (*c*) scutes of both sides is found a large patch of thin bone. All of these bones give evidence of more or less absorption and removal. On the outer border of each femoral scute area, at about its middle, is a thin bone (*d*) 30 or more mm. long. This appears to correspond to the anterior of the two bones found on the femoral areas of the specimen shown on plate 1. The hinder one had probably long before been absorbed. On the anal scute areas no similar bones are present, but a scar (*c*) on the one of the left side may indicate the former existence of a plate.

The most conspicuous feature of this shell is the numerous smaller plates scattered irregularly all over the surface of both the upper and the lower sides. Figure 8 shows some of these of nearly the natural size on the left side of the first vertebral scute area and on parts of the adjoining scutes. Here the little bones are yet present, each in a depression in the costal bone. Nearly all of these bones are polygonal. All over the shell are presented areas where there were evidently once little flakes of bone, but these are now gone, only little pock-like scars remaining. The figure of the plastron shows the number and size of

the bones (fig. 1). In two cases the depression holding the plate makes a hole through the shell, but this is only where they lie in the course of a sulcus where the bone is thin. These little bones have a yellowish appearance, being thus somewhat different from those of the other specimens. Nevertheless, they give the usual reaction with acid, and under the microscope they show the haversian canals and the lacunae.

What interpretation is to be put on these flakes of bone it is difficult to say. It has appeared possible that they are representatives of the mosaic of bony plates which are found between the keels in Dermochelys. So far as the writer now sees, the principal argument against this explanation is the irregularity of distribution. It has been suggested by some scientific friends that they are produced by parasites, but of this the writer has seen no evidence.

Still another shell of Chelys has been found in the collection of the U. S. National Museum. This has the catalogue number 8602 and is recorded only as having come from Amazon River. On this specimen there are no traces of either the plates of bone which underlie the center of growth of the various horny scutes, nor of those smaller plates which are scattered irregularly over the shell. How to account for the condition the writer does not know. Unless there is great variation in Chelys fimbriata, this specimen must belong to another species than that of the mounted one. It is possible that now and then an individual fails to reproduce such useless vestigial structures. At least the writer believes that this case does not invalidate his explanation of the presence of the bones found at the centers of growth of the scutes. If now and then a cat should fail to have the vestigial first upper molar, this would not prove that in other cases this molar had not been inherited from the original felids.

Our study of the shells of Chelys has therefore resulted in demonstrating the presence of epithecal bones which in the writer's opinion, correspond to those of the median, first lateral, and the marginal keels of the carapace and of the outer lateral keels of the plastron of Dermochelys; besides numerous smaller

flakes of bone which possibly correspond to the plates which form the mosaic between the keels of Dermochelys. No traces of the supramarginal and inframarginal keels are found. The presence of the bones of the marginal keels, as shown by distinct sutural surfaces and by the actual bones, suffices to prove that the peripheral bones of Cryptodira and Pleurodira are not epithecals, but belong to the same category as the costal plates, the neurals and the nuchal.

Dr. Otto Jaekel described in 1915 (Palaeont. Zeitschr., Bd. 2, S. 88–112) a remarkable and finely preserved turtle from the Trias of Germany. He is to be congratulated on having the opportunity to study such an important specimen and on his results. Unfortunately, the part of the Zeitschrift which contains the conclusion of his paper has not been received at Washington. Some remarks will be made here on that part at hand. Doctor Jaekel named this animal Stegochelys dux; but, inasmuch as this generic name was preoccupied, he later proposed instead the name Triassochelys (Abel, Die Stamme der Wirbeltiere, 1919, pp. 386–392, figs.)

In case Doctor Jaekel means, as he doubtless does, that he has been able to furnish corroborative evidence that the plastron of the Testudinata is composed of the clavicles and the interclavicle and of abdominal ribs (gastralia), his statement is readily accepted; but certainly there was previously little doubt about its composition. The present writer in 1898 (Amer. Naturalist, vol. 32, p. 934) assumed this view and made no claims of originality therefor. In the writer's paper referred to, he attempted (p. 946) to determine the number of gastralia that had entered into the formation of the plastron. This number, three or four pairs, is indeed small; and naturally, in case the number recorded by Jaekel, about twenty-five in each of the anteroposterior rows, is confirmed, the writer's calculations will be discredited.

The type of Triassochelys was evidently a fully mature, probably an old animal; and, like many of the ancient testudinates, it appears to have had most of the various bones of the shell thoroughly coossified. With the exception of the sutures between

the gastralia, none appears to be with certainty described. The plastron appears to have been solidly united with the carapace and no suture appears to separate the gastralia along the midline. Under such conditions, how can it be assumed that there were no hyoplastra, no mesoplastra, no hypoplastra, and no xiphiplastra? Is it probable that this turtle, which in most features resembles so closely other well-known forms, differed from them all in having none of the ordinary plastral bones, except the front ones, but instead of these a plastron composed of distinct and little modified gastralia?

Jaekel finds that the gastralia of Triassochelys diverged as they passed from the bridges toward the midline, and he gives an explanation of the divergence. If, now, this plastron represents a primitive condition from which, through segregation and consolidation of the gastralia, were produced definitive plastrals, how are we to explain the fact that in those turtles which possess mesoplastrals the sutures between the plastral bones converge as they are followed toward the midline? They appear, therefore, not to have followed the sutures between the gastralia, but to have struck across them at varying angles.

There can be no doubt that Triassochelys is closely related to Proganochelys. In this Triassic turtle Fraas (Jahresh. Ver. vaterl. Naturk., vol. 55, 1899, p. 416, pls. VII and VIII) convinced himself that there was present a pair of mesoplastrals, greatly expanded at the outer ends. It seems that later Doctor Jaekel (Placochelys placodonta, 1907, p. 59) succeeded in shaking Fraas's confidence in his determinations; but it appears to the present writer that the probabilities are in favor of their approximate correctness. How Jaekel's observations are to be harmonized with the views here expressed the writer does not at present comprehend. It may be noted in passing that Doctor Jaekel was in error when he stated that Fraas believed that there were in Proganochelys two pairs of mesoplastrals.

Doctor Jaekel concluded that in Triassochelys the pectoral scutes were missing. There appear to be no sufficient reasons for this conclusion. The great scutes which bound the notches for the fore legs are surely pectorals. In front of these scutes

there is abundant room for humerals, gulars, and even intergulars. The last-mentioned two pairs of scutes are applied to the epiplastra and the front of the entoplastron, as may be seen in figures of the Pleurosternidae and Baënidae (Hay, Fossil Turtles of N. A., 1908). These bones in Triassochelys were evidently small, and the gulars and intergulars were correspondingly small. To the writer it seems quite probable that the front of the plastron of Jaekel's specimen broke off along the humeropectoral sulcus.

Doctor Jaekel tells us (p. 106, fig. 9) that in his Triassochelys there are on each side of the carapace seventeen peripheral bones and that the marginal scutes correspond to these in number and in their boundaries. These are statements of such importance scientifically that they ought to be supported by unquestionable evidence. Although Doctor Jaekel states that these peripherals are very distinctly set off from each other and from the costals, he does not say that the bone sutures are present. Unless the sutures are to be seen, the limits of the bones are indeterminable. The condition of the shell in general indicates that the sutures are closed. What sets the areas off from one another is probably only the sulci between the marginal scutes. Indeed, Jaekel (p. 199, fig. 23) informs us that such is the case. If the reader will examine the figures in the writer's work of 1908, referred to above, which illustrate the structure of the Baënidae (apparently not distant relatives of Triassochelys), or will take a look at a shell of one of the Chelydridae or a shell of Chelys, he will find that the sulci between the marginal scutes cross the borders of the carapace at the notches, while the bone sutures cross between the notches. In the Baënidae there are often some small apparently supernumerary scutes at the front of the carapace. These appear to correspond to the little scutes which Doctor Jaekel has counted as the first and second in his series. At the rear of the carapace of Baëna the supracaudal scutes have been suppressed, along with the pygal bone. In Triassochelys these supracaudal scutes are present, but much reduced in size. In this way we may account for the unusual number of marginal scutes in Triassochelys. In that animal

there were, however, in all probability not more than eleven peripheral bones on each side.

Fraas (op. cit., p. 409, fig. 1; reproduced by Jaekel) has indicated the presence of twenty or more marginal scutes in Proganochelys; but if there were really present lines which marked out the boundaries between these areas, some of them were probably bone sutures; others sulci between the marginal scutes.

The results sought after in this paper may be summed up as follows:

1. The neck of the leatherback has not been inherited from the cryptodires, but has been independently developed.

2. The evidences relied on to connect the leatherback with the chelonioid sea-turtles, living or extinct, are by no means compelling.

3. Vestigial bones have been discovered in the Thecophora which correspond to those of the following keels in Dermochelys: the upper median (Toxochelys, Archelon, Chelys); the costal (Chelys); the supramarginal (Archelon), the marginal (Chelys), and the first lateral of the plastron (Chelys). The supramarginal keels are represented in many species by scute areas also. The inframarginal keels are known to us only from scute areas on the bridges. The lower median keel may be retained in the unpaired intergular of the Pleurodira, the intercaudal (Abel op. cit. p. 410, fig. 319) and occasional unpaired scutes in other turtles.

4. By the presence of vestigial bones on the peripherals at the points whence the marginal scutes expand it is shown that these peripherals are not to be homologized with the marginal bones of Dermochelys, but that they belong to the thecal armor.

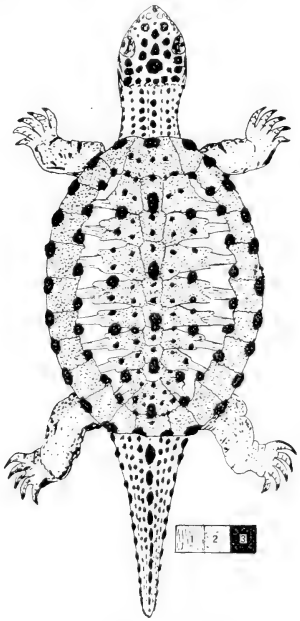
5. The occurrence of the various elements representing the epithecal armor in species scattered about in nearly all the large groups of turtles, and most of them provided with good solid shells, appears to show that these elements are vestiges of an armor of a common ancestor and not the beginnings of a new epithecal one.

6. The retention of the epithecal covering by Dermochelys, the loss of most of the thecal shell, and the possession of many

other structural peculiarities indicate that the ancestors of this turtle early parted company with the rest of the order.

7. The order of Testudinata is composed of two suborders, Athecae and Thecophora.

Doctor Versluys has presented a figure which was designed to show his conception of the composition of the carapace of the



Text Figure

primitive testudinate. The present writer has taken the liberty to modify the figure so that it shall present in a way his own views regarding the structure of the carapace of this interesting and theoretical animal. In addition to the epithecal bones shown on the carapace, the tail, and the neck, the writer has indicated a number on the head which underlay its horny plates. It appears evident that Jaekel's Triassochelys possessed a num-

ber of such bones scattered over its skull, but at its stage of life these had doubtless become consolidated with the underlying bones.

It may be that the costal plates ought to be represented as coming down to the peripherals. It appears to be assumed that fontanelles in the carapace are the result of reduction of the costal plates and peripherals and that this reduction, as well as a flattening of the whole body, is due to an aquatic existence; but we have lately learned that an African species of Testudo has suffered a nearly complete loss of its shell and has at the same time become excessively flattened (C. R. Acad. Paris, vol. 170, 1920, p. 263). It appears not unreasonable to suppose that in the most primitive turtles the costal plates had not yet joined the peripherals; perhaps not yet the neurals.

PLATE 1

EXPLANATION OF FIGURES

1 Shell of Chelys, seen from below. *a–e*, position of epithecal bones; *a*, scar on area of right gular scute; *b*, bone on area of pectoral scute; *c*, bone on area of abdominal scute; *d*, bone on area of femoral scute; *e*, scar on area of anal scute. $\times \frac{5}{16}$.

2 Fifth neural bone, showing epithecal plate. No. 7167, Amer. Mus. Nat. Hist. $\times$ 1.

3 Right fourth costal bone, showing presence of an epithecal bony plate. Same specimen as the preceding. $\times$ 1.

4 Left fourth peripheral, showing the upper surface. *a*, small, apparently partly absorbed, epithecal bone on the free edge of the peripheral. Its extension on the upper surface not well shown. Same specimen as that of figs. 2 and 3. $\times$ 1.

5 Free border of first and second right peripherals, showing inner face. *a. a.* surface for articulation of epithecal bones at focus of first and second marginal scutes; *b*, site of small epithecal bone. $\times$ 1.

6 Front of carapace showing nuchal bone and right first peripheral, loweer serface. *a*, epithecal bone at the focus of the nuchal scute; *b*, sites of buried epithecal plates. No. 6596, Amer. Mus. Nat. Hist. $\times$ 1.

7 Undersdie of parts of the right fifth and sixth peripherals of specimen no. 6596 (Amer. Mus. Nat. Hist.), showing numerous epithecal bones. $\times$ 1.

8 A part of the left half of first vertebral scute, with parts of second marginal and first costal. $\times$ 1.

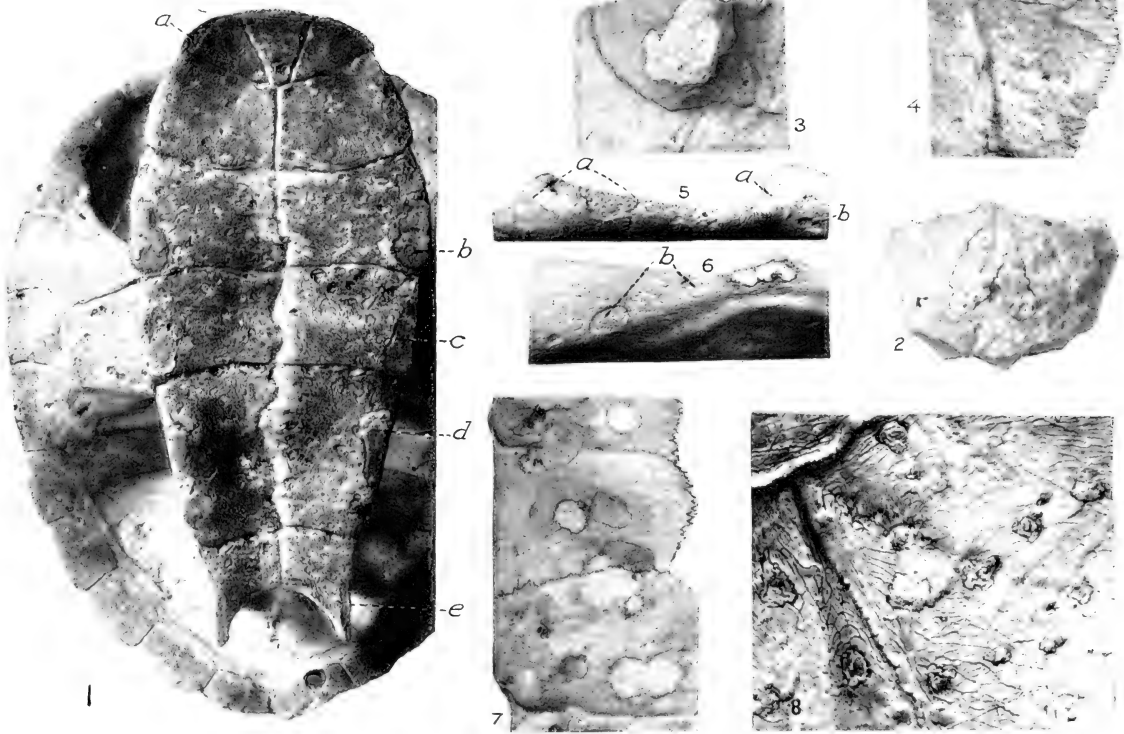
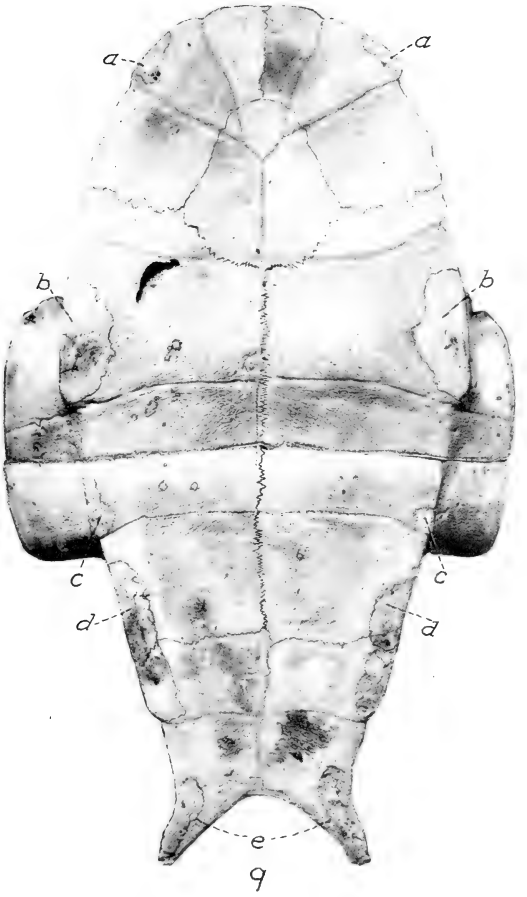

a
b
c
d
e
1
3
a
a
5
b
4
b
6
2
7
8

PLATE 2

EXPLANATION OF FIGURE

9 Lower surface of plastron of Chelys. No. 7167, Amer. Mus. Nat. Hist. Showing epithecal bones on the scute areas. *a, a,* on areas of gular scutes; *b, b,* on areas of pectoral scutes; *c, c,* on areas of abdominal scutes; *d, d,* on areas of femoral scutes; *e, e,* on area of anal scutes.


a
a
b
b
c
c
d
d
e
9

Resumen por el autor, Alden B. Dawson.

La topografía de la cloaca del macho de Necturus en relación con las glándulas cloacales.

El orificio externo de la cloaca del macho de Necturus es una hendidura longitudinal franjeada por dos labios poco desarrollados los cuales en su extremo caudal llevan un par de papilas blandas. Los labios están más modificados a consecuencia de la presencia de numerosas fisuras transversas. En posición inmediatamente dorsal al orificio cloacal está la cámara cloacal o vestíbulo que se continúa cranialmente en el tubo cloacal. El piso de este último tiene forma de artesa honda, con la mucosa surcada por crestas delgadas y paralelas las cuales se interrumpen caudalmente convergiendo en las papilas altas y delgadas presentes a los lados de la cámara cloacal. El techo está modificado también por la presencia de un surco medio profundo y a cada lado del tubo cloacal, entre esta depresión dorsal y la ventral, existen dos surcos longitudinales.

La cavidad cloacal está por completo rodeada por masas de glándulas tubulares largas y tortuosas. La gran masa media ventral se conoce con el nombre de glándula cloacal. Sus túbulos se abren en las cimas de las crestas paralelas y en los ápices de las papilas delgadas internas. Dos masas de túbulos, las glándulas abdominales pares, están situadas ventrolateralmente a la cámara cloacal y sus túbulos desembocan en la superficie media de las papilas externas pares. Dorsalmente existe una masa glandular media, la glándula media. Esta glándula presenta por lo menos cuatro diferenciaciones, que se distinguen histológicamente por el carácter del epitelio que tapiza sus túbulos. Existe una pequeña masa media cranial, una masa media caudal muy grande y dos masas laterales. Todos los túbulos de la masa de la glándula pélvica se abren en el techo del tubo cloacal. El autor considera un método posible de formación de un espermatóforo.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACTS OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MAY 1

THE CLOACA AND CLOACAL GLANDS OF THE MALE NECTURUS

ALDEN B. DAWSON

Department of Anatomy, Loyola University School of Medicine

THREE PLATES (SIXTEEN FIGURES)

INTRODUCTION

At present the mating habits of Necturus are not definitely known. Strong circumstantial evidence indicates (Kingsbury, '95) that fertilization is accomplished by the deposition of spermatophores and the reception of the spermatozoa which are borne upon the summits of the deposited spermatophores into the cloaca of the female. The time and the exact manner of insemination are not known. An abundance of spermatozoa was found by Kingsbury ('95) in the spermathecae of six females which he examined during the late fall and winter. Females examined by the writer in October and March were found also to have large numbers of sperms in their spermathecae. According to Smith ('11), fertilized eggs are deposited chiefly during May and June.

Although our information on the time and manner of fertilization is still incomplete, it seems highly probable that spermatophores are produced by the male Necturus. The matrix of the spermatophores is probably a product of the cloacal wall acting in conjunction with the surrounding masses of tubular glands. The degree of glandular activity in this region should furnish therefore some clue as to the probable time of spermatophore deposition. With this in mind, a study of the cloaca was undertaken. Owing, however, to the complexity of the internal configuration of the cloaca, the complicated relations of the cloacal wall to the tubules of the surrounding gland masses, and the many varying types of tubules encountered, the comparative study of the glandular activity at different times of the year had

to be postponed until the limits of the different masses of glands had been definitely determined. Accordingly, the present report deals primarily with the various masses of tubular glands in their relation to one another and to the topography of the cloaca. In a later communication it is planned to describe the variations which occur in the glands during the different seasons of the year and to follow the changes undergone by the several types of cells during the production of secretion.

Only adult males were used in this study. The material was dissected out and fixed in either formalin, Zenker's fluid, or Bouin's fluid. Serial sections, transverse and longitudinal, were made of the entire cloacal mass, including the cloaca proper and the surrounding glands. The tissue was stained with haematoxylin and eosin, Van Gieson's picro-acid fuchsin and Mallory's stain for connective tissue.

LITERATURE

We are indebted to Heidenhain ('90) for the first detailed and accurate description of the cloaca of a male urodele. He described three kinds of cloacal glands in the male Triton, the so-called cloacal gland, the pelvic gland, and the abdominal gland. Before this but two types of glands were recognized. Zur Mühlen ('93), who worked on Triton, Salamandra, and Siredon, confirmed in the main the findings of Heidenhain. Kingsbury ('95), in the course of an extended study of the cloacas of female Diemyctylus, Plethodon, Desmognathus, Amblystoma, and Necturus, discussed, incidentally for purposes of comparison, the structure of the cloacas and the adjacent glands of the males of these different genera. In Necturus, Kingsbury did not make a sufficiently careful study of the glands to enable him to determine whether the abdominal gland is present.

EXTERNAL APPEARANCE OF THE CLOACA

The external opening of the cloaca of the male Necturus is simply a longitudinal slit bordered by two inconspicuous lips which, at their caudal ends, give rise to a pair of low rounded papillae (fig. 1, *ext.p.*). The lips are modified further by

numerous transverse fissures and, immediately caudad to the paired external papillae, there is a distinct transverse crescentic groove. A ventral enlargement extending laterally along the cloacal slit and cranially toward the region of the pelvic girdle marks the extent of the large cloacal gland.

INTERNAL TOPOGRAPHY OF THE CLOACAL CAVITY

For purposes of description, the cavity of the cloaca may be considered as consisting of two portions, an enlarged caudal chamber or vestibule opening ventrally to the exterior by way of the cloacal slit and a narrower cephalic, tubular portion connecting the cloacal chamber with the rectum (figs. 4, 5, *cl.ch.*, *cl.t.*).

The internal configuration of the cloaca is decidedly complex, but in an undistended condition the cavity exhibits a very definite and constant form. The various depressions, folds, papillae, etc., which go to produce the complicated pattern of the cavity serve therefore as landmarks of the different regions into which the tubular glands discharge their secretion.

Before entering upon the more detailed description of the several regions of the cloaca, brief mention will be made of the most conspicuous modifications of the cloacal wall. The ventral side of the cloacal tube has the form of a deep, narrow trough, the mucosa of which is thrown into high, thin ridges (figs. 4, 5, 12, 13, 14, *v.tr.*, *v.r.*). Caudally, in the region of the cloacal chamber, the ventral ridges are interrupted and merge into tall, slender papillae (figs. 4, 5, 15, *int.pp.*). Dorsally the cloacal tube contains a deep median groove (figs. 4, 5, 13, 14, *md.gr.*) and on its sides between the dorsal groove and the ventral trough are two well-defined longitudinal furrows (fig. 13, *lt.fur.*).

The cephalic end of the cloacal tube presents the simplest condition, and the transition from rectum to cloaca occurs without any very evident change in structure. The urogenital ducts open dorsolaterally into the extreme cephalic end of the tube. They terminate separately in a pair of prominent papillae which project ventrally from the bottoms of two pit-like depressions (figs. 4, 5, 7, *ug.p.*). The urinary bladder opens

medially into the ventral side of the cloaca, almost opposite the more dorsal urogenital papillae (figs. 4, 5, 8, *ur.bl.o.*).

Caudad to the orifice of the urinary bladder a prominent longitudinal fold projects from the midventral wall, and on either side of it other smaller irregular folds can be distinguished (fig. 5, *mv.f.*, *v.f.*). The main fold continues caudally for a short distance as a single fold, but soon becomes doubled and is eventually broken up into the thin ridges which cover the walls of the ventral trough (figs. 5, 9, 10, *v.tr.*, *v.r.*). Laterally the wall of the cloacal tube is also modified by two low folds which extend, on either side, from the regions of the urogenital papillae caudally to the cephalic ends of the longitudinal lateral furrows, with whose dorsal walls they merge (figs. 4, 9, *lt.f.*). Furthermore, when the cavity of the cloaca is laid open by a longitudinal ventral incision so that the dorsal portion of the cloacal tube is exposed, the lateral folds, with the aid of the median dorsal groove and lateral furrows, are seen to mark off a Y-shaped area, the stem of which extends cephalad toward the region of the urogenital papillae (fig. 4, *Y*).

Another striking feature of the cephalic portion of the cloacal tube is the presence of large numbers of melanophores in the underlying connective tissue. No other portion of the cloacal cavity exhibits a like pigmentation, although a few scattered melanophores can occasionally be seen in other regions.

The ventral trough, longitudinal lateral folds, and median dorsal groove already referred to, are found in the more caudal portion of the cloacal tube. The ridges of the ventral trough are relatively high and thin. They run almost parallel, but diverge slightly as they approach the cloacal chamber. The number of ridges present is quite constant, the average being thirty-two, although thirty-four ridges can occasionally be counted (figs. 13, 14, *v.r.*). The longitudinal lateral furrows and median dorsal groove do not exhibit any conspicuous modifications and, gradually growing shallower as they pass caudally, are eventually obliterated in the region of the cloacal chamber.

The cloacal chamber itself is relatively simple in form, two rather deep ventrolateral recesses being the only modifications of interest in this study (figs. 4, 5, 15, *vl.rec.*). The long, slender papillae, found on the floor and ventral portions of the walls of the chamber, are also present in the ventrolateral recesses. The papillae in the recesses, however, are usually short. Both the internal papillae and the ventral ridges are highly vascular, being permeated by blood channels of considerable size.

THE WALL OF THE CLOACA

The wall of the cloaca, especially in its cephalic portion, closely resembles that of the rectum. Mucous and muscular layers are readily recognized (figs. 7, 8). No serous coat, however, is present, but the outermost layer consists of areolar tissue which blends with the connective tissue of the adjacent structures. The presence of large numbers of long tubular glands, which surround and open into the cloacal cavity, has resulted in a great thickening and extensive modification of practically the entire cloacal wall and of the three coats comprising it, but the tunica muscularis has suffered the greatest displacement.

a. The tubular glands

The grouping of great numbers of tubular glands in the cloacal wall has resulted in the production of a large glandular mass about the cloaca, which, for the lack of a better term, will be designated as the cloacal gland mass. This mass lies caudad to the pelvic girdle and occupies a large median ventral area. It is enclosed in a connective-tissue sheath which apparently is a modified portion of the median ventral septum which more caudally separates the hypaxial muscles of the tail (fig. 3, *m.v.s.*).

The dorsal portion of the mass extends close to the trunk-tail vertebrae and laterally is bounded in part by the unmodified trunk-tail myotomes and in part by three pairs of slender caudal muscles (mm. ischiocaudalis, caudalifemoralis, and caudalipuboischiotibialis, Wilder, '12) which are attached to the posterior appendicular skeleton (fig. 2). Cranially, the dorsal gland mass extends to the posterior ends of the mesonephroi and to the caudal

margin of the pelvic girdle. Dorsocaudally, the common sheaths of the three pairs of caudal muscles and the unmodified median ventral septum limit the mass.

The ventral portion of the gland mass is continued into the loose subcutaneous connective tissue, extending laterally beyond the median area bounded by the hypaxial muscles and, cephalically, to cover the surface of the caudal portion of the pelvic girdle.

In the cloacal mass of the urodeles studied (Heidenhain, '90; Zur Mühlen, '93; Kingsbury, '95) at least three distinct types of tubule have been recognized. They are arranged in definite groups and are known as the cloacal, pelvic, and abdominal glands, respectively. In Necturus both the cloacal and pelvic glands are greatly developed. The homolog of the abdominal gland can also be recognized, but it is relatively small and separated into two compact lateral masses (figs. 2, 3, 16, *abd.gl.*).

The cloacal-gland tubules form the large median ventral portion of the cloacal mass (figs. 11 to 15, *cl.gl.*). The tubules are long and straight. They extend in a cranial direction and end blindly. The mouths of the tubules open both on the summits of the thin ridges covering the ventral trough of the cloacal tube, and on the tips of the slender internal papillae which fringe the cloacal chamber. On the ridges the tubules terminate in low conical elevations which are arranged longitudinally to form two parallel rows. The terminal elevations which compose these double rows on each ridge are not placed opposite to each other, but have a regular alternating arrangement. The tubules which are connected with the internal papillae also exhibit a regular arrangement, usually two and occasionally three opening together at the tip of each papilla.

The large group of tubules comprising the dorsal portion of the cloacal mass has been designated as the pelvic gland. They are sharply separated from the ventral cloacal tubules by two lateral connective-tissue septa (fig. 2, *c.t.s.*). The pelvic-gland tubules, in contrast with the relatively straight cloacal tubules, are distinctly convoluted. They extend dorsocranially,

do not branch, and end blindly. In fresh material they appear opaque, while tubules of the cloacal gland usually appear clear. The difference in appearance is due to the different character of the secretion in their lumina.

All of the tubules of the pelvic gland open into the dorsal portion of the cloacal tube. They are arranged in four groups: a small cephalic medial group of short tubules (fig. 10, *plv.gl.''*); further caudad, two symmetrical, lateral groups of somewhat longer tubules (figs. 11, 12, *plv.gl.'''*), and a very large median caudal group of long, greatly convoluted tubules (figs. 11, 12, 13, 14, *plv.gl.'*). The caudal portion constitutes the greater part of the pelvic gland. The tubules which form the lateral differentiations of the pelvic gland are comparatively few in number. They are distributed cephalocaudally on either side of the cloacal tube and lie close to the lateral septa (*c.t.s.*) which separate the main mass of the pelvic gland from the more ventral cloacal gland.

The different groups of pelvic tubules are not distinctly separated from one another in any portion of the gland, but are distinguished by the character of their glandular epithelium. Owing to the great variety of secretory phases exhibited by the different tubules, it is not always easy to determine with certainty whether the tubules under consideration are of an entirely different character or are merely different phases of activity of the same kind of tubule. It is with some hesitation, therefore, that I have distinguished a median cephalic group, since cephalad to the main mass of the caudal division of the pelvic gland the tubules of its lateral differentiations approach the middorsal line, and in serial sections are seen to be intermingled with the more caudal tubules of the cephalic group. However, so far as my histological study has progressed at this time, there appears to be good evidence that the tubules of the groups under discussion, although intermingled where they come in contact, possess secreting cells of two distinct types. The tubules of the median caudal division, on the other hand, can be readily recognized at all times.

All pelvic tubules terminate in low papillae. At the bases of these papillae shallow circular depressions are usually observed recalling the structure of the circumvallate papillae of the tongue. In some regions papillae are indistinct and only barely recognizable. The tubules of the large median caudal division, for the most part, open upon the walls of the median dorsal groove (figs. 13, 14, 15). The more cranial tubules of the lateral pelvic differentiations open on the dorsal walls of the so-called longitudinal furrows (figs. 12, 13), while the most caudal ones are found to open upon a middorsal region, the caudal end of the stem of the Y-shaped area previously described (figs. 11, 12). The short tubules of the median cephalic group terminate on the middorsal region which forms the cranial portion of the stem of the Y-shaped area (fig. 10).

In comparison with the cloacal and pelvic glands, the abdominal gland in Necturus appears almost vestigial. It is divided into two masses which lie near the caudal end of the cloacal orifice and dorsolaterally to the paired external papillae (figs. 2, 3, *abd.gl.*). The tubules which compose this gland are short and greatly convoluted and possess a characteristic epithelium which distinguishes them definitely from the other tubules of the cloacal mass (fig. 16). They open mainly on the medial surfaces of the external papillae, but a few are also found to open along the inner margins of the cloacal lips.

b. Muscular layers and dorsal ganglion

The muscular coat consists of two layers of smooth muscle which, in the extreme cephalic portion of the cloacal tube, are sharply differentiated into an inner circular and an outer longitudinal layer (figs. 7, 8). Further caudad, however, this definite arrangement is more or less disturbed by the presence of a large, dorsal, ganglionated plexus and the numerous tubular glands.

The dorsal ganglion represents a local enlargement of a portion of the sympathetic nervous system, being apparently a caudal continuation of the myenteric plexus of the intestinal tube (figs. 9, 10). From the ganglion small bundles of nerve fibers pass caudally to the various cloacal glands.

Heidenhain ('90) observed a like mass of nerve tissue occupying a somewhat similar position in the male Triton and, according to him, it is found only in the males. He was in doubt as to the function of the ganglion, but interpreted it as being a terminal enlargement of the ganglionated plexus associated with the kidneys, and suggested that it might be a portion of the adrenal system which is more or less diffuse in urodeles. "Wohin diese Ganglienmassen zu rechnen sind (Nebenniere?), ist mir unbekannt" (p. 190). In some specimens of Necturus I have found scattered cells which exhibit a specific affinity for chromium.

The tubular glands extend deep into the cloacal wall, of which, as has been already stated, they form the most conspicuous part. In the dorsal portion of the wall the tubules of the pelvic gland obliterate the sharp differentiation of the muscularis into two layers, and the muscle fibers are irregularly arranged and interwoven, forming with the intermingled connective tissue a dense fibromuscular stroma in which the secreting tubules are imbedded. Some of the muscle cells of the stroma, however, are arranged circularly about the numerous tubules to form delicate muscular tunics.

The tubules of the cloacal gland, on the other hand, while as large and closely packed as those of the pelvic gland, do not produce such a decided rearrangement of muscle fibers, so that, in the ventral portion of the cloacal wall, a circular as well as a longitudinal layer can usually be distinguished. The tubules pierce only the circular muscle layer and are imbedded in a fibromuscular stroma similar to that described for the pelvic gland. Each tubule is also surrounded by a delicate layer of circularly arranged muscle cells. The ventral portion of the longitudinal coat of muscle fibers is not invaded by the cloacal tubules, but persists as a compact layer, arranged as a flat sheet to cover the ventral or external surface of the cloacal gland (figs. 11, 12, 13, 14). In the region of the cloacal chamber the definite arrangement of the smooth muscle into layers is gradually lost and the fibers are mingled with the connective tissue of the cloacal wall.

c. Epithelium of cloaca

The epithelium lining the cloaca of the male Necturus is not simple in any region. In the cephalic portion it is two-layered, consisting of a superficial columnar or cuboidal layer and a deep somewhat flattened replacing layer. In certain areas the outer layer of cells is ciliated; in others, the outer cells are of the tall mucous type, and in still others, they are unmodified. More caudad the number of cell layers is gradually increased until at the margins of the cloacal aperture a stratified epithelium similar to that of the external surface of the body is found. No Leydig cells, however, are present within the cloaca, although they occur in considerable numbers in the epidermis a short distance from the cloacal orifice.

Dorsally, in the region of the paired urogenital papillae, a small ciliated area is found. More cephalad, toward the rectum, the epithelium is non-ciliated and of the mucous type. Caudad, on the portions of the dorsal wall through which the pelvic tubules open, i.e., on the Y-shaped area, the median dorsal groove and the dorsal walls of the lateral furrows, the superficial cells, for the most part, are unmodified, resembling in their staining reactions the cells which in other areas possess cilia. Locally, however, groups of tall clear cells, typically mucous in appearance, are found. It seems possible, therefore, that the unmodified cells may be young or rejuvenating mucous cells.

Ventrally, in the region of the orifice of the urinary bladder, the cloacal wall is covered by a mucous epithelium, but more caudad ciliated cells are found, chiefly along the summits of the longitudinal folds. Also scattered patches of ciliated epithelium link up the ventral ciliated portions with the dorsal ciliated area. The high, thin ridges of the ventral trough are for the most part covered with a two-layered ciliated epithelium, but the conical elevations, on which the cloacal tubules terminate, have a mucous epithelium. The transition from one type of epithelium to the other is abrupt.

The slender internal papillae, through which the cloacal tubules open, with the exceptions of small areas at their bases, do

not have a ciliated epithelium. The more cephalic papillae are covered by a two-layered mucous epithelium, while those nearer the external aperture have a stratified epithelium of three to four layers similar to that covering the unmodified wall of the cloacal chamber and the cloacal lips.

DISCUSSION

In Necturus, spermatozoa are regularly found within the cloaca of the female. The transfer of spermatozoa from the body of the male to that of the female is supposedly accomplished by means of spermatophores. In Cryptobranchus, however, fertilization is external and the sperms are expelled into the water without the formation of spermatophores (Smith, '07). In Diemyctylus (Jordan, '01) and Amblystoma (Wright and Allen, '09) spermatophores are deposited and the female by her own activity must ensure the entrance of the spermatozoa into her cloaca. In the Tritons and in Desmognathus (Wilder, '13) the transfer of the spermatophore is accomplished by a venter to venter copulation.

Just what rôle the greatly developed cloacal glands of the male Necturus play in the mingling of the sexual products it is difficult to say. The success of the spermatophore method depends largely on the proximity of the female. In some urodeles specialized integumental glands are believed to attract the opposite sex. No such glands have been found in the integument of Necturus (Dawson, '20). Some of the cloacal glands, accordingly, may perform this function. The abdominal glands, on account of their superficial position, would not apparently enter into the spermatophore formation. They may, therefore, liberate chemicals which diffuse through the water and attract the female or, if the spermatophores are transferred directly to the female by a venter to venter copulation, these glands, together with the external papillae on whose median surfaces they open, may assist in overcoming the difficulties involved in sperm transfer in the water, the external papillae forming a kind of intromittent organ.

The configuration of the cavity of the cloaca, the arrangement of the glands and the positions of the ciliated areas make the theory of spermatophore formation in Necturus seem plausible and tend to stimulate speculation. The mucous secretion of the cloacal gland when liberated into the ventral trough would be gradually moved caudally by the cilia on the ridges and would eventually collect in the cloacal chamber and the ventrolateral recesses projecting from it. In this position the mass of mucous material would probably be increased by additional secretion from the cloacal tubules which terminate on the internal papillae. Dorsolaterally in the cloacal tube, the ripe sperm would be expelled from the urogenital ducts and the median dorsal ciliated area would carry them back until they became mingled, first, with the secretion of the median cephalic portion of the pelvic gland and later with the secretions of the lateral and caudal portions of this same gland. By this time the sperms would be in the dorsal groove and far enough caudad to be caught up by the moving mass of mucous secretion which is propelled caudad by the cilia of the ventral ridges. In this manner a spermatophore, having as a base a mass of mucous secretion and bearing on its dorsal surface spermatozoa mingled with secretion from the pelvic gland tubules, might be formed. The final solution of this problem must await direct observation in the field.

Early writers attempted to homologize the cloacal glands of urodeles with the prostate and bulbo-urethral glands of the higher mammals. Any such homology has been denied by Heidenhain ('90) and Kingsbury ('95). In attempting to discover homologies it seems unwise to begin with what are obviously specializations of some more simple arrangement, and the prostate and bulbo-urethral glands doubtless represent such specialization. A more primitive condition is seen in both monotremes and marsupials, in which urethral glands, tubular glands occurring in the wall of the urogenital canal, are abundant. In monotremes there is a common cloaca with a primitive penis projecting slightly from its ventral wall. From this simple organ it is believed the typical penis of mammals has been derived and it is also regarded as homologous with the intromittent organ of

turtles and crocodiles which develops from the ventral wall of the cloaca. The cloaca of urodeles is doubtless homologous with the cloaca of both reptiles and mammals, and from the ventral portion of this the special organ of copulation has been evolved. The cloacal glands of urodeles and the urethral glands of monotremes and marsupials perform the same function, i.e., furnish a fluid or semifluid vehicle for the spermatozoa, but this similarity of function is not sufficient to establish the homology, since we have many instances of similar structures performing the same function in different groups of vertebrates, but they are not homologous. However, even if the homology between the cloacal glands of urodeles and the urethral glands of lower mammals cannot be established, it is at least interesting to note that in such widely separated groups of vertebrates the same type of gland has been evolved in a similar position to serve apparently similar needs.

BIBLIOGRAPHY

Dawson, A. B. 1920 The integument of Necturus maculosus. Jour. Morph., vol. 34, pp. 487–589, 6 pls.

Heidenhain, M. 1890 Beiträge zur Kenntnis der Topographie und Histologie der Kloake und ihrer drüsigen Adnexa bei den einheimischen Tritonen. Arch. f. mikr. Anat., Bd. 35, S. 173–266, Taf. 10–13.

Jordan, E. O. 1891 The spermatophores of Diemyctylus. Jour. Morph., vol. 5, pp. 263–270.

Kingsbury, B. F. 1895 The spermathecae and methods of fertilization in some American newts and salamanders. Trans. Am. Micr. Soc., vol. 17, pp. 261–304.

Smith, B. G. 1907 The life history and habits of Cryptobranchus allegheniensis. Biol. Bull., vol. 13, pp. 5–39.

1911 Nests and larvae of Necturus. Biol. Bull., vol. 20, pp. 191–200.

Wilder, H. H. 1912 The appendicular muscles of Necturus maculosus. Zool Jahrb., Suppl. 15 (Festschrift für J. W. Spengel, Bd. 2), S. 383–424, Taf. 23–27.

Wilder, I. W. 1913 The life history of Desmognasthus fusca. Biol. Bull., vol. 24., pp. 251–341.

Wright, A. H., and Allen, A. A. 1909 Early breeding habits of Amblystoma punctatum. Am. Nat., vol. 43.

Zur Mühlen, Alex. v. 1893 Untersuchungen über den Urogenitalapparat der Urodelen. Dissert., Dorpat, 62 pp.

DESCRIPTION OF PLATES

ABBREVIATIONS

abd.gl., abdominal gland
c. mu., circular muscle layer
cl. ap., cloacal aperture
cl. ch., cloacal chamber
cl. gl., cloacal gland
cl.gl.a., cloacal gland, area of
cl.lp., cloacal lip
cl.lp.f., cloacal lip, fissures of
cl. t., cloacal tube
c.t.s., connective-tissue septum
d.g., dorsal ganglion
ext.p., external papilla
int.pp., internal papillae
lt.f., lateral fold
lt.fur., lateral furrow
l.mu., longitudinal muscle layer
md.gr., median dorsal groove
md.g.w., median dorsal groove, wall of
mv.f., median ventral fold
mv.s., median ventral septum
mes., mesonephros
m.cf., muscle caudalifemoralis, impression of
m. cpit., muscle caudalipuboischiotibialis, impression of
m.isc., muscle ischiocaudalis, impression of
pl.gl.' pelvic gland, median caudal division
pl.gl.'', pelvic gland, median cranial division
pl.gl.''', pelvic gland, lateral division
p., peritoneum
rectum, rectum
t.c.gr., transverse crescentic groove
ug.d., urogenital duct
ug.p., urogenital papilla
ur.bl., urinary bladder
ur.bl.cav., urinary bladder, cavity of
ur.bl.o., urinary bladder, orifice of
ur.t., urinary tubules
vl.rec., ventrolateral recess
v.f., secondary ventral folds
v.r., ventral ridges
v.tr., ventral trough
Y, Y-shaped area

PLATE 1

EXPLANATION OF FIGURES

1 Ventral view of the pelvic region of a male Necturus, showing the superficial topography of the cloaca. Drawn from a live animal in May.

2 Lateral view of the entire gland mass. Dissected from a specimen which had been hardened in alcohol.

3 Dorsal view of the entire gland mass. Dissected from a specimen which had been hardened in alcohol.

4 Dorsal view of the cloacal cavity. The cloaca was slit longitudinally, slightly to one side of the midventral line, and laid open. The cloacal gland is completely divided. Dissected from a specimen which had been hardened in alcohol.

5 Ventral view of the cloacal cavity, laid open by a longitudinal incision along the middorsal line. Pelvic gland is completely divided. Dissected from a specimen which had been hardened in alcohol.

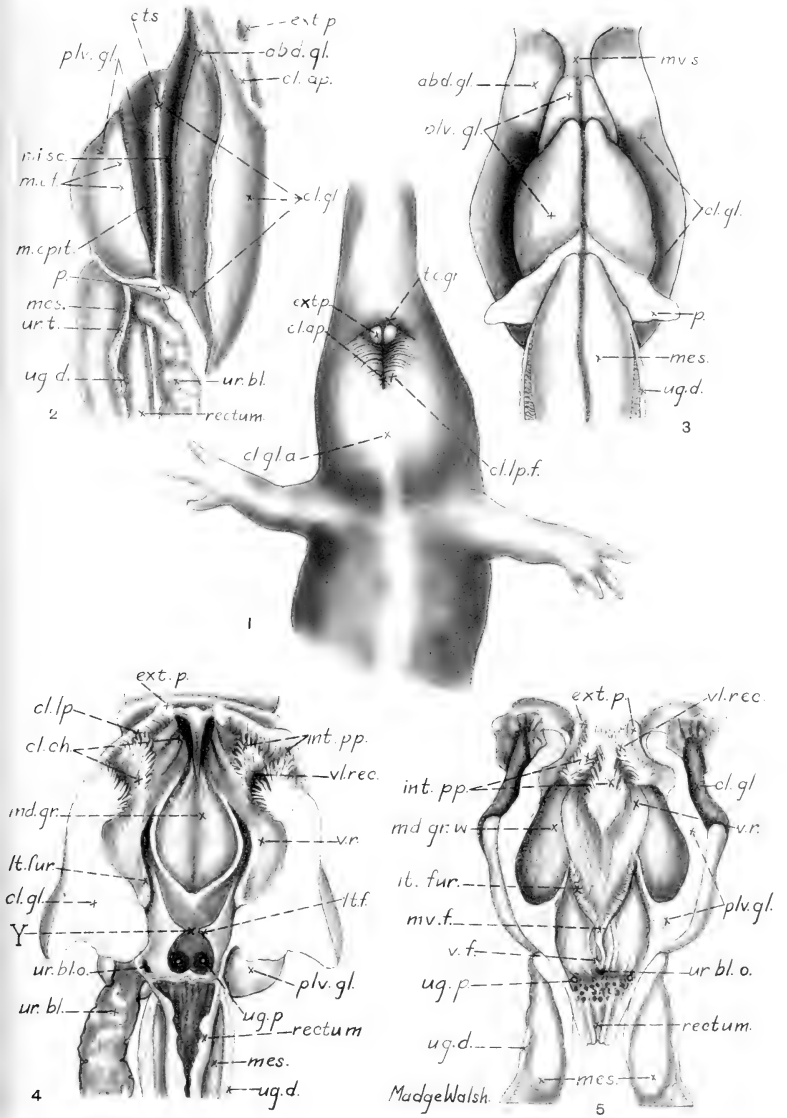

c.ts
ext.p
plv.gl.
abd.gl.
cl.ap.
m.i.sc.
m.v.f.
cl.gl.
m.cpit.
p.
mes.
ur.t.
ug.d.
ur.bl.
rectum
2
mv.s
abd.gl
plv.gl.
cl.gl.
p.
mes.
ug.d.
3
t.c.gl
ext.p
cl.ap.
cl.gl.a
cl.lp.f.
1
ext.p.
cl.lp
cl.ch.
int.pp.
vl.rec.
md.gr.
v.r.
lt.fur.
cl.gl.
lt.f.
Y
ur.bl.o.
ur.bl.
plv.gl.
ug.p
rectum
mes.
ug.d.
4
ext.p
vl.rec
int.pp.
cl.gl
md.gr.w
v.r.
lt. fur.
plv.gl.
mv.f.
v.f.
ur.bl.o.
ug.p
rectum.
ug.d.
mes.
Madge Walsh.
5

PLATE 2

EXPLANATION OF FIGURES

6 An outline sketch of figure 5, showing the position and plane of section of the sections represented in figures 7 to 17. The number at the end of each line corresponds with the number of the plate figure representing that level.

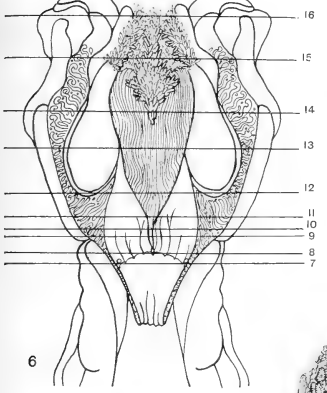
7 Transverse section cutting the cloaca at the level of the urogenital papillae.

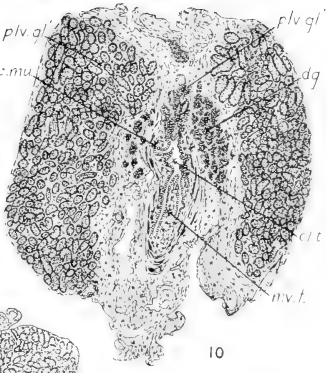
8 Transverse section cutting the cloaca at the level of the orifice of the urinary bladder.

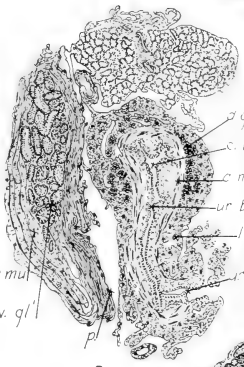
9 Transverse section of cloaca cutting the caudal end of the mesonephros and passing through the dorsal ganglion in the region of its greatest extent.

10 Transverse section of the cloaca, showing the tubules of the cranial differentiation of a pelvic gland.

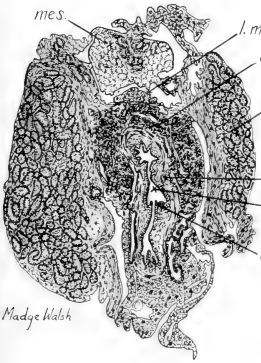
11 Transverse section of cloaca through the cranial ends of the lateral furrows and ventral trough, showing the tubules of the lateral differentiations of the pelvic gland.


16
15
14
13
12
11
10
9
8
7
6


plv. gl'
plv. gl'
c.mu.
dg
c.l.t
m.v.t.
10


dg
c. l
c. mu
ur. bl. c.
l. mu
str. mu
ur. bl. cav.
plv. gl'l
pl
8


mes
ug.p.
c. mu.
cl.t.
l.mu
ur.bl.
7


mes
l.mu
dg.
plv gl'
c. mu.
cl.t.
lt. f.
Madge Walsh
9

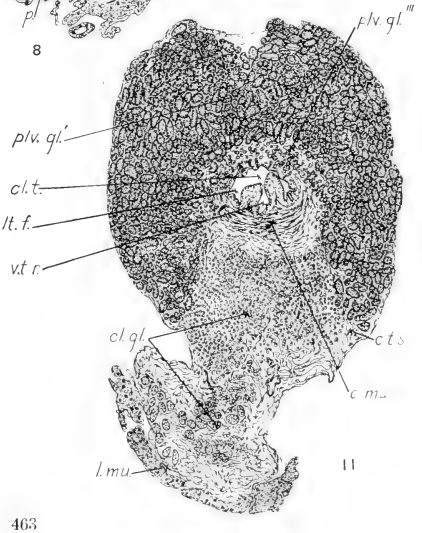

plv. gl.'''
plv. gl.'
cl.t.
lt.f.
v.t.r.
cl.gl.
c.t.s
c. mu
l.mu
11

PLATE 3

EXPLANATION OF FIGURES

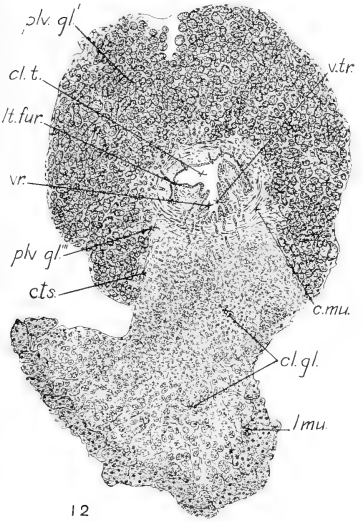
12 Transverse section of the cloaca immediately anterior to the cranial end of the median dorsal groove.

13 Transverse section of the cloaca showing the median dorsal groove, lateral furrows and ventral trough.

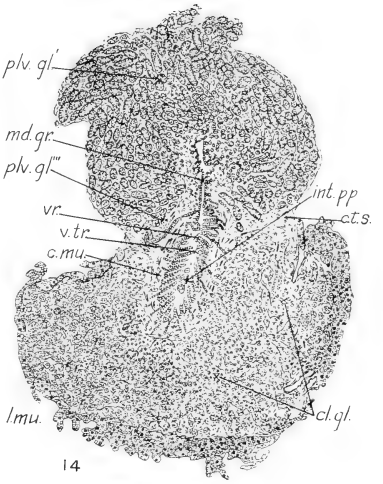
14 Transverse section through the cloaca caudal to the extent of the lateral furrows and immediately cranial to the chamber of the cloaca.

15 Transverse section of the cloacal chamber, showing the ventrolateral recesses.

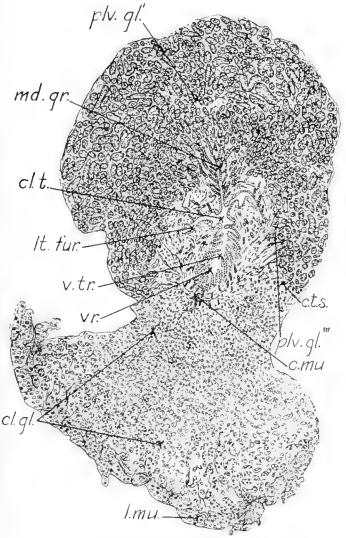
16 Transverse section through the caudal end of the cloacal slit showing the paired masses of abdominal gland tubules.



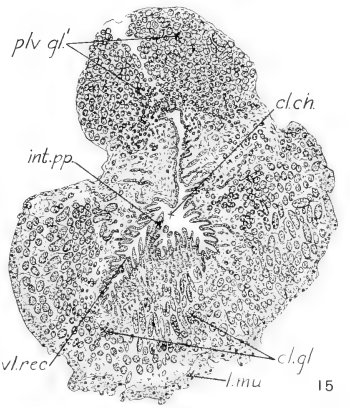
12



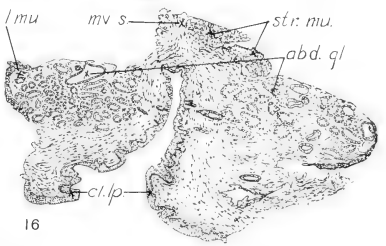
14



Madge Walsh. 13



15



16

Resumen por el autor, H. Hibbard.

Inclusiones citoplásmicas en el óvulo de Echinarachnius parma.

Una comparación entre el citoplasma de los huevos de Echinarachnius fecundados por sus mismos espermatozoides y los fecundados con espermatozoides de Arbacia no ha demostrado la existencia de diferencias visibles. El autor ha llevado a cabo un estudio del citoplasma del óvulo antes de la fecundación y en diferentes intervalos después de esta. Ha podido comprobar la existencia de tres tipos de inclusiones: 1) Deutoplasma en forma de gotitas de grasa situadas cerca del núcleo y también en forma de esferas vitelinas muy numerosas esparcidas por el citoplasma; 2) Mitocondrias, y 3) Grandes precipitados de material coloide coloreable con la hematoxilina ferruginosa después de la fijación en licor picroacético o en sublimado acético. Estos últimos corpúsculos pueden encontrarse en el óvulo no fecundado cuando su citoplasma está en estado soluble (sol) pero cesan de formarse cuando el citoplasma se transforma en una gelatina (gel) durante su preparación para la primera división. No se conoce nada más acerca de la naturaleza de estos precipitados. Existen pruebas que indican que las gotas de grasa situadas cerca del núcleo se fragmentan en pequeñísimas gotitas que se esparcen por la célula y producen las mitocondrias, y que estas a su vez son instrumentales en la formación de los corpúsculos vitelinos. Estos últimos desaparecen gradualmente cuando son absorbidos por el óvulo durante los procesos de la segmentación.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, MAY 22

CYTOPLASMIC INCLUSIONS IN THE EGG OF ECHINARACHNIUS PARMA

HOPE HIBBARD

Bryn Mawr College

ONE TEXT FIGURE AND FOUR PLATES (TWENTY-FOUR FIGURES)

CONTENTS

INTRODUCTION

The cytoplasm of the egg has attracted a great deal of attention among cytologists during the last few years, in contrast to the almost universal attention paid to the nucleus before that time. A great many observations on cytoplasmic inclusions have been made, but there is a distinct lack of coördination of the results of such work. Cowdry, in his valuable contribution to the literature on mitochondria, has summed up and correlated the observations and conclusions of various authors regarding these structures. Numerous other bodies occurring in the cytoplasm have been reported, but usually investigators have given merely a description of the morphology and staining reactions of these bodies. There have been, however, attempts to consider cytoplasmic inclusions in the light of the physiology of the

cell and to trace the interrelations of distinct bodies through different stages. The possible transformation of mitochondria into secretion granules, pigment, yolk, etc. (Cowdry gives a list of eighty such things into which mitochondria have been reported to change), and the cycle described by Schreiner ('15), in which fragments of the nucleolus wander into the cytoplasm, unite into vegetative threads, and break up into secondary granules which are ultimately transformed into fat drops, are instances of these attempts to consider visible structures as steps in the physiological processes of the cell.

The processes of metabolism in the cell necessarily include activities of both nucleus and cytoplasm. The part played by the nucleus is not fully understood. Some investigators have described particles of material passing out of the nucleus into the cytoplasm. These particles have been regarded as chromatin (Schaxel, '11; Danchakoff, '16), or as fragments of the nucleolus (Schreiner, '15; Nakahara, '17; Walker and Tozer, '09; Hargitt, '19, etc.). According to other investigators, the nucleus acts on the cytoplasmic substrate by liberating enzymes, which diffuse through the nuclear membrane and permeate the cytoplasm. Tennent ('20) has found in Arbacia eggs fertilized by Moira sperm, precipitates in the cytoplasm which are interpreted as the result of enzymes from the nucleus brought in by the foreign sperm.

The present work was undertaken in the hope of demonstrating more exactly the relation between the nucleus and the cytoplasm by comparing the cytoplasmic contents of an egg fertilized by sperm of its own species with that of an egg fertilized by sperm of another species. It was thought possible that the cytoplasm when acted on by two different types of nuclear enzymes might show visible differences. In the study of the particular cross made, Echinarachnius $\times$ Arbacia, no such visible differences between the self-fertilized and the cross-fertilized eggs have been found. This does not invalidate the conclusion that the nucleus gives out enzymes into the cytoplasm. It probably indicates that in the particular cross used here the enzymes of the foreign sperm were so much like those of the species sperm that no

visible differences in effect occurred. It should be remembered, however, that minor chemical variations may easily occur without giving rise to visible differences.

Attention was then directed to the cytoplasmic contents of the egg before fertilizaton and the changes which occur after fertilization and during the early stages of development. The study of the cytoplasm of the egg has certain peculiar advantages over the study of the cytoplasm of tissue cells. To be sure, one does not find secretion granules or other structures associated with specialized function, but there are instead those substances necessary for processes of development and differentiation. By the use of various methods, several types of inclusions have been demonstrated and certain conclusions regarding their part in the general metabolism of the cell have been reached.

The work was undertaken at the suggestion of Dr. David Hilt Tennent and pursued under his direction. It is a great pleasure to express my appreciation of his constant and stimulating supervision throughout the course of the investigation.

PREPARATION OF MATERIAL

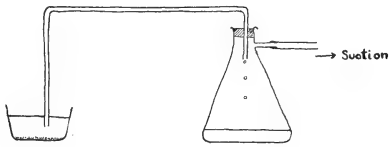
The material for this work was collected, fixed, and imbedded at Woods Hole during July and the early part of August, 1920. The particular eggs used were those of the sand-dollar, Echinarachnius parma. They were fertilized by Echinarachnius sperm or Arbacia sperm, and in all cases parallel series were kept of the self-fertilized and cross-fertilized eggs. Just ('19) has shown that when the eggs are normally shed into sea-water they may be cross-fertilized without special treatment. However, it is very rarely that Echinarachnius females can be obtained at Woods Hole which will shed their eggs. To stimulate them to shed, the test is clipped around the circumference with scissors and the animal placed aboral face down on a watch-glass. Although this was done with individuals from practically every lot of sand-dollars brought in from June 28th to August 4th, only one animal was obtained during that time which shed eggs. Therefore, it was necessary to open the test and shake out the ovaries in sea-

water. The eggs of this form are mature when shed or when they easily shake out of the ovary, and may be fertilized by species sperm as soon as they are clean, but eggs need further treatment before cross-fertilization. To clean from bits of ovarian tissue and coelomic fluid, the water in the finger-bowl in which the ovaries have been shaken is stirred, then allowed to settle for a brief period and the supernatant water poured off. The finger-bowl is then refilled with fresh sea-water and the process repeated several times. In this way the heavier eggs which settle are kept, while the lighter debris is poured off. Since these eggs were not normally fertilizable by Arbacia sperm, it was necessary to resort to some artificial means of breaking down the cortical resistance. As in other eggs, there are three methods of breaking down this resistance, namely, 1) by staling the eggs, 2) by over-insemination, and, 3) by the use of alkali. For the purpose of this work the third method was employed. To determine the optimum concentration of alkali, two experiments were performed. Varying amounts of n/10 NaOH in one case were added to sea-water, and n/10 KOH in the other. After fertilization by Arbacia sperm, the percentage of development was recorded. Very little difference was found between the NaOH and the KOH. The standard strength of twenty drops of n/10 NaOH per 150 cc. of sea-water was adopted as the proportion of alkali yielding the best results. The alkaline sea-water was added to the cleaned eggs from which most of the water had been poured, and as soon as possible the sperm suspension was added and the contents of the dish well mixed. As soon as the eggs had settled, the supernatant liquid containing excess sperm and alkali was drawn off by means of a suction flask connected as figured.

By this method all the liquid save about 8 cc. could be quickly withdrawn, leaving the eggs undisturbed on the bottom of the finger-bowl. Fresh sea-water was then added. After self-fertilization the supernatant water was similarly withdrawn and replaced by fresh sea-water.

In any one series of eggs preserved the eggs from only one female were used and they were fertilized by sperm from one

male of the same species or from one male Arbacia. In every case three finger-bowls were kept. In one, kept as a control, were placed eggs, 150 cc. of sea-water, and no sperm; in the second, eggs fertilized by species sperm, and in the third, eggs fertilized by Arbacia sperm by the aid of alkali. A sample of unfertilized eggs was preserved, and from the second and third finger-bowls samples were preserved at varying intervals after insemination. For example, series 4 was fixed every fifteen minutes, series 9 every twenty minutes, series 17 every ten minutes, etc. The original bowls were kept until the following day and examined to make sure that no contamination had occurred before actual insemination, as proved by the failure in every case of the eggs in the control to form fertilization membranes or to cleave. In



order to prevent chance fertilization, hands, instruments, and dishes were washed in fresh water before opening each animal. In addition, the animal itself was rinsed in fresh water and then in sterilized sea-water before being opened. Frequently all dishes, pipettes, and instruments were put into a large kettle and boiled.

The fixing fluids used were picro-acetic (saturated aqueous picric acid 95 parts, glacial acetic acid 5 parts), sublimate-acetic (saturated aqueous corrosive sublimate 100 parts, glacial acetic acid 5 parts), Bouin's fluid, Allen's warm modified Bouin (Bouin 50 cc. urea 1 gram, chromic acid 0.75 gram) made up immediately before using, Perenyi's fluid, Meves' fluid (Lee, Vade-Mecum, 7th ed., p. 328), Champy's fluid (3 per cent potassium bichromate 7 parts, 1 per cent chromic acid 7 parts, 2 per cent osmic acid 4 parts), Cajal's fluids, Helly's Zenker-formol, strong Flemming,

and Flemming without acetic acid. In the last-named fluid one series was fixed for one day and another for seven days. The fixation in Perenyi and Cajal was very poor, and therefore the material was discarded. All the material was imbedded at Woods Hole in soft paraffin, then taken to Bryn Mawr, reimbedded, sectioned, and stained. The sections were for the most part 4μ in thickness.

The stains employed were Heidenhain's iron hematoxylin, Auerbach's acid fuchsin-methyl green, lithium carmine and Lyons blue, basic fuchsin and methylene blue, Benda's alizarin and crystal violet, safranin, safranin and gentian violet and orange G, and for special tests, sudan III and Ziehl's carbol-fuchsin. Samples of material fixed in solutions containing osmic acid were also mounted unstained. Of these stains, the iron hematoxylin and the Benda stain proved the most satisfactory and were the most widely employed. In making up the alizarin the directions given in Guyer's Animal Micrology were followed rather than those given by Benda himself or by Cowdry in describing Benda's method. Guyer gives the following formula for Benda's solution of sulphalizarinate of soda: 1 part of saturated aqueous solution of stain to 80–100 parts of water. Benda's own directions are to add 1 part of a saturated alcoholic solution of the stain to 80–100 parts of water. Both methods were tried but Grübler's sulphalizarinate of soda was found to be practically insoluble in alcohol. The stain made from the saturated aqueous solution of the dye gave excellent results.

OBSERVATIONS

As has been mentioned above, there were no visible differences between the self-fertilized and the cross-fertilized eggs. Any given method of fixation followed by the same stains gave identical results in the two cases. In order to compare them the better, sections of self-fertilized eggs and sections of cross-fertilized eggs were mounted side by side on the same slide. This insured exactly the same degree of staining.

A number of structures were found in the cytoplasm following different methods of fixation and staining. All the bodies

found could be demonstrated in the unfertilized egg, but some of them changed or disappeared during subsequent stages of development. Where there were progressive changes in the cleavage stages, a whole series was mounted on one slide. Thus any variation due to differences in technique of staining was eliminated, since all stages of the same series received the same treatment.

Gatenby ('19 b) and others have classified cell inclusions in two main groups: first, inert inclusions like deutoplasm and, second, active or living inclusions like mitochondria. As deutoplasm are classed fat, glycogen, yolk, etc. The egg of Echinarachnius parma contains a considerable amount of fat. The glycogen, if there had been any, would have been dissolved out by the technique employed in preparing this material. The cytoplasm is packed with spherical or plate-like bodies of nutritive material which is identified as yolk. Active inclusions in the form of mitochondria have been demonstrated. There is still another type of structure found in these eggs. It is an inert inclusion and yet is not deutoplasm. It will be considered under the heading 'Precipitations.' The occurrence of these substances will be considered more in detail.

A. Deutoplasmic inclusions

1. Fat. It is known (Partington and Huntingford, '21) that fat droplets reduce osmic acid to osmium dioxide and assume, therefore, a dense black appearance after the use of a fixing fluid containing osmic acid. Accordingly, eggs which had been fixed in Fleming, Flemming without acetic, or Meves' fluid were mounted unstained and examined for fat. No fat was found in the material which had been fixed for seven days, but in the Flemming and in the Flemming without acetic material which had been fixed for eighteen to twenty-four hours there were numerous black bodies. Figure 1 shows an unfertilized egg fixed in Flemming without acetic and mounted unstained. There are in the cytoplasm large blackened masses surrounded by fine droplets of blackened material of uniform size, and in some cases there are clumps of fine droplets without any central larger drop.

This emulsified condition is a characteristic of fats. It seems probable that the original large drop of fat is being split up into smaller parts and that the scattered fine particles of blackened material throughout the cell have been formed by such emulsification of larger masses.

Sudan III, a specific stain for fat, was used on this material, but gave no decisive results because the drops had been previously blackened and naturally could not be stained red. A further proof of their fatty character was obtained by soaking the sections for twenty-four hours in oil of turpentine. After this treatment the black droplets were completely dissolved out. Since turpentine is a fat solvent, the material which was removed was probably fat.

The large groups of fat droplets are slightly more numerous in the region of the nucleus than they are in the more distant parts of the cytoplasm. This is of interest in the light of the views of Schreiner, Popoff, and others, that granules of nuclear origin pass through the membrane and give rise to fat droplets. The granules are believed to come from the nucleolus. The evidence given here of the accumulation of fat near the nucleus shows nothing more than the fact that they are associated with some kind of nuclear activity. It is also true that an occasional oocyte has been found on the slides in which there is always a large nucleolus present which is entirely absent in the ripe egg.

The continued splitting of the large drops into minute droplets and the dispersal of the latter through the cytoplasm is illustrated in figures 2 and 3. These show eggs from the same series from which figure 1 was drawn, in stages twenty-five minutes and one hour and forty minutes, respectively, after insemination. As development proceeds, there is a gradual decrease in the amount of blackened material present. As the cell prepares for the first division the fat droplets are much fewer, and in the two-celled stage none are visible. It is highly probable that these cells do not show as much fat as is present in the living egg, because some of it must have been dissolved out in the processes of preparation, but the fact that a definite series of changes can be demonstrated is a true indication of what actually occurs.

2. Glycogen. As was mentioned formerly, no glycogen was demonstrated in these eggs. No material was fixed by any of the methods for the preservation of glycogen.

3. Nutritive plates (yolk). By far the most conspicuous and unusual inclusions are those which are best demonstrated after fixation in Flemming without acetic, Meves' or Champy's fluids, and staining in iron hematoxylin or Benda's alizarin and crystal violet. These bodies are shown in figures 1 to 6, 12, or 19 to 24. In figure 12, drawn from an egg fixed in Champy's fluid, they are distinctly plate-like and much larger than in any other lot of material. The more usual appearance is shown in figures 19 to 24, where they are smaller and less distinctly plates. Their fate indicates that they are nutritive in function. They do not, however, respond to all the usual tests for yolk. At first their staining reaction seems to mark them as mitochondria, for in the series illustrated in figures 19 to 24 the fixation is Flemming without acetic and they are stained a deep violet with the Benda stain. They do not always give this reaction, however. The series described above was fixed for seven days. If it be compared with the series shown in figures 4 to 6, which is also Flemming without acetic, but fixed for eighteen to twenty-four hours only, it will be seen that the large bodies are there in both cases, but they differ in staining capacity. They take the violet stain strongly after seven days' fixation, but are pink after one day's fixation. The behavior of these two series when stained with iron hematoxylin shows great dissimilarity also. In the first case the plates are black and in the second they do not stain. Since they are so striking in appearance in some series of eggs, their apparent absence after other fixatives was unaccountable until it was found that in practically every case the material of which these plates are formed could be shown in the cytoplasm even though not in the form of such distinct separate bodies. For instance, when stained in iron hematoxylin after fixation in modified Bouin, the cytoplasm had a decidedly reticular appearance with minute black granules throughout (fig. 18); in the same material stained in Benda's stain (fig. 15), or in iron hema-

toxylin and basic fuchsin, the cytoplasm had a mottled appearance. A distinct difference between the cytoplasm of the center of the egg in which the division figure lies and the peripheral cytoplasm may be seen. This regional differentiation corresponds to the distribution of the nutritive bodies fixed so distinctly in Flemming without acetic. This shows that the nutritive material is present after modified Bouin fixation. Similar results were found after sublimate-acetic fixation. After strong Flemming followed by safranin, gentian violet, and orange G, this nutritive material takes up the orange stain more strongly than any other cell constituent. Therefore, while certain fixing fluids are decidedly more favorable for the demonstration of these plates, the material is not dissolved completely by the other reagents used.

Yolk is the only substance ordinarily found in the cytoplasm of the egg in great quantities comparable to this material. But these plates do not respond to all the tests for yolk given by Miss Beckwith ('14). For instance, yolk turns black after Flemming fixation and iron-hematoxylin stain. These bodies are not black. Yolk is definitely fixed by picro-acetic and sublimate-acetic. Gatenby ('19 b) states that in some animals yolk discs take a deep violet after Benda's stain. He points out that this staining reaction may be due to protein in the yolk in addition to lecithin. The yolk plates in the egg of Echinarachnius, however, do not invariably stain a deep violet, but do so only after prolonged fixation. Some proof of the nature and function of these bodies may be obtained by tracing them through the early cleavage stages to the blastula. They become gradually fewer in number and in the blastula they are almost gone. Figures 19 to 24 show progressive stages in which they have become fewer. Figure 24 illustrates a stage four hours after insemination in which there are spaces left which the large plates formerly occupied and in which many of the minute granules are stained violet. This may be due to the breaking up of the larger masses by a process of digestion. The disappearance of this material is not confined to those series which have been fixed in Flemming without acetic Meves, and Champy. Figure 11 (picro-acetic) shows a much

more spongy and vacuolated cytoplasm than the earlier stages of the series, which indicates a loss of some substance. Figures 16 and 17 show a similar phenomenon in modified Bouin material. Therefore, these plates are of a nutritive character and are used up by the cell during processes of development.

They contain a certain amount of fat, as is shown by staining with sudan III. Fifteen minutes' staining with alcoholic sudan III gives them a very decided salmon-pink color. After soaking for twenty-four hours in oil of turpentine, the sections stained in sudan III gave no color whatever, even when the stain was allowed to act for thirty minutes. There was evidently some fat there which was removed by the turpentine. After treatment with turpentine the plates retained their characteristic form and appearance except that they lacked the capacity to take up sudan III. The nutritive plates are probably of complex chemical structure.

While the nutritive plates do not in all cases behave like yolk with regard to staining reactions, yet their obvious function and their shape and distribution point to the conclusion that they must be yolk.

From their method of origin these yolk plates may be linked up with the other cell inclusions. This point will be considered after the discussion of mitochondria.

B. Living inclusions

Mitochondria. In addition to the above deutoplasmic inclusions, there are also active, living constituents which are distinct from the ground cytoplasm. These are the mitochondria. The stain which differentiates them most successfully from other cell inclusions is Benda's alizarin and crystal violet. They show up a deep violet against a background of neutral pink. Iron hematoxylin blackens them, but it also blackens other cell granules. With basic fuchsin and methylene blue they are red, with safranin they are red. It is possible to demonstrate mitochondria after fixation in Flemming, Flemming without acetic, Meves, Champy, and modified Bouin. They are not found

after ordinary Bouin fixation. The shape and distribution of mitochondria in the eggs of Echinarachnius are shown in figures 13 to 17 which were drawn from a series of eggs fixed in modified Bouin and stained in Benda's alizarin and crystal violet, and in figures 4, 5, and 6 which were drawn from a series fixed in Flemming without acetic followed by the Benda stain.

The study of this material indicates that the mitochondria bear a definite relation to the fine uniform fat droplets described above. In figure 4 there is shown an unfertilized egg fixed in Flemming without acetic and stained according to Benda's method in which the mitochondria were deep violet. They are shown as black granules in the figure. The egg also contains granules which appear the same in size, shape, and distribution but which take the violet stain with varying degrees of intensity or which may be quite pink like the ground cytoplasm. There is also a continuous variation in color from the small fat droplets which are brown from osmic-acid impregnation, through similar granules which are less and less brown, to pink granules. It seems probable, therefore, that the small fat droplets which are formed by emulsification of larger fat drops change gradually, as indicated by differential staining, into the bodies which take a deep violet stain after Benda's method and are identified as mitochondria.

In the modified Bouin material, the mitochondria are stained a deep violet with the Benda stain. They are the granules illustrated in figures 13 to 17. These same eggs when stained with iron hematoxylin show many more black granules than can be identified as mitochondria. Figure 18 shows such an egg thirty minutes after insemination which contains a larger number of black granules than the number of mitochondria shown in figures 14 and 15. No granules stain with sudan III after this fixation.

The relation between the mitochondria and the nutritive plates may be considered here. The great mass of plates is already formed by the time the egg is ripe, but there is some indication of how they may be formed. In the eggs from which figures 4, 5, and 6 were drawn the mitochondria were stained violet and the nutritive plates pink. In figure 4 and more especially in figures 5 and 6 some of the mitochondria show pale centers. In fact,

sometimes the inside distinctly took the pink alizarin stain. This is interpreted as showing the formation of the nutritive plates, or yolk, from mitochondria. The nutritive material accumulates at the center and increases until it is nearly as large as one of the numerous yolk plates. When this occurs the mitochondrial remnant is found as a delicate violet rim around the surface of the plate, or there may be in addition a larger bit of the violet-stained material clinging to one side.

There have thus been followed the fat drops which are emulsified into minute fat droplets and are distributed through the cytoplasm where they are probably transformed by some kind of synthesis into mitochondria. These mitochondria in turn build up within themselves the large nutritive plates which furnish energy for the cleavage processes. The line which is drawn between active and inactive inclusions is, in the case of the egg of Echinarachnius parma, purely arbitrary. All transitional stages between deutoplasmic granules like fat, and mitochondria have been demonstrated.

C. Precipitations

After the use of picro-acetic and sublimate-acetic fixatives, striking bodies which stain strongly in iron hematoxylin are found in the cytoplasm. These are the large black masses surrounded by clear areas shown in figure 7. They are invariably at the centers of open spaces, which leads to the conclusion that they are condensed or precipitated from material once occupying the entire space. After fixation in sublimate-acetic they tend to assume a slightly more elongated form than after picro-acetic as figured. Such irregular precipitations are found in all unfertilized eggs fixed either in picro-acetic or sublimate-acetic. Proof that eggs containing them are normal is found in the subsequent history of some series where practically 100 per cent development followed fertilization. It may be argued that these precipitations are the result of the action of the fixing fluids, since, as has been pointed out by Mathews (Physiological Chemistry, p. 120 and p. 1086), the salts of metals and picric acid have the power to precipitate proteins. The following facts prove that this cannot

be the entire explanation of the matter. These masses are actual precipitations of material in the normal cell, for in later stages these fixing fluids do not form such precipitations. Figures 7 to 11 show that the bodies have become fewer as development has proceeded. By the time the first division is well under way all the large masses have disappeared and there is no indication of the larger spaces in which they lay. They do not again reappear after the first division.

Similar precipitated masses may be found following other methods of fixation, but the precipitations do not actively take up any of the stains employed; they are always like the diffusely stained ground cytoplasm. Figures 13, 14, and 15 illustrate their appearance following modified Bouin fixation. In these eggs, stained in alizarin and crystal violet, they were of the pale pink color of the great mass of cytoplasm, and therefore not so conspicuous as when stained in iron hematoxylin after picro-acetic or sublimate-acetic fixation. In some eggs they looked almost like bacteria, but they failed to stain with Ziehl's carbol-fuchsin. Also their behavior in later stages could not be accounted for if they were bacteria.

As to the nature of these bodies, nothing is known beyond the fact that they are precipitations of colloidal material in the cytoplasm. It is probable that these bodies are of the same character as the rods observed by Tennent ('20) in the egg of Arbacia fertilized by Moira sperm, after fixation in sublimate-acetic.

In the unfertilized egg there is no uniformity in the orientation of the precipitated masses, but as soon as the spindle for the first division begins to form, they begin to be oriented parallel to the astral radiations extending through the cytoplasm. They appear as if swept about by the currents of more fluid protoplasm flowing in toward the focus of the aster, until they present the least surface in opposition to the direction of flow. Such a flowing in of more fluid protoplasm to the centers of the hyaline areas (such as are shown in figure 10) as the protoplasm goes into a state of gelation at division, was demonstrated by Chambers ('19). In this connection, Bowen's observations on the division

of mitochondria in Hemiptera ('20) may be mentioned. He states that the fact that the mitochondria are oriented with definite relation to the centrosome proves that they are under its directive influence, and therefore there is a mechanism for exact division of mitochondria in mitosis as well as for chromosomes. Since the flowing of material described by Chambers occurs at this time, it is inevitable that any inert masses lying in the cytoplasm should be swept into line, and their orientation by this means need have no connection with any attractive force exerted on them by the centrosome.

DISCUSSION

All of the inclusions found in the egg of Echinarachnius parma have been found in the unfertilized egg and they have decreased during cleavage. This is wholly in accord with the nature of the processes going on in the egg at this time. Its growth period is past and it is about to start on a series of changes involving great energy expenditure. While these changes are occurring there is no opportunity for the aquisition of food material either from maternal tissue or from the medium in which the cells are living. All energy then must come from materials stored up within the egg at the time it is set free from maternal tissues. This does not in any way preclude transformations of some materials in the cytoplasm into others, and it is believed that such transformations have been demonstrated. Here there are reduced to their lowest terms the problems of the biochemist concerning the synthesis of proteins, carbohydrates, and fats, for the large amounts of the substances present in an animal body are merely the sum total of all the minute particles of proteins, carbohydrates, and fats synthesized and transformed in the single cells. The exact steps in the synthesis of the complex chemical compounds which serve as sources of energy in the physiology of the cell are wholly undetermined. There are no reliable microchemical tests for many of these substances and our interpretation of them according to their staining reactions may be quite erroneous.

Regaud has demonstrated that mitochondria are made up of phospho-lipin and albumen. The transformation of mitochondria into fat or of fat into mitochondria has been discussed by a number of investigators, but the intermediate steps are unknown. Dakin states that fatty acids are not directly transformed into amino acids, but the evidence leaves open the possibility of such a transformation through carbohydrates. In that case, is there a carbohydrate stage in the formation of mitochondria or of yolk? Evidence of such a stage would be removed by all ordinary methods of technique in preparing sections. Perez ('03) found in the adipose cells of Formica rufa a transformation of fat globules into albuminous bodies by the digestion of the former.

A number of other authors have touched on this problem of synthesis in the cytoplasm, but their conclusions are not in agreement. According to Popoff ('10), chromidia from the nucleus pass out and change to fat. Saguchi ('20) working on the islet cells in the pancreas, describes lipoid granules being formed from mitochondria. Hollande ('14) observes fat formed from granules near the nucleus and then a transformation of part of the fat into albuminoid bodies. Beckwith ('14) found pseudochromatin granules which developed directly into yolk spheres; Schaxel ('11) found chromatin emitted from the nucleus which formed yolk spheres; Danchakoff ('16) found chromatin emitted from the nucleus which synthesizes more chromatin in the cytoplasm. Ludford ('21) in Patella oogenesis found yolk to be formed by Golgi bodies which were entirely distinct from the mitochondria and the fragments of the nucleolus which he described as being emitted from the nucleus. Nakahara's results ('17) on Pieris, and Perez' work on Formica rufa both point to a transformation of fatty bodies into albuminous bodies. From these many observations it is evident that there are transformations of visibly distinct bodies into one another in the living cell. The great need at present is for some technique which will demonstrate accurately the intermediate steps.

In this paper an attempt has been made to interpret those chemical compounds in the cytoplasm of the egg of Echinarachnius parma which form distinct visible bodies and which can be

stained with ordinary reagents, and to show the part played by them in the processes of development initiated by fertilization.

SUMMARY

1. The cytoplasm of eggs of Echinarachnius parma when fertilized by sperm of the same species shows no visible differences from that of eggs fertilized by Arbacia sperm.

2. Fat drops occur in the unfertilized egg. These drops are emulsified and the fine droplets of fat thus formed gradually become used up or transformed during the early cleavage stages.

3. Small spherical mitochondria are found scattered throughout the cytoplasm and there is evidence to show that they are the direct products of the fine fat droplets.

4. The cytoplasm is packed with plates of nutritive material which have some fatty component in their makeup. They are probably yolk. They are in some cases closely associated with mitochondria, and it is probable that the mitochondria are instrumental in their synthesis. These plates gradually become fewer in number and in the blastula the cytoplasm is quite spongy and full of vacuoles once occupied by the plates.

5. After certain fixatives, picro-acetic and sublimate-acetic, large precipitations of colloidal material in the cytoplasm are stainable with iron hematoxylin. Other fixing fluids preserve them, but do not mordant them so that they actively take up the stain. These precipitations are found in the unfertilized egg when its cytoplasm is in a sol state. They cease being formed as the cytoplasm becomes a gel during its preparation for the first division.

BIBLIOGRAPHY

Allen, E. 1919 A technique which preserves the normal cytological conditions in both germinal and interstitial tissue in the testis of the albino rat. Anat. Rec., vol. 16.

Arnold, J. 1914 Über Plasmastrukturen und ihre funktionelle Bedeutung. Jena.

Beckwith, C. J. 1914 Genesis of plasma structures in Hydractinia. Jour. Morph., vol. 25.

Benda, C. 1910 Färbung der Mitochondria. Enzyklopädie Mik. Technik. Urban und Schwarzenberg.

Berlese, A. 1901 Vorgänge welche während der Nymphosis der metabolischen Insecten vorkommen. Zool. Anz., Bd. 24.

Bowen, R. H. 1920 Studies on insect spermatogenesis. I. The history of the cytoplasmic components in the sperm in Hemiptera. Biol. Bull., vol. 38.

Cowdry, E. V. 1918 The mitochondrial constituents of protoplasm. Pub. Carnegie Inst. Wash., No. 271.

Chambers, R. 1919 Changes in the protoplasmic consistency and their relation to cell division. Jour. Gen. Physiol., vol. 2.

Dakin, H. D. 1912 Oxidations and reductions in the animal body. Longmans, Green & Co.

Danchakoff, V. 1916 Studies in cell division and cell differentiation. I. The development of the cell organs during the first cleavage of the sea-urchin egg. Jour. Morph., vol. 27.

Gatenby, J. B. 1917 Cytoplasmic inclusions of germ cells. Parts I and II. Quart. Jour. Micr. Sci., vol. 62.

1918 Cytoplasmic inclusions of germ cells. Part III. Quart Jour. Micr. Sci., vol. 63.

1919 a Cytoplasmic inclusions of germ cells. Parts IV and V. Quart. Jour. Micr. Sci., vol. 63.

1919 b Identification of intracellular structures. Jour. Roy. Micr. Soc.

1920 On the relationship between the formation of yolk and the mitochondria and Golgi apparatus during oogenesis. Jour. Roy. Micr. Soc.

Guyer, M. F. 1917 Animal micrology. Univ. of Chi. Press

Hargitt, G. T. 1919 Germ cells of coelenterates. VI. Jour. Morph., vol. 33.

Hollande, A. C. 1914 Formation endogènes des crystalloids. Arch. Zool. Exp. Gen., T. 53.

Just, E. E. 1919 The fertilization reaction in Echinarachnius parma. II. Biol. Bull., vol. 36.

Ludford, R. J. 1921 Contributions to the study of the oogenesis of Patella. Jour. Roy. Micr. Soc.

Mathews, A. P. 1920 Physiological chemistry. Wm. Wood & Co.

Nakahara, W. 1917 Physiology of nucleoli in silk gland cells in insects, Jour. Morph., vol. 29.

Partington and Huntingford 1921 The reduction of osmic acid by lipoids. Jour. Roy. Micr. Soc.

PEREZ 1903 Contribution à l'étude des métamorphoses. Bull. sci. France et Belgique, vol. 37.

POPOFF, M. 1910 Ein Beitrag zur Chromidialfrage. Festschrift zum sechzigsten Geburtstag, R. Hertwig. Bd. I. Arbeiten aus dem Gebiet der Zellenlehre und Protozoenkunde. Jena.

SAGUCHI, W. 1920 Cytological studies of Langerhans' islets. Am. Jour. Anat., vol. 26.

SCHREINER 1915 Über Kern und Plasma Veränderung in Fettzellen. Anat. Anz., Bd. 48.

SCHAXEL, J. 1911 Das Zusammenwirken der Zellbestandteile bei der Eireifung, Furchung und ersten Organbildung der Echinodermen. Arch. für mik. Anat., Bd. 76.

1910 Die Eibildung der Meduse Pelagia noctiluca. Festschrift zum sechzigsten Geburtstag. R. Hertwig. Bd. I. Arbeiten aus dem Gebiet der Zellenlehre und Protozoenkunde. Jena.

TENNENT, D. H. 1920 Evidence on the nature of nuclear activity. Proc. Nat. Nat. Acad. Sci., vol. 6.

WALKER AND TOZER 1909 Observations on the history and possible function of the nucleoli in the vegetative cells of various animals and plants. Quart. Jour. Exp. Physiol., vol. 2.

DESCRIPTION OF PLATES

All drawings, except figure 12, were made at table level by the aid of a camera lucida, using a Zeiss 1.5-mm. oil-immersion objective, and a compensating ocular no. 4. This gave a magnification of 1250 diameters. Figure 12 was made slightly above table level and its magnification was 1170 diameters.

Plates 1 and 3 were then reduced to $\frac{2}{5}$ their size, giving a final magnification of 500 diameters.

Plates 2 and 4 were reduced $\frac{1}{2}$, making a final magnification of 625 diameters, except for figure 12, which is 585 diameters.

PLATE 1

EXPLANATION OF FIGURES

(All eggs illustrated on this plate are from the same series.)

1 Unfertilized egg. Flemming without acetic, unstained.

2 Twenty-five minutes after insemination. Flemming without acetic, unstained.

3 One hour and forty minutes after insemination. Flemming without acetic, unstained.

4 Unfertilized egg. Flemming without acetic, alizarin and crystal violet.

5 Twenty-five minutes after insemination. Flemming without acetic, alizarin and crystal violet.

6 One hour after insemination. Flemming without acetic, alizarin and crystal violet.

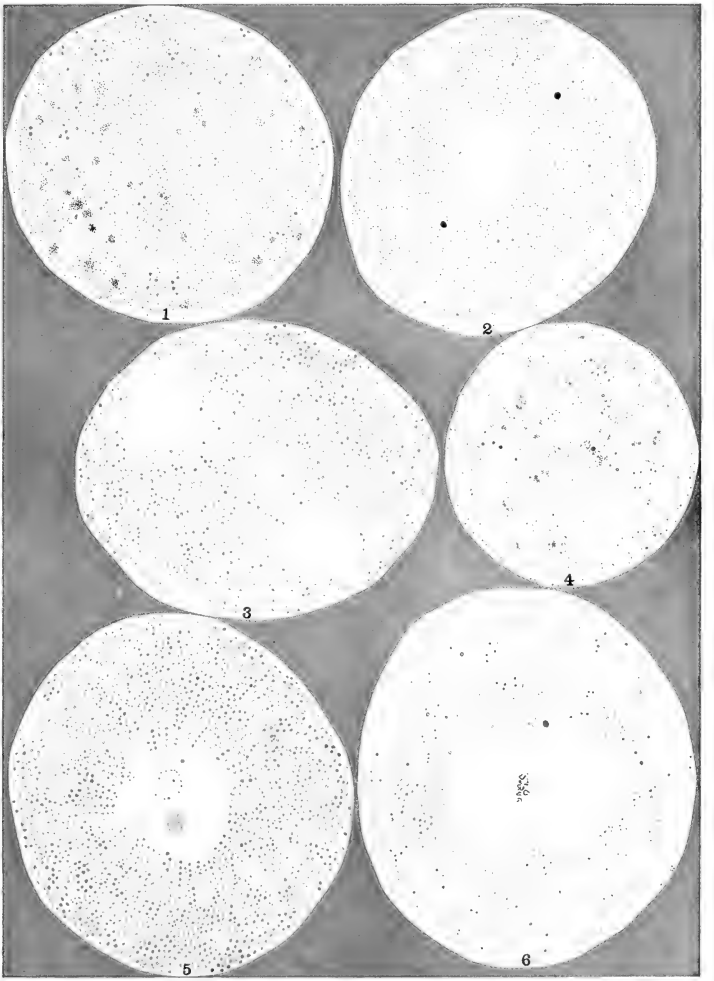

1
2
3
4
5
6

PLATE 2

EXPLANATION OF FIGURES

(Figures 7 to 11 are from the same series.)

7 Unfertilized egg. Picro-acetic, iron hematoxylin.

8 Twenty minutes after insemination. Picro-acetic, iron hematoxylin.

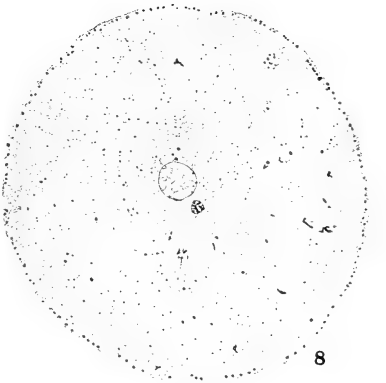
9 Fifty minutes after insemination. Picro-acetic, iron hematoxylin.

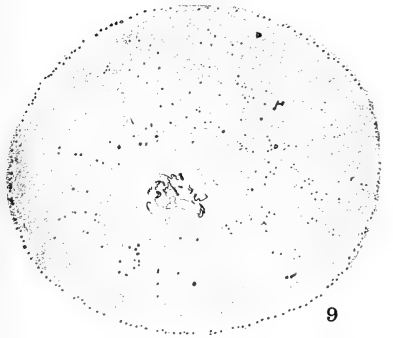
10 One hour after insemination. Picro-acetic, iron hematoxylin.

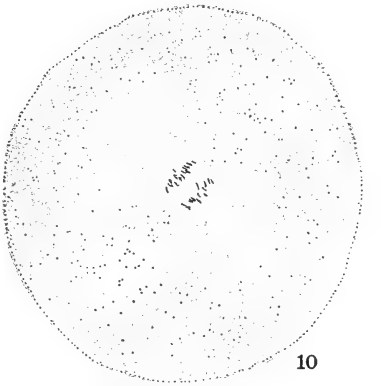
11 Three hours and one-half after insemination. Picro-acetic, iron hematoxylin.

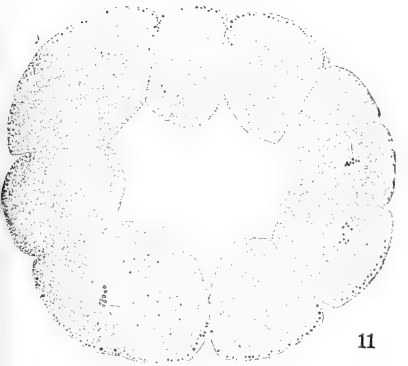
12 One hour and ten minutes after insemination. Champy, iron hematoxylin


7


8


9


10


11


12

PLATE 3

EXPLANATION OF FIGURES

(All eggs illustrated on this plate are from the same series.)

13 Unfertilized egg. Modified Bouin, alizarin and crystal violet.

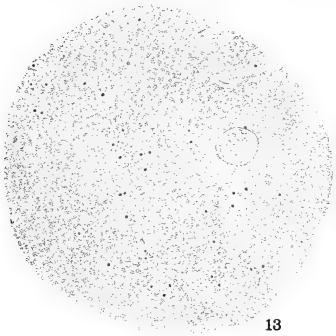
14 Fifteen minutes after insemination. Modified Bouin, alizarin and crystal violet.

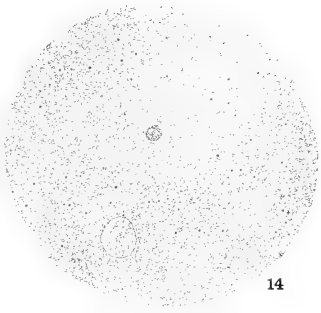
15 Forty-five minutes after insemination. Modified Bouin, alizarin and crystal violet.

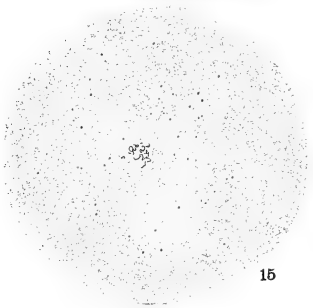
16 Two hours and forty-five minutes after insemination. Modified Bouin, alizarin and crystal violet.

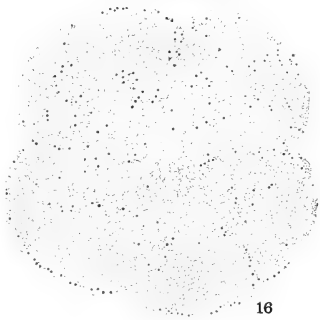
17 Eight hours and fifteen minutes after insemination. Modified Bouin, alizarin and crystal violet.

18 Thirty minutes after insemination. Modified Bouin, iron hematoxylin.


13


14


15


16


17

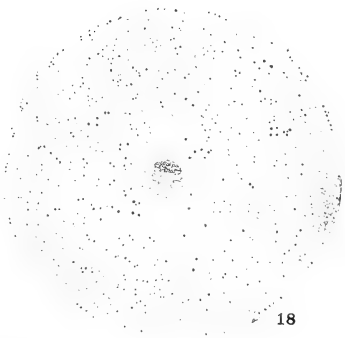

18

PLATE 4

EXPLANATION OF FIGURES

(All eggs illustrated on this plate are from the same series.)

19 Unfertilized egg. Flemming without acetic, alizarin and crystal violet.

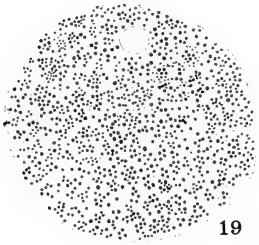
20 One-half hour after insemination. Flemming without acetic, alizarin and crystal violet.

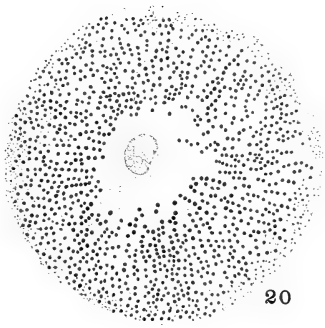
21 One hour after insemination. Flemming without acetic, alizarin and crystal violet.

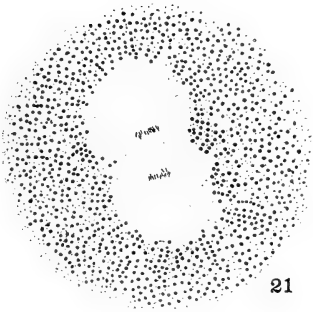
22 One hour and one-half after insemination. Flemming without acetic, alizarin and crystal violet.

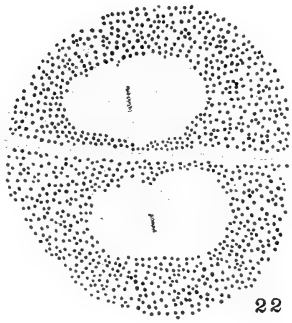
23 Two and one-quarter hours after insemination. Flemming without acetic, alizarin and crystal violet.

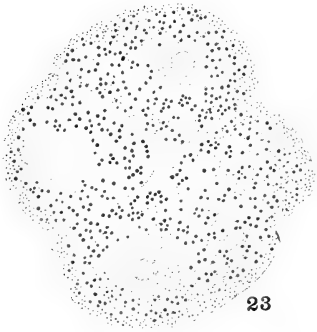
24 Four hours after insemination. Flemming without acetic, alizarin and crystal violet.

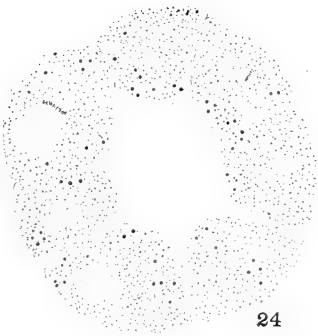

19


20


21


22


23


24

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, SEPTEMBER 11

THE CASTES OF TERMOPSIS[1]

CAROLINE BURLING THOMPSON
Department of Zoology, Wellesley College

NINE TEXT FIGURES AND TWO PLATES

CONTENTS

INTRODUCTION

Termopsis angusticollis and T. nevadensis are found along the Pacific slope of the United States and in British Columbia, and the latter species extends into Montana (Banks and Snyder, '20). The two species frequently occur in the same locality and in close proximity; at Pacific Grove, California, the writer has found several colonies of the two species, less than a foot apart, in one log. Termopsis is the largest North American termite and also the least injurious. The nests or galleries are found in partly decayed wood in forests, usually just beneath the bark, and not in

[1] Owing to the death of the author, proof of this article has been read by the editor and Dr. T. E. Snyder.

the deeper heart wood; very rarely in the woodwork of buildings, and never in the earth.

In normal colonies of T. angusticollis and T. nevadensis four stable types or castes may be found: 1) the first-form adults, with long wings at the time of swarming, and later with the scales or bases of the broken-off wings; 2) the second-form adults, with very short wing vestiges which are much shorter than in second-form individuals of other genera; 3) the wingless third-form adults; 4) the soldiers. The first and third forms and the soldiers are of common occurrence, the second forms are comparatively rare. In addition to these four stable castes, three additional types or variations are occasionally found. These are: soldiers with wing pads (fig. 9), second-form individuals with very minute wing vestiges (fig. 7), and individuals closely resembling the third form, but with wing vestiges that are merely narrow lateral borders of the thoracic segments (fig. 8). The nymphs of all castes occur in different stages of development according to the season of the year. No true sterile worker caste is known to occur in Termopsis, although the wingless third-form individuals have been frequently described by different writers as 'worker-like forms' and even as 'workers.' The worker functions are performed by the developing nymphs, especially those of the third forms, and it is well known that the older reproductive forms do not survive long in captivity unless in the company of nymphs. The same is true of soldiers.

MATERIAL AND METHODS

Most of the material for this paper was collected by the writer in the pine forests of Pacific Grove, California, in April and May, 1919, while a guest of the Hopkins Marine Station of Leland Stanford University. I wish here to express my thanks for the hospitality of the station during this time. For the identification of my collections and for additional material the writer is indebted to Dr. T. E. Snyder, of the Bureau of Entomology, U. S. Department of Agriculture.

This study of Termopsis angusticollis has been made chiefly by means of serial sections and dissections of organs after stain-

ing the body in bulk and partly decolorizing. Stained whole mounts of the heads, showing the brain and eyes, are fairly successful, and some whole mounts of nymphs have been made, but the stained whole mounts of the abdomen or entire insect that have proved so useful in studying other termite genera have been rarely successful with this genus. This may be due to the large size, to the thicker skin, and the more abundant fat-body, but I am inclined to attribute it in large part to the different chemical composition of the Termopsis tissues, with consequent different staining reaction from other termites.

In the following pages the material described is Termopsis angusticollis, but, although T. nevadensis has been less thoroughly studied, enough has been done to state that the morphology of the two species is very similar.

THE CASTES

Termopsis angusticollis Hagen

T. angusticollis is the largest North American termite, the body length of young first-form individuals, from the tips of the mandibles to the end of the tenth abdominal segment, ranging from 10 to 14 mm., and the head width from 2.1 to 2.8 mm. The old enlarged first-form females attain a length of 15 to 17 mm., and the males 14 to 15 mm. These figures show that the post adult growth which takes place after mating is very slight in this genus. The first-form males and females are active and of nearly similar size throughout life, for the Termopsis queens never acquire the relatively huge bulk attained by the queens of the smaller genera of the higher termites, such as Reticulitermes, Nasutitermes, etc. It seems surprising at first that the egg-laying queens of such a large termite should be of relatively smaller size and greater activity; this, however, is merely an evidence of the primitive character of the genus. The small bulk and the activity of Termopsis queens may be due partly to the more or less exposed habitat in wood above ground, partly to the lack of the true work caste, but chiefly to the fact that Termopsis, in common with other lower termites, still retains some of the ancestral independence of the non-social insects.

The second form of T. angusticollis (fig. 6) with very short wing vestiges extending only to the first[2] abdominal segment, and some specimens with even shorter wing vestiges, is of infrequent occurrence. It is possible that this form has been overlooked by collectors on account of its inconspicuousness and that it may be more common than is now supposed. Among the many specimens of reproductive adults collected from about fifty colonies in Pacific Grove, Californinia, I have only six individuals of the caste, two of which were taken for third forms until my attention was called to their very short wing vestiges by Dr. T. E. Snyder, and four which were at first considered young first-form nymphs.

The wingless third-form individuals have a wide range in size, seen not only in the abdomen, which increases but slightly with age, but also in the head and thorax. The body length of young egg-laying females of my collection varies from 11 to 13 mm., and the width of the head of both sexes ranges from 2.5 to 3.7 mm. Older third-form females with slightly enlarged abdomens measure from 15 to 17 mm. long. A number of individuals closely resembling the third form in structure, but with very minute wing vestiges (fig. 8), have been found and will be referred to again.

The soldiers have a still greater size range, the body length, from tip of mandibles to the end of the last abdominal segment, varying from 15 to 25 mm., and all sizes may be found in a single colony. In a colony taken on May 12, 1919, a number of young white soldiers, evidently just molted, were present. Examining the material later, three of these young soldiers, one female and two males, were found to have wing pads. The other young soldiers of the same lot were normal. The histology of these soldiers with wing vestiges will be described below. It will be recalled that in the related genus Kalotermes soldiers with wing vestiges are not uncommon, and occur in many species.

The soldiers with wing vestiges and the second- and third-form individuals with minute wing vestiges are to be regarded as evidences of the high degree of variability of this genus, especially in the direction of the retention of primitive characters.

[2] The sternite, or the ventral part, of the first abdominal segment of termites is not developed, so that the true second segment appears to be the first, when viewed from the ventral surface, and for convenience will be so termed in this paper.

DEVELOPMENT

The eggs of T. angusticollis are long, slender, and reniform. In living eggs, and after fixation, two sizes may be noted: the smallest eggs are about 1.3 mm. long; the larger ones range from 1.5 to 1.7 mm. I am unable to state what these size differences may imply.

The youngest nymphs of T. angusticollis that I have examined—2.2 to 2.5 mm. long, with eleven and twelve antennal segments—are all alike in external appearance, but with a lens the two types of fertile or reproductive nymphs and sterile or soldier nymphs may be seen. The heads are of similar size, but the large brain, almost filling the head cavity of the reproductive nymph, is clearly distinguished from the smaller brain of the soldier nymphs, and correlated with the brain structure is the whiter denser abdomen of the reproductive nymphs and the more transparent abdomen of the soldier nymphs. In stained and mounted specimens the larger sex organs of the reproductive nymphs are in marked contrast to the smaller ones of the soldier nymphs (Thompson, '19, p. 385).

The origin of the soldier, in late nymphal life, from a wingless, rounded, worker-like nymph, has been described by several writers. Lespès ('55) described this origin of the soldier of L. lucifugus, and Snyder ('15) saw it in L. flavipes, L. virginicus, and T. angusticollis. Knower ('94) saw the nasutus of Eutermes rippertii (?) (Nasutitermes pilifrons) molt from a worker-like skin. The writer can add one more example of the late origin of the soldier from a previous work-like phase in the species Termopsis nevadensis. In May, 1919, a deälated first-form male and female of T. nevadensis, together with about fifteen wingless nymphs, which were thought to be young third forms, were collected at Pacific Grove, Californinia, and placed in a small glass vial with fragments of pine wood. The vial was brought to Wellesley in July of the same year, and when the contents were examined a white newly molted soldier was found in place of one of the wingless nymphs.

Fuller ('20), p. 248, writing of the origin of the soldier, states:

It is even very difficult to state exactly when the distinguishing characters of soldiers and workers first become discernible. In certain species examined it would appear that slight differences can be detected

in the third instar. The transformation of a soldier takes place during the third period of quiescency, so that after ecdysis the differentiation is most decided. A difference between majors and minors of the same caste is observable at the beginning of the fourth instar. The smaller grow but little and become adult minors. The larger increase to twice their length and several times their bulk and become adult majors.

THE FIRST FORM OF T. ANGUSTICOLLIS

The first form has three well-defined phases of development: *a*) nymphs of the first form, with long wing pads and creamy white bodies; *b*) winged adults of the first form, with long wings and dark brown bodies, length variable, 11 to 13 mm; *c*) the older males and females the post adults of the first form, with enlarged abdomen and the 'scales' or bases of the shed wings, average length 15 mm., greatest length observed 17 mm.

The nymph of the first form

According to their age, these nymphs present two different appearances, which are so diverse that they at first seem to belong to two different castes. The younger, immature, nymphs of the first form—(fig. 2) 10 to 12 mm. and under, with probably two molts to undergo—may be distinguished from the mature nymphs —(fig. 1) 11 to 13 mm., and with only one more molt—by the appearance of the wing pads and the size and color of the compound eyes. The immature nymphs have small colorless or pale brown eyes and thin transparent wing pads, extending back to the third abdominal segment; the mature nymphs have larger dark brown eyes and thick wing pads with greatly convoluted tissue and indistinct venation, extending back likewise to the third abdominal segment. The mesonotum and metanotum are narrow from side to side in the mature nymphs, a character found in the first form adult, but are broad in the younger immature nymphs. Both immature and mature nymphs of the first form may be distinguished from mature second-form nymphs by the slightly smaller compound eyes of the latter and its very short wing pads, which extend only to the first abdominal segment.

The mature nymphs of the first form are found shortly before the period of swarming, which occurs in California from May to

October. The body color of the mature nymphs (fig. 1) is creamy white, except for two dark areas at the base of the clypeus and at the tips of the mandibles. The head has almost the typical adult form, broadest behind the eyes, and tapering forward to the

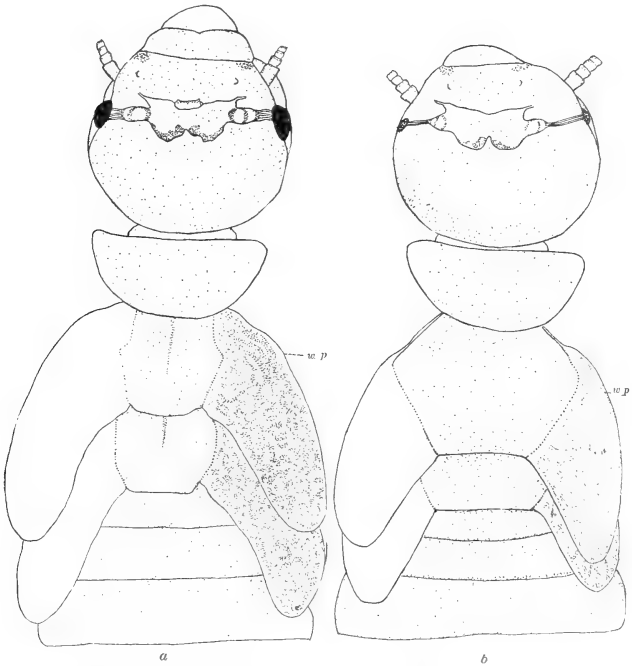


Fig. 1 Termopsis angusticollis. *a*, mature nymphs of the first form, with thick wing pads, *wp.*, surface view; *b*, immature nymph of the first form, with thin wing pads, *wp.*, Spencer oc. 6, obj. 32 mm., stage level, reduced one-half.

clypeus. A depressed area on the frontal surface marks the position of the 'frontal gland,' but no external opening, or fontanel, is visible. The large compound eyes are dark brown and slightly reniform (fig. 2, *a*). Twenty-five and twenty-six antennal segments have been observed. The wing pads are thick and

opaque, slender, though rather short in comparison with the first-form nymphs of other genera—a characteristic of this genus. In stained whole mounts (fig. 1, *a*) the thickened and greatly convoluted embryonic tissue of the future wings, *w.p.*, is clearly seen, the veins and tracheae in process of formation. The meso- and metathoracic tergites are narrow from side to side as in the adult. The notable features of the legs are the five tarsal segments, a primitive character, and the pulvillus or onychium

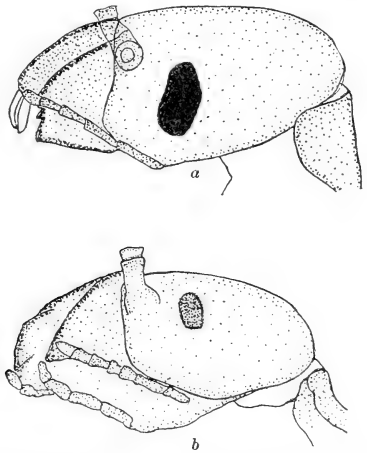


Fig. 2 Termopsis angusticollis. *a*, mature nymph of the first form, lateral view of head; *b*, immature nymph of the first form, lateral view of head. Spencer oc. 6, obj. 32 mm., stage level, reduced one-third.

between the claws. The lateral tibial spines (fig. 3, *b*) are almost as large as in the adult, and are variable in number, from five to one. Female nymphs are clearly recognized from males by the larger seventh abdominal segment which, on the ventral surface, practically covers the eighth. Styles or genital appendices are present on the ninth segment in both sexes. The anal cerci consist of five segments.

Other external points of difference between the mature and immature nymphs, besides the differences in eyes, wing pads, and

shape of thorax already mentioned, are the relatively broader head and the smaller lateral tibial spines of the younger nymphs.

The most interesting features of the internal anatomy are in the nervous system—brain, eyes, and frontal gland—and in the digestive and reproductive systems.

The brain of Termopsis has the form characteristic of the lower termites, namely, a great extension from side to side, caused by the size and lateral position of the optic lobes, and the slighter

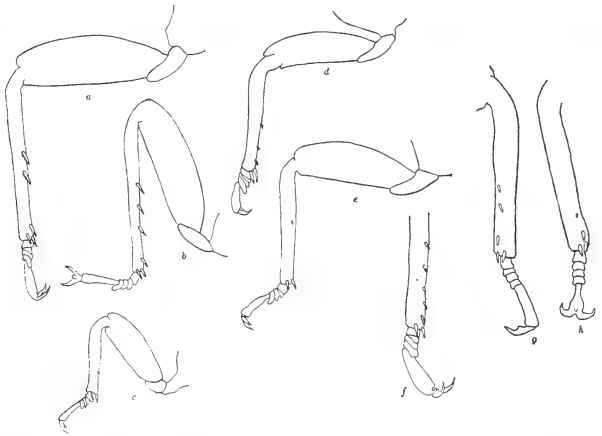


Fig. 3 Termopsis angusticollis, a comparison of the metathoracic legs of the four castes. *a*, first-form adult; *b*, mature first-form nymph; *c*, immature first-form nymph; *d*, second-form adult; *e*, *f*, third-form adults; *g*, *h*, adult soldiers. Spencer oc. 6, obj. 32 mm., stage level, reduced three-fifths.

development of the mushroom bodies. The smaller mushroom bodies of lower termites with their smoother rounded surfaces may be recognized at a glance from the relatively larger and convoluted bodies of a higher termite, e.g., Reticulitermes.

As in other termites, the brain of Termopsis attains its greatest size and complexity in the mature nymph and young adult of the first form, these two phases being practically alike in brain structure. Figure 4, *a*, represents an optical section of the brain of a first-form adult; the huge optic lobes are correlated with the

large compound eyes, and in sections the cell layers and the three fiber masses are plainly seen. The mushroom bodies, though relatively somewhat smaller than in the higher termites, attain their greatest size in the individuals of this caste. The smooth

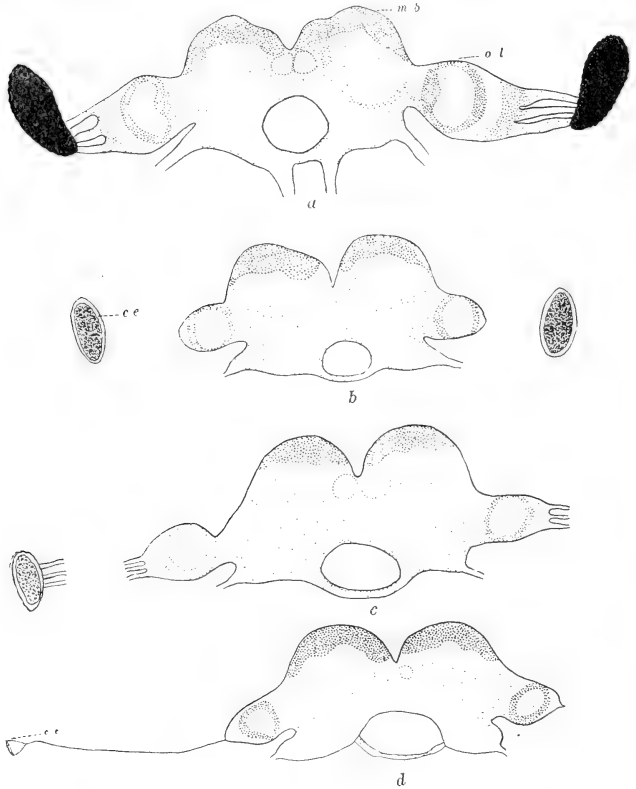


Fig. 4 Termopsis angusticollis. A comparison of the brains and compound eyes of the four castes. *a*, first-form adult; *b*, second-form adult; *c*, third-form adult; *d*, adult soldier. *mb*, mushroom body; *ol*, optic lobe; *ce*, compound eye. Spencer oc. 6. obj. 32 mm., stage level, reduced two-fifths.

rounded surfaces seen in the mushroom bodies of all the other castes (fig. 4, *b,c,d*) are slightly convoluted in the first form, the outer lobe together with part of the inner, curving upward to a level above the inner lobe. Sections show that the three cell groups of the mushroom bodies described in the brain of Reticulitermes, Thompson ('16), are also present in Termopsis, and that similarly all the cells are small and of equal size. The relations of the anterior and posterior roots of the mushroom bodies are also the same as in Reticulitermes and the other termites studied by the writer. The protocerebral lobes are connected by a broad ventral, and two slender dorsal commissures. The central body is largest in individuals of the first form. A frontanel nerve is present in Termopsis, homologous with the frontanel nerve described in Reticulitermes, Thompson ('16), running from the basement membrane of the frontal gland down to the upper surface of the ventral protocerebral commissure, in the same sections with the posterior roots of the mushroom bodies. There is nothing essentially peculiar in the other parts of the brain.

The brain in the younger first-form nymphs (fig. 1, *b*) differs from that of the mature nymphs (fig. 1, *a*) in the smaller optic lobes and the smooth rounded contour of the mushroom bodies. In this caste these characters are due to the age of the individuals, but the same characters occur as castal differences, as will be seen in the description of the brains of the other castes and in figure 4.

The frontal gland. The 'frontal gland' of Termopsis is of the type termed by Holmgren a non-glandular 'Fontanel-platte.' It consists, in the first form nymph (fig. 5, *fg*) of a broad but shallow depression of modified hypodermal cells covered above by their own cuticle and resting upon a base of connective tissue which tapers to a point, and from which the frontanel nerve (Thompson, '16) takes its way downward and inward between the mushroom bodies to the protocerebral tissue. The hypodermal cells (fig. 5, *hy*) of the head of Termopsis are tall and slender epithelial cells, the supporting cells distinctly columnar in form and interspersed with occasional gland cells, but on the frontal surface within the area of the frontal gland the height of the supporting epithelial cells is very greatly increased, and similarly

the occasional gland cells are tall and slender, but the majority of the cells are non-glandular. The same non-glandular structure of most of the frontal gland cells may be noted also in the adults of Termopsis, so that, together with the absence of an external opening or fontanel, the frontal gland of Termopsis may be said to be non-functional and merely a primitive form of the elaborate sac-like frontal glands of the higher termites. To draw an embryological comparison, the frontal glands as seen in the first forms of Termopsis and Reticulitermes, respectively, may be compared in form to the neural-plate and neural-tube stages of an embryo chick.

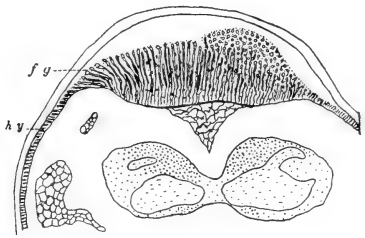


Fig. 5 Termopsis angusticollis. First-form adult, frontal section of the head through the frontal gland and anterior part of the brain. *fg*, frontal gland; *hy*, hypodermal cells. Spencer oc. 6, obj. 16 mm., stage level, reduced one-third.

The noteworthy features of the abdomen are in the digestive and reproductive systems and in the fat body.

In the digestive system, four enteric caeca, or outpocketings between the gizzard and the mid-intestine, may be noted. They are short slender tubes, two dorsal and two ventral, and all of equal size in the reproductive castes, but unequal, two large and two small, in the soldier. Imms ('19) has described five such enteric caeca in Archotermopsis, and states, page 165, that "these structures have not so far been detected in any other Termite." The number of malpighian tubules in Termopsis is eight—the same number as in Archotermopsis.

In a young immature female nymph of the first form with thin wing pads, about 10 to 12 mm. long, the reproductive organs present the following appearance, as seen in figure 11. The ovaries contain many egg tubes, consisting of numerous eggs in linear rows, the two ovaries being connected with each other by threads of connective tissue, the terminal filament, not shown in figure 11, as it is easily broken in dissection. A few of the proximal ova are enlarged, showing that the growth period has begun. The oviducts are narrow as yet, but bear at their junction a small trilobed seminal receptacle, or spermatheca. The colleterial gland is much convoluted, but its tubules are of narrow diameter.

The reproductive organs of a mature female nymph of the first form with thickened wing pads, 12 to 13 mm. long, are shown in figure 12. The largest ova are nearly three times the size of those of the immature nymph just described; many more enlarged eggs are present, and the oldest, or proximal ones, are surrounded by a pellicle of small cells. The oviducts are broader, the seminal receptacle, *sr*, has grown, and the colleterial gland, *cl*, is larger as a whole and has tubules of greater diameter.

In male nymphs of the first form a similar correspondence between age and size of the reproductive organs may be observed in the two phases of young immature nymphs with thin wing pads and mature nymphs with thick wing pads. In figure 16 the reproductive organs of a mature male nymph of the first form are shown. The testes, *t*, consist of many short rounded lobes, the vasa deferentia are slender and open into the basal part of the paired seminal vesicles, *sv*, which are greatly branched and convoluted, the convolution increasing with age. The seminal vesicles after their junction with the vasa deferentia open into the ejaculatory duct which ends in a short muscular penis. In stained whole mounts of the male reproductive organs, which have been dissected out from the surrounding fat-body, zones of developing sex cells in the testes are clearly seen even with a low-power lens.

Sections of the male reproductive organs of a mature nymph of the first form, made by sectioning the posterior end of the abdo-

men, show that these organs are closely invested by the masses of the fat body. The testis is divided into many lobes opening at their lower or proximal ends into the central space which leads into the upper enlarged end of the vas deferens. The testis lobes are enveloped by a delicate outer layer of connective tissue which also extends as septa into the interior, dividing each lobe into separate portions, in which lie the groups or cysts of the male sex cells. The youngest sex cells, the spermatogonia, form a terminal zone at the upper, distal, ends of the lobes; proximal to these may be seen zones of cells that are evidently in the first maturation division, but with the chromosomes so massed together that no exact determination of their number could be made; a still more proximal zone of smaller cells in mitosis probably represents the second maturation division, and toward the center enlarging spermatids in groups of four are recognizable. In the central space many spermatids and a few evidently nearly mature spermatozoa are present.[3] The vas deferens is lined by slender columnar cells, surrounded by a thin layer of connective tissue and muscle fibers. The tubes of the seminal vesicles are lined with an epithelium of tall slender cells with clear basal nuclei and prominent nucleoli. These cells are evidently glandular, for dark secretion granules occur in the cytoplasm and a fluid secretion, staining yellow with iron haematoxylin and orange G, is found in the lumen. Muscle fibers and connective tissue form the outer part of the walls of the seminal vesicles, and no spermatozoa have been observed within the vesicles.

The fat-body in the mature first-form nymph forms a nearly solid mass between the viscera and the body wall, completely enveloping the sex organs.

[3] Stevens ('05) has made a brief study of the spermatogenesis of Termopsis angusticollis, presumably of the first form. She states that the spermatogonial number of chromosomes is twenty-six, and that there is one unique feature in the development, namely, that there is only a nuclear division and no division of the cell body of the first and second spermatocytes, so that the four spermatids resulting from a primary spermatocyte actually develop as four nuclei within a single cell body, and appear throughout development in groups of fours.

The winged adult of the first form

After the last nymphal molt the individuals of this caste have a colorless body, but rapidly assume the dark brown adult pigmentation. The large black compound eyes, the dark body pigment, and the long filmy wings are the distinguishing characters of this phase.

The wings of T. angusticollis are dissimilar in size, the fore wing being slightly larger; there are also slight differences in venation and in the humeral suture in the two wings.

Comstock ('18, p. 143) states: "In Termopsis there is a complete humeral suture in the fore wing, as in Mastotermes, and in the hind wing the anal area is crossed by a suture that appears to be the beginning of a humeral suture." The writer finds that in some specimens of T. angusticollis the humeral suture is incomplete in the fore wing, extending toward the costal margin only as far as vein R 3 of Comstock, and not reaching the anal margin. In this respect Termopsis resembles Archotermopsis, as, according to Imms ('19, p. 99), in Archotermopsis the "basal suture" "is frequently incomplete in that it does not always extend to the costal margin." The completeness or incompleteness of the humeral suture in different individuals is evidently one more example of the great variability already noted in the genus Termopsis.

As in other termites, the veins of the anterior or costal part of the wing are fully chitinized, those of the posterior part are but faintly chitinized.

After the swarming or 'colonizing flight' (Snyder, Banks and Snyder, '20) is over and mates are chosen, the wings are shed, breaking usually behind the humeral suture in the fore wings, and leaving the bases or 'scales' attached to the thorax. The short aerial life is then ended forever and the life within the galleries of wood is begun.

Except for the increase in the size and maturity of the sex organs, the internal anatomy of the winged adult is very similar to that of the mature nymph.

The enlarged adults of the first form

The young deälated first-form individuals have a dark chestnut-brown head and thorax, and an abdomen with bands of light brown alternating with white, which bands become more marked as the abdomen enlarges with age. The head is broadest between the eyes, tapering forward to the clypeus; the frontal surface between the eyes and caudal to the brain bears a wide and shallow depression, plainly seen in profile view of the head, made by the sinking in of the cuticle above the large but non-functional frontal gland, which, in this species, has no external opening or fontanel. The compound eyes are black and large, 0.48 by 0.16 mm., and slightly reniform. Just behind the suture of the clypeus and nearly in line with the bases of the antennae are two light crescentic areas which, in the opinion of the writer, represent vestiges of the two lateral ocelli, present in most other lower termites, but not hitherto described in Termopsis. The view that these are vestigial ocelli and not merely spots or flecks in the chitin is based upon the study of dissections and sections of the head, and upon the similar position of the ocelli in the related genera Kalotermes and Hodotermes. In the latter genus, Jorschke ('14, p. 219) describes, in a similar position, in the worker of Hodotermes vagans, two white spots or areas which he concludes are rudimentary ocelli.

The antennal segments are frequently broken at the tip, but in some apparently uninjured specimens twenty-six segments have been noted.

The meso- and metathoracic segments are narrow from side to side with a heavy median line and bear throughout life the stubs, 'scales,' of the broken-off wings.

The tibiae of all three pairs of legs bear lateral as well as terminal spines, which are large and serrate. The lateral spines are arranged on different tibial surfaces in the three pairs of legs, always being found on the outer or anterior surface of the prothoracic legs, on both outer and inner surfaces of the mesothoracic legs, and on the inner surface of the metathoracic legs. The number of spines is very variable, from five to one, and usually

different in the legs of a pair. Imms ('20, p. 97) notes a similar arrangement and variability of the lateral tibial spines of Archotermopsis. The size of the lateral tibial spines of Termopsis varies with age, and to some extent in the different castes, as may be seen in figure 3. The first-form adult (fig. 3, *a*) has the largest lateral spines with one margin serrate; in the mature first-form nymph (fig. 3, *b*) they are slightly smaller and not serrate, and very small in the immature first-form nymph (fig. 3, *c*). In the second-form adult (fig. 3, *d*) the lateral spines are very small, and and still smaller in some third-form individuals (fig. 3, *e*) and in some soldiers (fig. 3, *h*). In other third-form individuals and in other soldiers (fig. 3, *f*, *g*) the spines are larger, though always smaller than those of the first-form adult, and not serrate. Five tarsal segments are distinctly seen on the inner surface of the legs, but the second segment is usually not seen on the outer surface. A large pulvillus or onychium is seen between the claws. In these two points also Termopsis agrees with Archotermopsis.

The sexes of the adults are seen with even greater clearness than in the nymphs, as the seventh sternite of the adult female completely covers the eighth, and the styles, genital appendices, of the ninth segment are absent in the female, but present in the male. The anal cerci of the tenth abdominal segment are dark brown and consist of five segments. The abdomen is more enlarged in older females than in males, but the activity and the relatively slight postadult enlargement of this, the largest American termite, cause surprise and disappointment to those first acquainted with the greatly distended abdomens of the queens in the smaller genera, Reticulitermes and Nasutitermes.

The internal anatomy of the sex organs and fat-body only will be described.

In enlarged deälated females, 13 to 15 mm. long (fig. 10), the largest eggs observed in the egg tubes were only slightly smaller than eggs that have been laid and had a similar yolk content. The oviducts and especially the vaginal duct are very broad and thick walled; the seminal receptacle, *sr*, has attained its greatest size; the tubules of the colleterial gland, *cl*, are thick and dilated and contain a fluid secretion. In the very oldest females fewer and shorter egg tubes are noted.

In the enlarged deälated males, 12 to 14 mm. long (fig. 15), the testes, *t*, are slightly smaller, especially in the very oldest individuals, than in the mature nymphs of the first form (fig. 16). This is due to the shrinkage of the organ as most of its component sex cells become transformed into spermatozoa and pass out into the vas deferens, leaving behind eventually only connective-tissue cells. The vasa deferentia are firm though slender, with a definite lumen and are connected with the base of the seminal vesicles on their dorsal surface. The seminal vesicles, *sv*, are greatly branched and convoluted, each tubule being broader and more expanded than in the mature nymph. No spermatozoa were found within the seminal vesicles of males of any age or caste, and the lumen is filled by a fluid secretion, staining yellow with iron haematoxylin and orange G. This shows that the seminal vesicles of Termopsis are glandular in function, and doubtless homologous with the colleterial glands of the female.

Sections of the testes of the older males of the first form show the central space and the proximal portions of most lobes filled with masses of metamorphosing spermatids and mature spermatozoa in tangled clumps, not in packets. In the oldest individuals very few spermatogonia remain in the distal ends of the lobes, and there are many empty spaces crossed only by strands of connective tissue. The tubes of the seminal vesicles have a larger lumen and a more copious secretion than in the nymphs.

The fat-body in very old individuals is still large, but is no longer a solid mass, and contains more empty spaces than in the nymphs and young adults.

THE SECOND FORM OF T. ANGUSTICOLLIS

Second-form individuals of T. angusticollis, with very minute wing vestiges (fig. 6), are at present considered rare, and specimens have been recognized with certainty only in the young adult and enlarged adult phases. The writer is inclined to believe, however, that this caste is less rare than has been supposed, and that it has been mistaken on the one hand for a young nymphal phase of the first form and on the other for an adult of the third form. Specimens frequently have one or more of the tiny wing

vestiges broken off, the scars remaining, and such scars or mutilations are very commonly found on individuals which have been thought to belong to the third form.

The nymphs of the second form

The nymphs of the second form are not known to me with absolute certainty, although one specimen in my material might

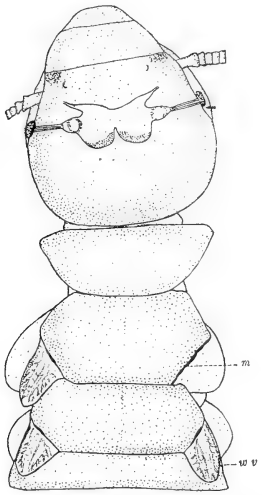


Fig. 6 Termopsis angusticollis. Second-form adult, head and thorax, surface view. *wv*, wing vestige; *m*, scar or mutilation. Spencer oc. 6, obj. 32 mm., stage level, reduced one-half.

be either a nearly mature nymph or a young adult of this caste. This specimen is about 10.5 to 11 mm. long. The body is creamy white, the eyes pale pink with white rims, oval and small. The meso- and metathoracic segments are broad from side to side and bear very small wing vestiges, the second pair extending only over part of the second abdominal segment. The internal anatomy of the second-form nymph has not been studied,

but, as in the case of the first form, is probably similar to that of the young adult described below.

The young adult of the second form

The body length of the young adult of the second form is about 11 to 12 mm. The color of the body is pale yellow with darker chitinized areas on the clypeus and mandibles. The head is slightly broader than that of the first-form adult and tapers less in front of the eyes. The eyes are pinkish with a white rim, oval and not reniform, 0.2 by 0.1 mm., slightly smaller than the eyes of young first-form nymphs of similar length with which

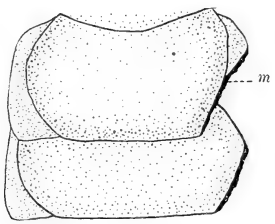


Fig. 7 Termopsis angusticollis. Second-form adult with unusually short wing vestiges, thorax, *m*, scar or mutilation. Spencer oc. 6, obj. 32 mm., stage level, reduced one-third.

these young adults are sometimes confused. No fontanel is present. Two light crescentic areas, vestiges of the lateral ocelli, are seen on the frontal surface of the head, in a position similar to that in first-form individuals. The meso- and metathoracic segments are very broad from side to side, evidently a primitive character, as it will be recalled that this condition was noted in the younger first-form nymphs. The wing vestiges are very short and are variable in length, in some specimens (fig. 7) appearing merely as heavy lateral borders to the thoracic segments, in others (fig. 6) as small scale-like vestiges that extend over part or all of the second abdominal segment. The venation of these tiny wing vestiges is similar to that of the adult in that the main trunks of the homologous veins are present. Very often some

wing vestiges are broken off, leaving jagged scars on the edge of the thoracic segments.

The legs of the second-form adult (fig. 3, *d*) are not larger and heavier than those of the first form, as in the genus Reticulitermes, the tibiae indeed are shorter, and the lateral tibial spines are markedly smaller, varying in number from four to one. Five tarsal segments are present, the second segment reduced in size, and a pulvillus, onychium, is found between the claws.

The enlarged adult of the second form

The body length of the largest second-form queen of my collection is 14 mm.; the males are smaller, 10 to 11 mm. The color of the body is pale yellow and the enlarged abdomen has the characteristic bands of yellow and white. The compound eyes are dull pinkish with white rims, and the vestiges of the lateral ocelli may be noted. No fontanel is present. Some of the wing vestiges are frequently broken off, leaving jagged scars (fig. 6, *m*). The enlargement of the abdomen is as much as, or more than, in first-form queens, and the caudal end is blunt, not pointed. The styles, genital appendices, of the ninth abdominal segment are present in both sexes. The anal cerci consist usually of four segments, and rarely of five.

Internal anatomy. The brain (fig. 4, *b*) is smaller than in the first form, differing chiefly in the reduced optic lobes and in the smaller mushroom bodies with smooth contours, lacking the convolutions of the first form. In the few heads sectioned no frontal gland could be detected.

The female reproductive system is evidently well developed, but owing to scarcity of material no preparations have been made.

The male reproductive organs are intermediate in size between those of the first and third forms. Sections of the testes show the male sex cells in the different phases of development, but except for size the testes of the three reproductive castes appear much alike.

The fat-body is large and fills most of the abdominal space but appears loose and vacuolated with age as in the other castes.

THE THIRD FORM OF T. ANGUSTICOLLIS

Like the other reproductive castes, the third form of T. angusticollis has the three developmental phases of nymph, young adult, and older adult, but these phases are less marked, owing to the negative structural characters of the caste, such as the lack of wings, the paler body pigment, the less distended abdomen of older individuals, etc.

Like the other castes again, the third form is very variable, the body length of mature third forms ranges from 9 to 17 mm. the width of the head from 2.5 to 3.6 mm., the form of the abdomen from flat to distended. The true color of the head and body is light straw colored, or almost colorless, but the woody contents of the intestine give the abdomen of most specimens a dark muddy appearance. The compound eyes are small, 0.2 by 0.1 mm., either colorless or pale pinkish with white rims. The number of antennal segments is twenty-six or twenty-seven. The head is much broader than in either first or second forms. The thoracic segments are either wholly wingless or, in many specimens (fig. 8, *wv*), with narrow marginal thickenings which may be interpreted as very minute vestiges of the ancestral wing pads common to the species—another instance of variability.

The legs are not relatively larger than in the first form, as might be expected with the broader head and body. In some individuals (fig. 3, *e*) the legs are actually smaller, with slender tibiae and tiny lateral tibial spines; in other larger individuals (fig. 3, *f*) the legs slightly surpass the size of the larger first forms, but the spines are smaller. Five tarsal segments and an onychium are present as in the other castes.

The abdomen is sometimes distended, though less so, relatively, than in the first- and second-form adults; sometimes flat, as in the soldier. It was at first thought that the third-form individual with flattened abdomen might be sterile, or true workers, but a careful dissection of many flattened specimens has proved that the collapse of the abdomen frequently comes with age, after many eggs have been laid, and after the fat-body deteriorates, and that the rounded abdomen is usually, although not always, characteristic of young individuals with large fat-masses and

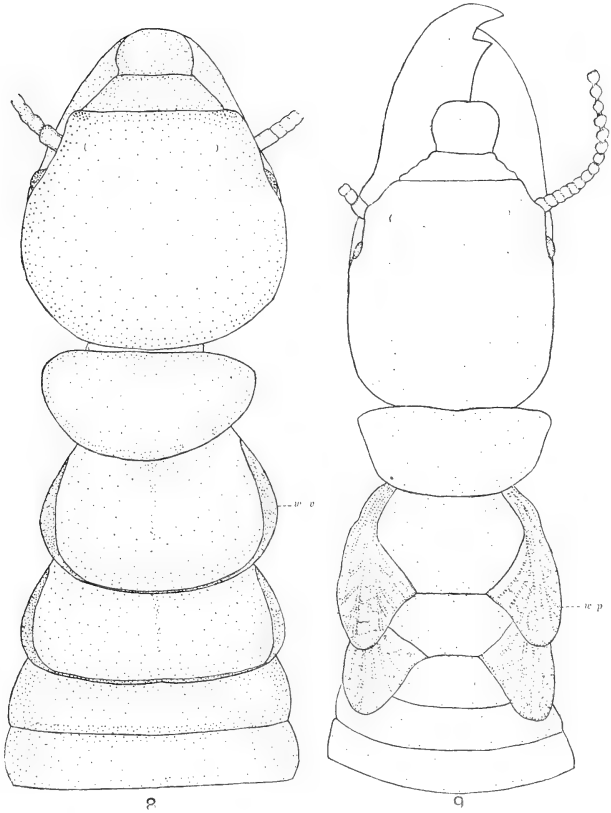


Fig. 8 Termopsis angusticollis. Third-form adult with minute wing vestiges, *wv*, head and thorax, surface view. Spencer oc. 6, obj. 32 mm., stage level, reduced one-half.

Fig. 9 Termopsis angusticollis. Young white soldier with wing pads, *wp.*, head and thorax, surface view. Spencer oc. 6, obj. 32 mm., stage level, reduced one-half.

many unlaid eggs. The wingless condition and active habits of the third form of Termopsis have led many writers to refer to it as a 'worker,' or at best as a 'worker-like' form, but because true workers are sterile the term should not be applied to this invariably fertile caste. Females are recognizable from males by the larger seventh abdominal sternite; the styles, genital appendices, are present in both sexes. The pale yellow anal cerci of the tenth abdominal segment have frequently four segments, but sometimes five, as in the first form.

Internal anatomy. The brain (fig. 4, *c*) varies with the individual in size, but even in the largest specimens is smaller then that of the first form, on account of the reduction of the optic lobes; the mushroom bodies are large, but lack the curved surfaces of the first form. The hypodermis of the frontal surface is not differentiated to form a frontal gland in the specimens sectioned.

The four intestinal caeca are of equal size as in the other reproductive castes.

The female reproductive system of a young third-form queen is shown in figure 13. There are practically no differences in any of the organs from those of a young first-form adult. A nearly mature egg from an older third-form queen is shown in figure 14.

The male reproductive system (fig. 17) shows a great increase in size in this caste. The huge testes, *t*, consist of a multitude of short rounded lobes; the vasa deferentia, as seen in whole mounts, are filled with spermatozoa, *sp.*, the tubules of the seminal vesicles, *sv*, are very long, stout, and convoluted. The testes sectioned were very young, though of large size, and most of the sex cells were in the condition of spermatogonia and spermatocytes, with no spermatozoa as yet developed. All of the whole mounts of older individuals showed spermatozoa in the testes and vasa deferentia.

The fat-body is smaller in third-form individuals than in the other reproductive castes, not always filling the abdominal spaces.

THE SOLDIER OF T. ANGUSTICOLLIS

The adult soldier of T. angusticollis is very variable in size and color. Banks and Snyder ('20) state that the body length of this caste ranges from 15 to 19 mm. and the writer has collected some specimens 25 mm. long. In the darkest individuals most of the head is black, the remainder of the body shading backward to a light brown abdomen. In lighter individuals the color ranges from dark brown on the head to the whitish abdomen. The head is greatly elongated; the compound eyes are small, 0.08 by 0.04 mm., but are larger in young soldier nymphs; crescentic areas are present on the frontal surface of the head similar to those found on the second and third form, but even smaller, and like them probably representing vestigial ocelli; the fontanel is lacking in the soldier, as in all other castes of Termopsis angusticollis. Twenty-five antennal segments have been counted; this number, however, is probably variable.

Although the thorax is normally wingless, in one colony, taken at Pacific Grove, California, in May, 1919, the writer found three young white soldiers with short wing pads (fig. 9), together with normal wingless young soldiers. It will be recalled that Heath ('03) also found a few soldiers with wing pads in this genus.

The abdomen is elongated and round in cross-section in some individuals, short and much flattened in others. The legs are large and stout; the lateral tibial spines are variable in number, five to one, and in size, some individuals (fig. 3, *g*) having quite large lateral spines, those always smaller than those of the first form, other specimens (fig. 3, *h*) have very minute spines; five tarsal segments are visible, and a small onychium. The anal cerci have either four segments, like the second form, or five, as in the first form. Styles, genital appendices, occur in both sexes, and the sexes are readily distinguished by the larger seventh abdominal sternite of the female.

Internal anatomy. The brain of the soldier is smaller than that of the first and third reproductive forms, but is as large if not larger than that of the second form. By comparing figure 4, *a*, *b*, *c*, *d*, it will be seen that the brains of the four castes of Termopsis differ chiefly in the size of the optic lobes; a correlation

with the different-sized compound eyes, and that the most significant parts, the mushroom bodies, are of nearly similar bulk in the second and third forms and in the soldier, and are only slightly larger in the first form. The brain of the soldier of Termopsis, slightly smaller than two reproductive castes and about equal to another, is, therefore, when compared with the soldier brains of higher termites, a relatively large brain. In short, this soldier brain has not varied far from the primitive type of the genus—the first form—and lacks the degenerate or specialized features characteristic of the soldiers of higher termites. The interesting correlation between the size of this soldier brain and that of the almost sterile reproductive organs will be pointed out below.

The hypodermis of the frontal surface of the head of the soldier of Termopsis is not differentiated into a frontal gland as in the nymphs and adults of the first form. The specimens sectioned were young white soldiers, but with well-developed heads covered by a fairly thick cuticle. No sections were made of adult soldier heads on account of the very thick cuticle.

In the abdomen, the four enteric caeca of the soldier are not of similar size, as in the reproductive forms, but the two dorsal caeca are slightly larger than the ventral pair.

The female reproductive system. The reproductive system of the female soldier of Termopsis angusticollis presents some of the most interesting features of this study. In long-headed but colorless soldier nymphs, with probably only one more molt to undergo, the ovaries are smaller than in the nymphs of any of the reproductive castes of about similar age. The egg tubes are, however, numerous, but the eggs are small, only three or four eggs at the lower or distal ends of the tubes having begun to enlarge (fig. 20). These slightly enlarged eggs are surrounded by distinct egg follicles.

The egg tubes of each ovary are continued forward in threads of connective tissue which unite and form a delicate strand, the terminal filament, which in turn runs to the anterior end of the abdomen, spreading out into a brush of fibers at its point of attachment. The terminal filament occurs in the ovaries of all the castes of this species, but is seen to best advantage in the

soldier, and is shown in figure 19, *tf*. The oviducts are broad and, in some individuals, are completely fused with a small but well-formed seminal receptacle; in other individuals the oviducts have not grown together with the seminal receptacle. The colleterial gland is greatly convoluted, but the individual tubules are slender. It will be seen from the foregoing description that many of these young soldiers seem to give promise of fertility in the adult phase. The adult female soldier is, on the contrary, undoubtedly infertile. The reproductive organs of a large number of adult females have been dissected out after staining and other individuals have been sectioned. In every case there is evidence of the arrested development of the female reproductive organs, either of the ova alone or of the ova and the ducts. Instead of the well-rounded ova, regularly spaced and with large central nuclei, that were present in the soldier nymphs (fig. 20), we note in the adult soldiers, shrunken eggs, irregularly spaced with respect to one another and with smaller nuclei at one end of the cell body (fig. 21). Furthermore, many adult individuals were found in which the three embryonic fundaments of the oviducts, the seminal receptacle, and the colleterial gland, had failed to unite during development (fig. 18), so that the three parts were entirely separate, which is always the case in the sterile soldiers and workers of Reticulitermes and Prorhinotermes where reproduction is impossible (Knower, '01; Thompson '20). Two degrees of infertility, therefore, exist among the adult female soldiers of Termopsis angusticollis: 1) individuals in which the oviducts, the seminal receptacle, and the colleterial gland are fully fused, so that sexual intercourse might be possible, but whose ova have undergone an arrest in development (fig. 19); 2) individuals without fusion of the above-mentioned ducts, so that sexual intercourse would be impossible, and in addition an arrested development of the ova (fig. 18).

In the young female soldier with wing pads found at Pacific Grove, the ova were as yet normal, but the three fundaments of the reproductive system were not united, so that this specimen, if it had lived, would have fallen into the second category of infertile female soldiers.

These observations are not in agreement with the work of Heath ('03), who has recorded the case of a Termopsis soldier. I quote below Heath's account, ('03, p. 58):

> In colonies where either the king or queen persists the substitute royal individual is usually, so far as I know, an immature perfect insect, but, where both have perished the substitute royalty may sometimes contain a worker or a nymph or even a soldier capable of laying eggs. Such monstrous forms are not infrequent in large orphaned nests, but never apparently in colonies headed by the true royal pair. We may also find winged soldiers, soldiers with mandibles of varying size, and, as just mentioned, soldiers with wing pads, the straw-color characteristic of substitute forms and with functional reproductive organs. These last named insects are comparatively rare. I have had but three in my possession. All of them laid eggs in captivity and in one case I followed the development for a long period of time, but the young and the nymphs and workers into which they became transferred, appeared in all respects perfectly normal.

The male reproductive system. The testes of young white soldier nymphs are smaller than those of the adult soldiers and the lobes are shorter and stouter. Prominent zones of dividing cells may be noted in stained whole mounts. In sections, groups of cells which are evidently spermatogonia are found at the tips of the lobes, proximal to these are masses of cells in division with the chromosomes in tetrad form. Other groups of much smaller cells in mitosis are doubtless in the second maturation division, and in the more central part of some lobes spermatids developing in groups of four may be recognized. In the central space of the testes a few apparently mature spermatozoa are present. There are no visible signs of degeneration or arrested development in the testes of these young soldier nymphs. The seminal vesicles however, are not normally developed, consisting of a few very short and slender tubules whose epithelial cells lack the height of the homologous cells of the three reproductive castes.

In the adult soldier the testes are larger than in the first-form adults and nymphs, although much smaller than those of the second and third forms, but the vasa deferentia are slender and contracted, almost without lumen, and the seminal vesicles are vestigial in structure. Few of the testes lobes are broad and rounded, as in the reproductive castes, but are long and usually

slender (fig. 22), several small lobes merging into one larger lobe, and finally all uniting at the base of the testis into one common space, with which the vas deferens is connected. The vasa deferentia are attenuated lobes with thin walls, the same is true of the small and poorly developed tubules of the vestigial seminal vesicles. The paired character of the seminal vesicles is especially well seen in the soldier.

Sections of the testes of the adult soldier show that there is a complete breaking down of the inner ends of the lobes into a central chamber or space, so that the base of the testis makes one big sac with slender shrunken lobes leading to it, the remaining spermatogonia and spermatocytes occupying a few cysts at the tips of the lobes. In some adults the central space is filled by masses of developing or possibly degenerating spermatids interspersed with a few spermatozoa. In other, older, individuals the spermatozoa predominate over the spermatids. A very few swollen spermatids and some spermatozoa were noted in the vas deferens of one adult soldier. No spermatozoa were found in the seminal vesicles. The slender tubules of the seminal vesicles of the adult soldier, as seen in sections, consist of an outer layer of slender epithelial cells less than one-half as tall as the similar cells of the reproductive castes, and lacking the dark staining secretion granules that are so abundant in the latter. Only a very small amount of yellow staining secretion (iron haematoxylin and orange G) is found in the lumen of the seminal vesicle tubules of the soldier, in contrast to the copious secretion of the reproductive forms.

From the facts just stated, it seems evident that, although the testes of the adult soldier produce spermatozoa that are apparently normal, the lack of the secretion of the seminal vesicles may render these spermatozoa non-functional and thus cause the sterility of the male soldier. A detailed cytological study of the soldier spermatozoa may prove that they lack some morphological feature or that they are entirely normal, but the attenuated vasa deferentia and the degenerate seminal vesicles indicate that these parts at least of the male reproductive system are vestigial. An examination of the gonads of the young male soldier with

wing pads shows the same conditions as in the young wingless soldier nymphs. My conclusion is, therefore, that the male soldier of T. angusticollis, as well as the female soldier, is actually sterile, although near the ancestral state of fertility.

Current literature affords other instances of sterility, although spermatozoa are formed by the testis.

Boring and Pearl ('18), in a study of hermaphrodite birds, have noted a case of sperm in the testis without correlated sex behavior. In reference to the bird known as 1426, they say: "This is the most interesting of the Holland birds, absolutely indifferent as to its sex behavior and yet with sperm in the testis, and at least one corpus luteum remnant in the ovary, and the ovary of a laying hen."

Safir ('20), referring to the cause of sterility in the XO males of Drosophila, states that the XO males do not inject sperm into the female during copulation, but that when the XO males were dissected the testes appeared perfectly normal in shape and color, though of smaller size. When the testes were teased open the bundles of sperm remained compact, and when separated artificially it was found that the sperm was non-motile. After many dissections "it became apparent that the immediate cause of the sterility was the non-motility of the relatively scanty sperm." Safir also notes that the cell bodies of the primary and secondary spermatocytes of Drosophila often fail to divide after the nuclear divisions, forming giant multinuclear cells, the spermatids, which, he believes, often die and disintegrate without the formation of spermatozoa, but, in other cases, develop into the bundles of non-motile spermatozoa. The resemblance of this spermatogenesis to that of Termopsis, as described by Stevens ('05), gives rise to interesting speculations.

The fat-body of the abdomen of soldier nymphs, though copious, is far less developed than in the nymphs of the first and second reproductive forms. In the adult soldiers the fat-body is reduced to a thin layer beneath the skin.

DISCUSSION

To review briefly the more significant facts stated in the preceding sections we find that the colonies of T. angusticollis have commonly four stable types or castes of individuals, the first, second, and third forms, fertile; and the soldier, wholly or almost sterile, with occasional and rare deviations or variations from one or all of the castes. We find, further, that each caste is a complex of evidently correlated characters which, on the whole, are well defined, although the range of variability in Termopsis is unusually great.

The first form, with long wings or stubs, has, as a rule, the highest type of structure, so that in general there is a gradation of structure from the first form down through the other castes. Examples of this are: the brain, the compound eyes, the frontal gland (present only in the first form), the wings, the size of the lateral tibial spines, the body pigment, the anal cerci. In fewer cases there is gradation up from the first form, e.g., the size of the head, of the testes, and of the soldier mandibles and legs.

The genus Termopsis is remarkable for the retention of many primitive characters, both in habits and in structure. Among habits may be mentioned the activity of the old egg-laying queens; the relatively strong powers of flight of the winged first forms in comparison with other termites genera; the lack of a true nest except for the galleries in wood; the frequent presence of several parent first forms in a colony.

The list of primitive structures is a long one. The great size; the large number of antennal segments, twenty-six to twenty-seven; the hypodermal plate that forms the non-glandular frontal gland of the first form; the vestiges of the lateral ocelli; the slightly reniform condition of the compound eyes of the first form, especially in the nymphal phase; the incomplete humeral suture of the wings; the primitive type of venation; the wing vestiges of third form and soldier; the arrangement of the lateral tibial spines on the three pairs of legs, similar to that of Archotermopsis; the five tarsal segments; the very slight post-adult growth of the abdomen of the egg-laying queens; the four enteric caeca of all castes; the eight malpighian tubules of the digestive

tract; the lack of the sterile worker caste found in the higher termites the large brain and the nearness to fertility noted in both male and female soldiers.

Two views are held today as to the origin of these complexes of characters that we call the castes. In Italy, the classic theory of Grassi and Sandias ('93–'94)—that the castes are the product of their environment, the result of special feeding and the action of protozoa—finds an ardent advocate in Dr. Carlo Jucci. Jucci ('20–'21) has made certain interesting experiments in cutting off the wings of developing nymphs, and has analyzed the excretory products of the different castes, and, in a preliminary note before the Academy dei Lincei, claims to have demonstrated the existence of the particular diet by which caste production was brought about by Professor Grassi in his experiments.

The view that termite castes are hereditary, the product of the germ plasm, found its first support in France from Prof. E. Bugnion ('12–'13), who advanced strong evidence drawn from observations on the mushroom-feeding and other termites of Ceylon. In England, the morphological work of Dr. A. D. Imms ('19) on the primitive genus Archotermopsis gives valuable evidence which will be quoted more fully below. In America, support for this view has come from the field observations and the breeding experiments of Dr. T. E. Snyder ('15, '16), from Dr. Alfred Emerson, whose work on South American termites is still unpublished, and from the writer, whose opinions are based upon the differentiations found in newly hatched termites, and morphological data from the adult castes. Valuable indirect evidence is derived from the studies of Dr. C. A. Kofoid and Miss Olive Swezy on the protozoa of the intestinal tract of termites. Kofoid and Swezy ('19) state that all the castes of T. angusticollis are infested by protozoa, and in this connection it should be remembered that Grassi's hypothesis postulates the absence of protozoa in the reproductive forms, as the cause of their fertility, and, conversely, the presence of protozoa in the sterile workers and soldiers only.

The biologist who believes that termite castes are of hereditary origin will next ask the mode of origin.

Imms ('19, p. 144) writes in regard to this question as follows: "I maintain that there is no satisfactory evidence conclusively proving that any particular type of nutrition, or the absence thereof, is capable of producing such fundamental changes in the external and internal morphology which characterize the soldier caste. It has also been shown that the reduction in the gonads is not an invariable attribute of the soldier, and that caste production is not in any way related to the presence of intestinal protozoa."

On pages 147 to 149 Imms ('19) discusses his view of the origin of termite castes by mendelian inheritance from the winged sexual forms:

I propose to consider first a typical species of Termite comprising monomorphic soldier and monomorphic worker castes and the ordinary winged sexual forms. Let the symbols ASF represent the various allelomorphs which express themselves collectively in the winged sexual forms; F standing for the fertility determinant, and f for the absence of that factor. The worker mutation I would explain as having arisen by the loss of certain correlated allelomorphs, which we represent by S and their absence by s. Similarly, by means of a second mutation, involving the loss of another group of characters A, the soldier caste is accounted for; the absence of A we will represent by a. The parental forms will have the constitution AaSsFf, but the formula may be simplified by omitting the fertility determinant, which will be considered at a later stage, since it bears no relation to the origin of caste. Now the cross AaSs × AaSs affords an ordinary case in which two kinds of differentiating characters are united, and the series contains nine different forms among sixteen individuals (Bateson, '13, pp. 355 and 345). These may be classified as follows:

Soldiers	*Winged sexual forms*	*Workers*
2 AaSS	2 AASS	2 AASs
1 aaSS	4 AaSs	1 AAss
2 aaSs		2 Aass
	Sterile gametic union	
	1 aass	

' The above hypothesis, involving the Mendelian inheritance of two analogous mutations, appears to offer a simple explanation of the origin of polymorphism among Termites. It, furthermore, accounts for the persistence of castes, which are in themselves mostly sterile, securing their representation in the germ-plasm of the species in each succeeding generation.

Thompson and Snyder ('19), attempting to answer the question of the mode of origin of the termite castes, suggested that the castes might be interpreted either as a series of fluctuating variations or as mutations "comparable to the series of mutations found in Drosophila." To-day, the writer, influenced by the recent work of Morgan and his school, especially by their interpretation of the genetic behavior of Oenothera lamarckiana, believes that termite castes should be interpreted as comparable to the offspring of Oenothera, as arising by segregation from a heterozygous parent form. In modern terminology, therefore, the termite castes are not mutants, in the sense of the progeny of Drosophila, arising once for all from a mutating parent, and then breeding true, but are rather segregants, in the sense of the offspring of Oenothera lamarckiana, arising generation after generation by the splitting and recombination of the genes of a heterozygous parent form. My views on this point therefore, are in general agreement with those of Imms, except in the use of the term mutant, which cannot to-day be applied with exactness to the recurrent termite castes.

With another theoretical point advanced by Imms I am unable to agree. Imms ('19, p. 146) says of the wingless third form of Archotermopsis, which he terms the 'worker-like' form: "I consider that they exhibit the first step in the evolution of the worker caste." . . . . "At the same time they afford a clue to the possible origin of the worker, which appears to have arisen as a mutation *of the nymphal stage and not of the winged adult.*" (Italics mine.) The view that the wingless sterile worker is merely a physiological phase of the wingless fertile third form, and one a step to the other, has also tempted the writer (Thompson and Snyder, '20), but a careful study of any termite genus with both castes gives strong evidence that the two castes are morphologically distinct. The fertile third form of higher termites, like the first form, is probably heterozygous, and produces among its offspring sterile workers, but we lack as yet actual proof of this. We do know, however, that the first form gives rise to both third forms and workers. Imms' statement, that workers may have arisen as mutations of the nymphal stage, and

not of the winged adult, has a flavor of the neoteinic or 'substitution' idea, which seems in disharmony with his other views and which I am unable to support.

We know also to-day from the work of Morgan and his school that many characters are controlled by a single factor and, conversely, that a character may be affected by several factors. The many characters of a termite caste are undoubtedly correlated, and probably linked in heredity; indeed a caste, like sex, may depend upon one factor. We may make hypothetical mendelian formulae, but as yet there are no exact data from breeding to test by the formulae. We may talk about the expected results, but we need the actual ones. We do not know the ratio between the number of fertile and sterile forms. We are not absolutely sure whether the second and third forms arise from parent first forms or from parents like themselves, nor whether each reproductive type is capable of producing the sterile workers and soldiers. A few breeding and field observations exist; for example, Feytand ('12) states that the first brood in a new colony consists only of sterile forms, workers; Snyder ('15) observed in several genera that the first brood is composed of both workers and soldiers, and in a definite ratio, according to the species or genus; but more data are needed, and must be obtained to complete the evidence for the hereditary origin of the termite castes.

SUMMARY

1. The two species angusticollis and nevadensis of the genus Termopsis are found on the Pacific slope and in the northwestern United States.

2. The habitat is the decaying wood of forests, very rarely in buildings, and never in the earth.

3. Four stable castes are of common occurrence; the first form, the second form, and the third form are the fertile reproductive castes; among the soldiers the females are sterile, the males are probably also sterile, though near fertility. There is no true sterile worker caste.

4. The sexes are differentiated externally in all castes.

5. Three additional variations or types are of occasional occurrence. These are: second and third forms with very small wing vestiges and soldiers with wing vestiges. There is no correlation between the presence of wing vestiges and fertility, but brain size and fertility are invariably correlated.

6. A plate-like non-functional frontal gland, without fontanel, is present in first-form nymphs and adults.

7. Vestiges of the lateral ocelli are found in all the castes, except, possibly the third form.

8. There is great variability in all organs, and even in the degree of infertility of some female soldiers.

9. Termopsis is considered a very primitive genus, on account of its many ancestral characters and its close resemblance to the even more primitive genus Archotermopsis.

10. The castes of termites are regarded as segregants, arising by mendelian inheritance from a heterozygous parent form.

Wellesley, Massachusetts
July, 1921

BIBLIOGRAPHY

Banks, N., and Snyder, T. E. 1919 Revision of Nearctic termites with notes on biology and geographic distribution. U. S. National Museum, Bull. 108.

Boring, Alice M., and Pearl, Raymond 1918 Sex studies. XI. Hermaphrodite birds. Jour. Exp. Zoöl., vol. 25.

Bugnion, E. 1912 Observations sur les termites. Differentiation des Castes. Comp. Rend. Soc. Biol. Paris, I, T. 72.

Comstock, J. H. 1918 The wings of insects. Ithaca, New York.

Feytaud, J. 1912 Contribution à l'étude du termite lucifuge. Arch. d'anat. micros., T. 13.

Fuller, Claude 1920 Studies on the post-embryonic development of the antennae of termites. Annals Natal Museum, vol. 4.

Grassi, B., and Sandias, A. 1893–94 Costituzione e sviluppo della società dei Termitidae. Atti Acad. Gioenia di sci. nat., Catania.

Heath, Harold 1903 The habits of California termites. Biol. Bull., vol. 4.

Holmgren, N. 1909 Termitenstudien 1. Anat. K. Svenska Vetensk. Akad. Handl., Bd. 44.

1911 Termitenstudien 2. System. K. Svenska Vetensk. Akad. Handl., Bd. 46.

Imms, A. D. 1919 On the structure and biology of Archotermopsis, together with descriptions of new species of intestinal Protozoa, and general observations on the Isoptera. Phil. Trans. Royal Soc. London, Series B. vol. 209.

JÖRSCHKE, HERMANN 1914 Die Facettenaugen der Orthopteren and Termiten. Inaug. Dissert. Leipzig.

JUCCI, C. 1920–21 Sulla differenziazione delle caste nella Società dei Termitidi. Rendicont. R. Accad. Naz. Lincei, vols. 29, 30.

KNOWER, H. McE. 1894 Origin of the 'Nasutus' (soldier) of Eutermes. Johns Hopkins Univ. Bull., vol. 13.

1901 A comparative study of the development of the generative tract in termites. Johns Hopkins Hospital Bull., vol. 12, nos. 121–123.

KOFOID, C. A., AND SWEZY, O. 1919 Studies on the parasites of the termites. I to IV. Univ. of Cal. Publ., vol. 20, nos. 1 to 4.

LESPÈS, C. 1856 Recherches sur l'organisation et les moeurs du Termite lucifuge. Ann. Sci. Nat. Zool., 4e ser., T. 5.

MORGAN, T. H. 1919 The physical basis of heredity. Philadelphia and London.

MULLER, H. J. 1918 Genetic variability, twin hybrids and hybrids, in a case of balanced lethal factors. Genetics, vol. 111.

SAFIR, S. R. 1920 Genetic and cytological examination of the phenomena of primary non-disjunction in Drosophila melanogaster.

SNYDER, T. E. 1915 Biology of the termites of the eastern United States, with preventive and remedial measures. U. S. Dept. Agric., Bur. Ent., Bull. no. 94, pt. II.

STEVENS, N. M. 1905 Studies in spermatogenesis with especial reference to the 'accessory chromosome.' Carnegie Institution of Washington, Publ. 36.

THOMPSON, C. B. 1916 The brain and the frontal gland of the castes of the 'white ant,' Leucotermes flavipes Kollar. Jour. Comp. Neur., vol. 26.

1917 Origin of the castes of the common termite, Leucotermes flavipes Kol. Jour. Morph., vol. 30.

1919 The development of the castes of nine genera and thirteen species of termites. Biol. Bull., vol. 36.

THOMPSON, C. B., AND SNYDER, T. E. 1919 The question of the phylogenetic origin of termite castes. Biol. Bull., vol. 36.

1920 The 'third form,' the wingless reproductive type of termites: Reticulitermes and Prorhinotermes. Jour. Morph., vol. 34.

DESCRIPTION OF PLATES

ABBREVIATIONS

cl, colleterial gland
ov, oviduct
sp, spermatozoa
sr, seminal receptacle
sv, seminal vesicle
t, testis
tf, terminal filament

PLATE 1

EXPLANATION OF FIGURES

All figures are drawn from semitransparent whole mounts of dissections of Termopsis angusticollis. Spencer oc. 6, obj. 16 mm., stage level.

10 Female reproductive organs, first-form adult.
11 Female reproductive organs, immature first-form nymph.
12 Female reproductive organs, mature first-form nymph.
13 Female reproductive organs, third-form adult.
14 Nearly mature egg, third form.

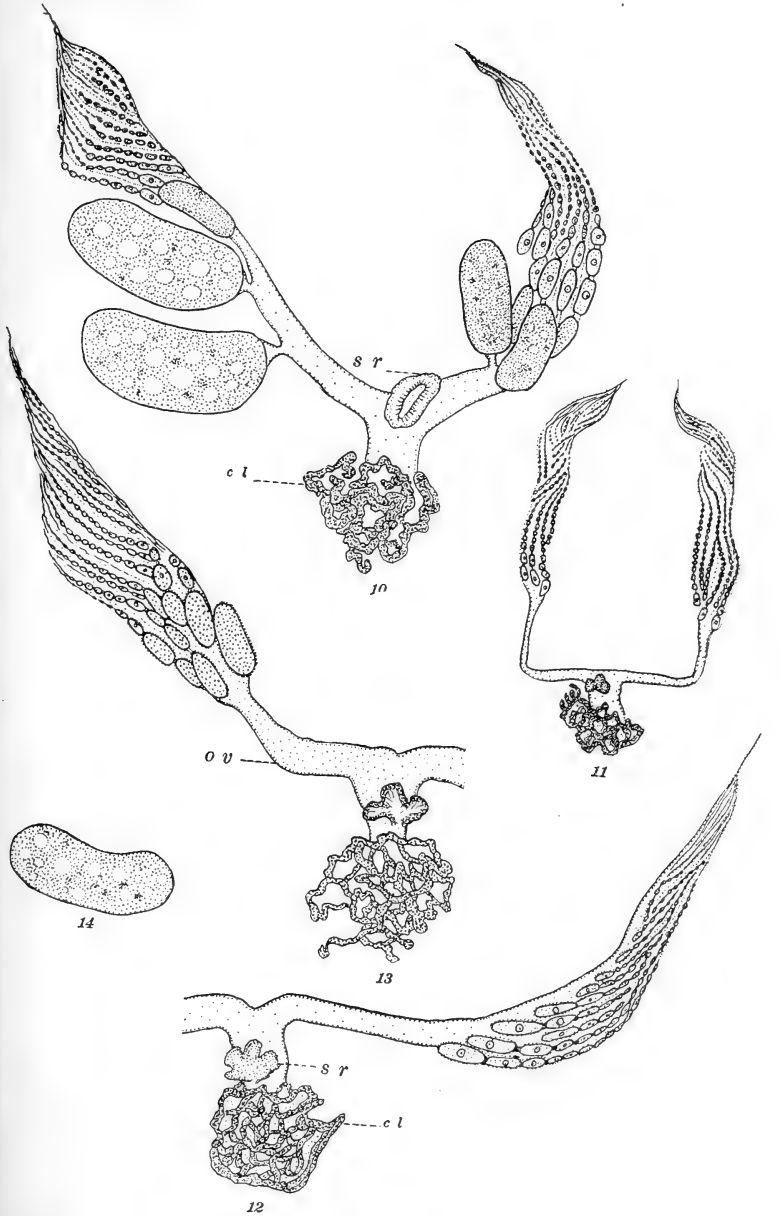
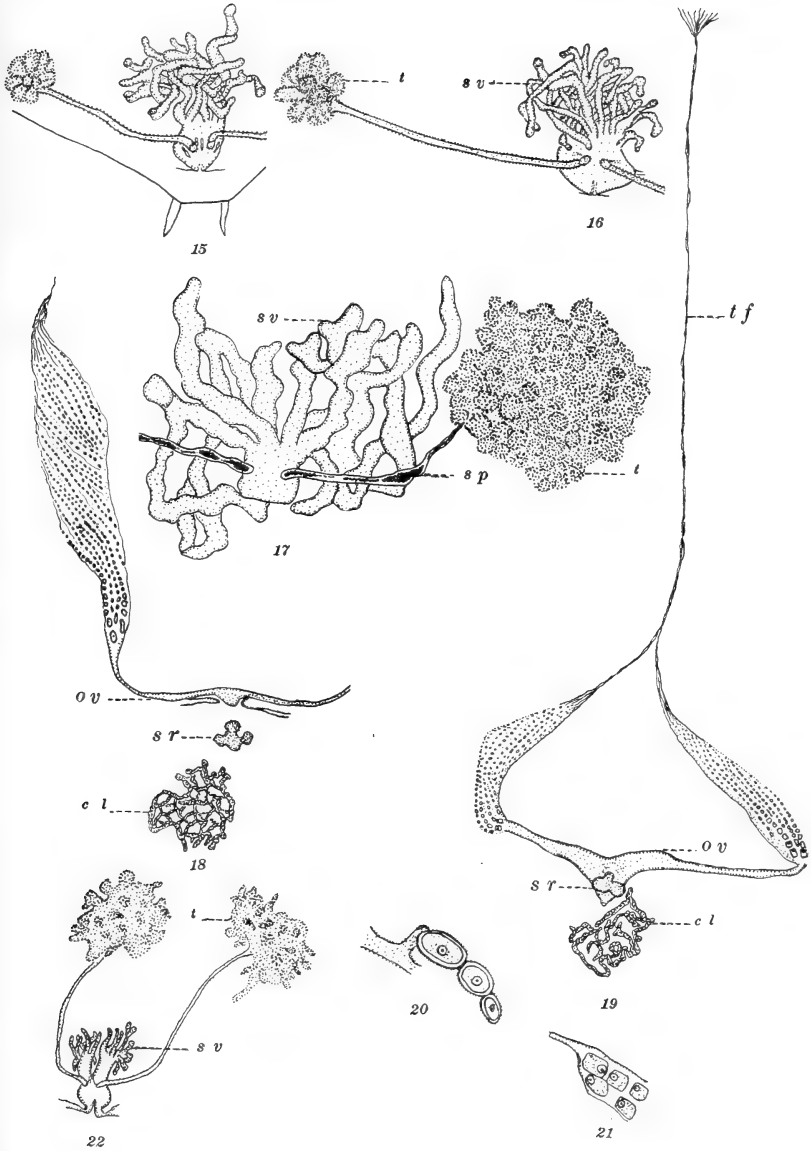

s r
c l
10
11
o v
14
13
s r
c l
12

PLATE 2

EXPLANATION OF FIGURES

All figures are drawn from semitransparent whole mounts of dissections of Termopsis angusticollis. Figures 15 to 19 and 22, Spencer oc. 6, obj. 32 mm., stage level. Figures 20 and 21, Spencer oc. 6, obj. 16 mm., stage level.

15 Male reproductive organs, first-form adult.
16 Male reproductive organs, mature first-form nymph.
17 Male reproductive organs, third-form adult.
18 Female reproductive organs, adult soldier.
19 Male reproductive organs, adult soldier.
20 Ova from the proximal end of an egg tube, soldier nymph.
21 Ova from the proximal end of an egg tube, adult soldier.
22 Female reproductive organs, adult soldier.


15
t
s v
16
t f
s v
s p
t
17
O v
s r
c l
18
O v
s r
c l
19
t
s v
22
20
21

Resumen por el autor, Dean L. Gamble.

La morfología de las costillas y procesos transversos de Necturus maculatus.

En la mayor parte de los urodelos la cabeza ventral o capitular de la costilla se inserta sobre un proceso del arco neural, dorsalmente a la arteria vertebral. En Necturus, lo mismo que en los elasmobranquios, la cabeza ventral de la costilla se inserta, en todas las vértebras, excepto en las dos o tres primeras, en el nódulo basal, ventralmente a la arteria vertebral. En la porción extrema anterior de la columna vertebral, el tabique horizontal y las costillas están opuestas a la base del arco neural. En esta región cada costilla se inserta en un proceso del arco neural (el "portador de la costilla" de Goeppert) y no presenta connexión alguna con el nódulo basal. Esta prueba presta apoyo á la explicación de Rabl sobre la inserción de las costillas de los urodelos, la cual es opuesta a la explicación más generalmente aceptada de Goeppert. Las costillas de la segunda y tercera vértebras de Necturus se insertan sobre la columna vertebral de un modo exactamente semejante al que se observa en los otros urodelos. Además, los bastones dorsales y ventrales de los procesos transversos en las primeras vértebras de Necturus son homólogos de semejantes estructuras en otros urodelos. El nódulo primitivo basal de Necturus, (el elemento basi-ventral de Gadow) desarrolla un proceso lateral, la parapófisis, y una hemapófisis ventral. En la región del tronco, en un estado más joven, ambas existen. En estados ulteriores, en la región del tronco, la parapófisis persiste y se une con la cabeza ventral de la costilla, mientras que la hemapófisis desaparece. En la región de la cola la hemapófisis se alarga para formar el arco hemal y la parapófisis desaparece. En este respecto Necturus corresponde casi exactamente a Polypterus.

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 17

THE MORPHOLOGY OF THE RIBS AND TRANSVERSE PROCESSES IN NECTURUS MACULATUS[1]

D. L. GAMBLE

THIRTY-ONE FIGURES

A summary of the literature upon the transverse processes and their relations to the ribs and haemal arches in the urodeles results in contradictions with regard to the morphology of these parts. An attempt to verify statements by a study of the development of the parts concerned has resulted in the present communication. Necturus was chosen as the type, partly for convenience and partly because it was believed that it might exhibit conditions primitive enough to serve as a basis for interpreting the morphology of these structures in urodeles generally.

For a clear conception of the problem, the following observations are pertinent: the ribs of urodeles exhibit several differences when compared with the dorsal fish ribs. In elasmobranchs the ribs are connected with the centrum by means of the basal stump, which is located ventrad of the vertebral artery. In most urodeles, however, the ventral head of the rib is attached to a process of the neural arch above the vertebral artery. If this latter attachment were brought about by the simple dorsal shifting of the rib and basal stump, the relations of the vertebral artery in regard to these structures would be unchanged.

Two interpretations of this condition obtain at present. One is that, due to the dorsal shifting of the horizontal septum and with it the rib, the latter structure has simply lost its connection with the basal stump and has become attached to the neural arch (Rabl, '92).

[1] This problem was suggested by, and carried on under the direction of Dr. H. D. Reed. The writer is not only indebted to him for his help and advice throughout the work, but also for the use of his splendid series of Necturus larvae.

The interpretation most generally accepted is that of Goeppert ('96), who attempted to show that the attachment of the rib to the neural arch has come about in a complicated fashion. Goeppert was an advocate of the view that the rib is an outgrowth of the basal stump, while Rabl thought of the same structure as an independent element, having nothing to do genetically with the vertebra. Goeppert therefore offered another explanation to account for the new rib attachment, which was not incompatible with his belief in the morphological relations of the two structures.

The following is a brief summary of Goeppert's work on this problem. In Necturus, the basal stump, at about the middle of its length, sends off a dorsal process which he called the 'rib-

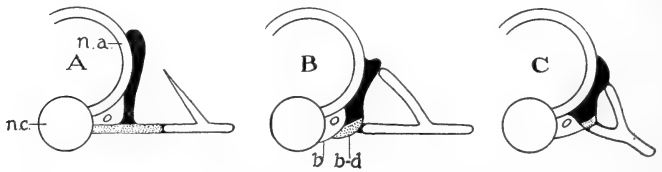


Fig. 1 A series of diagrams to illustrate Goeppert's interpretation of the change in the vertebral attachment of the rib in urodeles. Black, rib-bearer; stippled, parapophysis (basal stump of Goeppert); clear, rib; *b*, basal portion of basal stump; *b-d*, distal portion of same; *n.a.*, neural arch; *n.c.*, notochord. A, Necturus; B, Salamandra; C, Triton.

bearer' (fig. 1A, black). This passes dorsally to the neural arch, laterad of the vertebral artery, and then continues dorsocaudally over the surface of the arch. The rib-bearer is separated from the cartilage of the neural arch by a sheath of connective tissue and later bone. Laterad of the rib-bearer the basal stump (fig. 1A, stippled) continues horizontally, and the rib (fig. 1A, clear) forms as a mere prolongation of this element. Further laterad still the rib develops a dorsal process which extends mesally toward the vertebra and becomes the dorsal head of the rib.

In the larval Salamandra, according to Goeppert, the basal portion of the basal stump has disappeared, and its place is taken by a slender bar of bone (fig. 1B, *b*). The distal end of the basal stump (fig. 1B, *b-d*), that is, the portion which projects laterad

of the rib-bearer in Necturus, is much shortened and is indistinguishable from the rib-bearer. Now, the rib is attached directly to the neural arch, although it has never actually lost its connection with the distal end of the basal stump. According to this interpretation of the dorsal shifting of the rib attachment, the rib-bearer is substituted for the proximal portion of the basal stump in attaching the rib to the vertebral column.

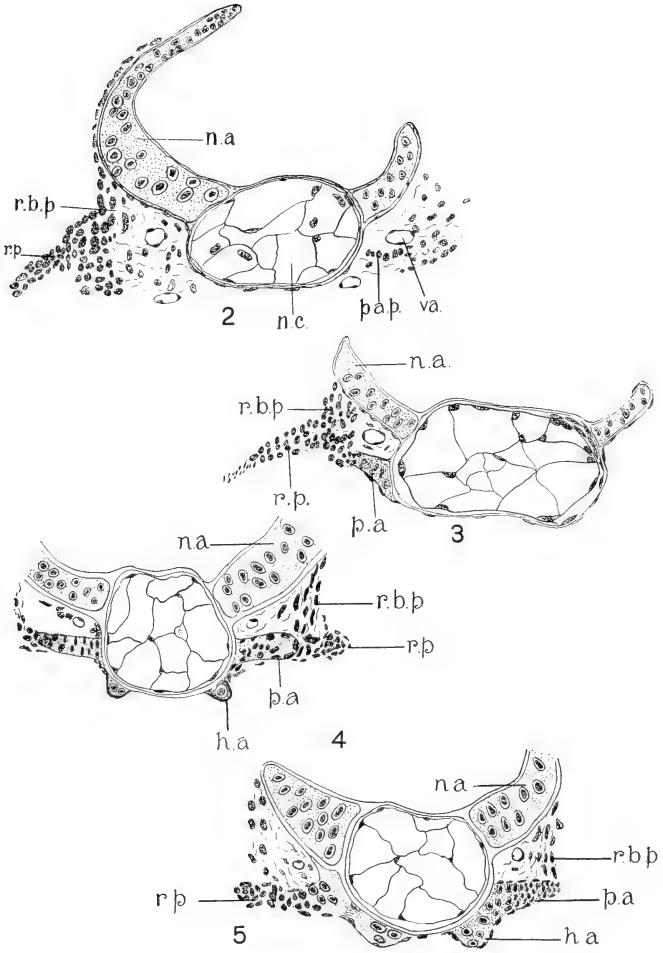
In view of these conflicting opinions regarding the independence of the rib and the way in which its attachment changes, and because of the possibility that the two stages (24 and 43 mm.) studied by Goeppert failed to furnish all the facts, it was deemed advisable to undertake a study of the development of the ribs and transverse processes in a more complete series. Accordingly, Necturus larvae of the following stages were studied: 20, 21, 22, 23, 24, 25, 26, 30, 40, 50, and 70 mm., respectively, and in addition various stages of other species.

After a study of these stages, it is believed that the dorsal shifting of the ribs in urodeles is not the complicated process that Goeppert supposed and that all the steps in this change in attachment can be illustrated in the single form, Necturus.

In discussing the development of these structures the following order will be followed: 1) Fourth to antepenultimate trunk vertebrae. 2) First three vertebrae. 3) Definitive estate of trunk vertebrae. 4) Last trunk and first and second tail vertebrae.

DEVELOPMENT OF TRUNK VERTEBRAE (FOURTH TO ANTEPENULTIMATE)

In the 20-mm. larva (fig. 2) the only cartilage present is that of the neural arch which is not yet complete dorsally. The neural arches of consecutive vertebrae are separated by quite an interval. The sheath of the notochord is made up of an outer skeletogenous layer, the elastica externa, within which is a thinner layer, the elastica interna, while the outer cells of the notochord are arranged in an epithelial-like layer, the notochordal epithelium. In the middle of the future centrum the notochord sheath is thin and all three layers are close together. Intervertebrally the sheath is much thicker, due to the great increase in the num-


n.a
r.b.þ
r.þ
2
n.c.
þ.a.þ.
va.
n.a.
r.b.þ
r.þ.
þ.a
3
n.a
r.b.þ
r.þ
þ.a
h.a
4
n.a
r.b.þ
r.þ
þ.a
h.a
5

ber of cells between the elastica externa and interna. Between the notochord and lateral muscle mass is found loose mesenchymal tissue, in which at this stage a condensation is appearing laterally, which extends outward into the horizontal septum marking the position of the future rib (fig. 2, *r.p.*) and also upward to the neural arch along the inner edge of the transverse septum in the position of the future rib-bearer (fig. 2, *r.b.p.*). In tracing this condensation mesally toward the notochord, it will be seen, in the greater number of vertebrae, to become much weaker and gradually dissolve into what appears to be typical mesenchymal tissue. In some vertebrae the basal stump is indicated by a few slightly modified mesenchymal cells which line up between the notochord and the rib proton[2] (fig. 2, *p.a.p.*). In either case the greatest condensation of mesenchyme appears laterally at the inner margin of the lateral muscles, and weakens toward the notochord.

Goeppert's belief in the rib as a lateral outgrowth of the basal stump and the rib-bearer as a dorsal outgrowth of the same element were based apparently upon the mistaken observation that these structures are continuous in the proton stage. The examination of a number of vertebrae at this stage of development shows that there is a difference in the time of appearance of the protons of these structures. The rib and rib-bearer protons are well marked out in many of the vertebrae before the proton

Fig. 2 Transection through the fourth vertebra of a 20-mm. larva. *n.a.*, neural arch; *n.c.*, notochord; *p.a.p.*, proton of parapophysis; *r.b.p.*, rib-bearer proton; *r.p.*, rib proton; *v.a.*, vertebral artery.

Fig. 3 Transection through the fourth vertebra of a 20-mm. larva slightly more advanced in development than that shown in figure 2. *n.a.*, neural arch; *p.a.*, parapophysis; *r.b.p.*, rib-bearer proton; *r.p.*, rib proton.

Fig. 4 Transection through the fifth vertebra of a 21-mm. larva. *h.a.*, haemapophysis; *n.a.*, neural arch; *p.a.*, parapophysis; *r.b.p.*, rib-bearer proton; *r.p.*, rib proton.

Fig. 5 Transection through the eighth vertebra of a 21-mm. larva. *h.a.*, haemapophysis; *n.a.*, neural arch; *p.a.*, parapophysis; *r.b.p.*, rib-bearer proton; *r.p.*, rib proton.

[2] The term 'proton,' introduced by Prof. B. G. Wilder, is used in the present communication in preference to the word 'anlage.' It has the same meaning and has the added advantage of being an English term.

of the basal stump appears. When it does form it has a distinctly different appearance; that is, it is weaker and made up of fewer cells and stains much less intensely with haematoxylin.

This difference between the proton of the rib and rib-bearer on one hand and that of the basal stump on the other, points to the former as elements formed in the muscle septa and seems to indicate that the basal stump only is a lateral product of a vertebral element.

In a slightly more advanced larva which also measured 20 mm. the basal stump makes its first appearance (fig. 3, *p.a.*). It is seen here as a cartilaginous process projecting laterally from the notochord. This cartilage in the fourth trunk vertebra does not lie in a horizontal plane, but projects slightly dorsad as well as laterad.

At its tip the hyaline matrix disappears and it becomes continuous with a strand of procartilage cells (the proton of the rib-bearer) which extends upward to the neural arch. At a level with the base of the neural arch this strand of cells is also continuous with the rib proton (fig. 3, *r.p.*) which passes into the horizontal septum. Farther back in the trunk region the proximal end of the rib proton appears on a level with the middle of the notochord and here the basal stump lies horizontally (fig. 4, *p.a.*) and is directly continuous with the rib proton (fig. 4, *r.p.*). When this is the case the distal end of the basal stump is connected with the neural arch by a strand of procartilage cells as in the anterior region (fig. 4, *r.b.p.*).

In the 21-mm. larva the neural arch is completed dorsally, but the basal stump appears no further advanced than in the 20-mm. larva. The first rib chondrification is to be noticed in the anterior trunk region of the 21-mm. larva. This first cartilage is developed distally, and between it and the cartilage of the basal stump procartilage is found.

On the ventral side of the notochord just ventrad of the base of the basal stump are found in many of the trunk vertebrae small knobs of cartilage (fig. 4, *h.a.*). Occasionally two are found in a single vertebra, one lying on either side of the aorta. Generally, however, only one is present. The hyaline matrix of

these ventral cartilages stains as deeply as that of the neural arch, the lacunae are large and contain large cells. This is in sharp contrast to the matrix of the lateral cartilage ('basal stump' of Goeppert) which stains very faintly and gives every indication of having been formed more recently.

In the 5th, 6th, 7th, 8th, 11th, and 12th vertebrae the ventral cartilages are entirely independent of the lateral cartilages (fig. 4) but in the 9th, 13th, 14th, 16th, and 17th they are joined with the bases of the lateral cartilages, from the proximal ends of which they appear as ventral outgrowths (fig. 5, *h.a.*). These elements are absent in the remaining trunk vertebrae, but in several of these there is to be noted a slight tendency of the base of the lateral cartilage to bulge downward. This possibly locates the position of the ventral cartilage. It is seen that anteriorly the greater number of the vertebrae possess separate ventral elements, but that posteriorly the ventral and lateral cartilages are joined. In several the ventral elements have lost their identity entirely.

These ventral knobs of cartilage were not observed by Goeppert in Necturus, but he saw them in Salamandra ('96). Ventrad of the slender bar of bone (fig. 1, *b*), which he interpreted as the reduced basal stump, ventral cartilages were located which disappeared at the onset of bone formation. Goeppert observes: "There occurs in Salamandra an unhitherto described peculiarity. On the ventral side of the notochord, cartilage elements are found belonging to the haemal arch system. These are present in the young animal. In the tail region they elongate to form the haemal arches. There is no connection between the bony strand of the rib-bearer and these elements. This separation must be considered as a secondary condition." The way in which Goeppert believes this separation comes about will be described when the vertebrae of the tail-trunk region of Necturus are discussed.

In a 30-mm. Polypterus larva Budgett ('01) found a metameric series of cartilages resting upon the notochordal sheath. There was a dorsal row, the bases of the neural arch, a lateral row, forming the foundation of the transverse processes and ribs

and a still smaller series of ventral cartilages forming the foundations of the ventral ribs. He found all three series well developed in the anterior region. The lateral series were developed out into the horizontal septum as long processes, but at that stage had no connection with the ribs which he saw forming independently in the lateral portion of the horizontal septum, but having no connection with the lateral cartilages. The ventral series were also well developed and passed out between the ventrolateral muscles and the kidneys, never reaching, however, the peritoneum at this stage. In the caudal region the lateral series was not found and the ventral series had elongated to form the haemal arches.

Budgett stated in conclusion: "In the possession of three pairs of vertebrally placed cartilages resting upon the notochordal sheath, before the commencement of bone formation, Polypterus differs from all living vertebrates."

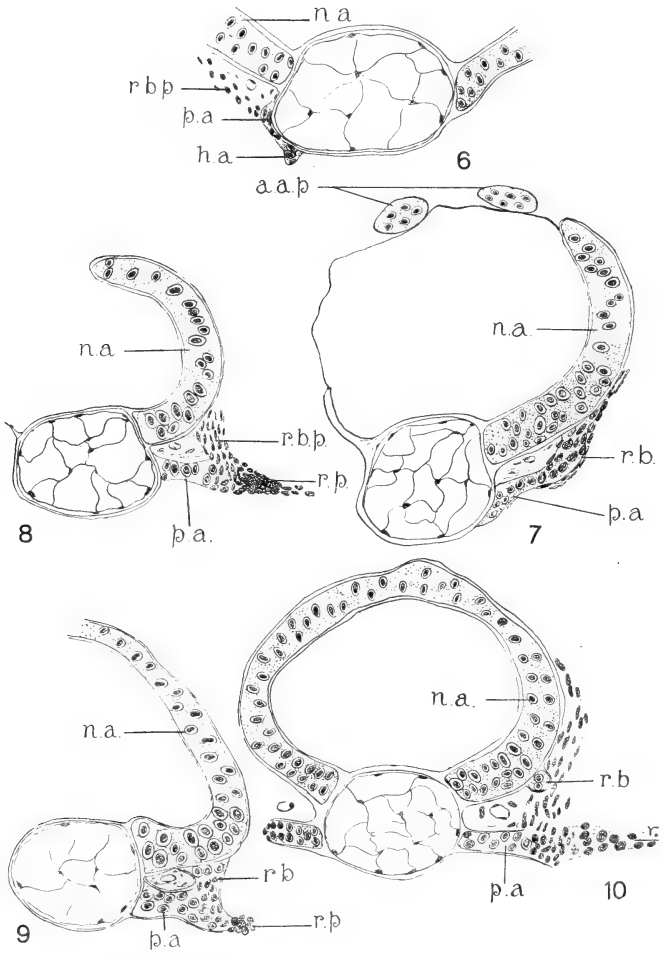
It is interesting to note that in the possession of these cartilages the 21-mm. Necturus larva at this stage corresponds very closely to Polypterus. Furthermore, cartilage is appearing in the rib distally, apparently as it does in Polypterus. The difference between the two forms lies in the fact that in Necturus the ventral cartilages, in some of the trunk vertebrae, are joined with the bases of the lateral cartilage and in the posterior trunk region become more or less indistinguishable from them. In the tail region this basal portion which represents ventral cartilage grows down to form the haemal arch while in Polypterus the haemal arch is formed by the ventral cartilages which have remained independent of the lateral cartilages throughout the trunk region. In Polypterus the ventral cartilages are present throughout the life of the individual and elongate to form the ventral ribs, while in Necturus they disappear when bone formation begins.

Salamandra, in so far as the ventral cartilages are concerned, also corresponds closely to Polypterus. Here at an early stage they are found in the trunk region and in the tail form the haemal arch. Salamandra differs from Polypterus, however, in the loss of the lateral cartilage.

The ventral cartilages were first seen in a 20-mm. Necturus larva (fig. 6, *h.a.*). Here a lateral outgrowth of the ventral cartilages is just beginning to appear (*p.a.*). Although these two processes are continuous, it is very evident that the cartilage of the ventral element is better developed and that the cells of the lateral process have just begun to secrete a hyaline matrix. This shows clearly that the primitive basal stump is growing in two directions, ventrally giving rise to a haemapophysis and laterally becoming the parapophysis. When bone forms in the skeletogenous layer of the notochord the haemapophyses of the trunk disappear. From this point on the terms of Owen will be used while discussing these 'derivatives' of the basal stump. The lateral cartilage will be called the parapophysis and the ventral, the haemapophysis.

In the 22-mm. larva the cartilage of the rib has developed mesally so that the area of procartilage between it and the parapophysis is much less extensive. Caudad of the third vertebra the ventral cartilages or haemapophyses do not appear, the reason for this being apparently that bone is beginning to make its appearance in the outer layer of the notochordal sheath. In this stage (fig. 7) in the fourth and fifth vertebrae the parapophysis makes a cartilaginous connection with the rib-bearer for the first time. Because of the fact that in these vertebrae the basal stump tends to project dorsolaterally, no distinction can be made between it and the rib-bearer. In passing caudally, however, it will be seen that as the parapophysis comes to lie in the horizontal plane, the cartilage of the rib-bearer disappears and in its place appears a strand of procartilage cells (fig. 8).

In the 24-mm. larva structures are considerably more advanced. The dorsal portion of the neural arch has begun to develop caudally into a median posterior articular process and cephalad into two anterior articular processes, one on either side of the middorsal line. The parapophysis in the midtrunk region projects laterally and in the strand of cells which connects it and the neural arch in the preceding stage, cartilage appears (fig. 9, *r.b.*).


n.a
r.b.p
p.a
h.a
6
a.a.p
n.a
r.b
p.a
7
n.a
r.b.p
r.p
8
p.a.
n.a.
r.b
r
p.a
10
n.a
r.b
r.p
9
p.a

Following the law of cephalocaudal growth and differentiation, structures nearer the anterior end of the body have differentiated further than those which are located more caudally, so that in passing backward through the trunk region successively younger stages in the formation of ribs and transverse processes are encountered.

This law applies to all the trunk vertebrae except the first three. Here, as will be discussed later, the development of the various parts of the transverse process and ribs seems to lag behind the trunk vertebrae immediately caudad. This condition may be interpreted as the beginning of a process which in higher forms leads to a reduction of ribs in the cervical region.

Beginning with the fourth vertebra, the rib-bearer is seen connecting the neural arch and the distal end of the parapophysis, while farther caudad this connection has not yet been made and the rib-bearer appears as a knob of cartilage attached to the neural arch (fig. 10). Still farther caudad the cartilage of the rib-bearer has not yet made its appearance.

In the 43-mm. larva studied by Goeppert this cartilage of the rib-bearer next to the neural arch is found, but he concluded that the lack of continuity between it and the basal stump was due to a secondary degeneration of the cartilage. When intermediate stages are studied, however, it is shown very clearly that cartilage between the two may never have been formed. The study of older stages shows that in the greater number of the trunk vertebrae this connection is finally made, but that in a few in which development is arrested the two are separated. In such an event the result is that of the relations seen by Goeppert and interpreted by him as a secondary degeneration.

Fig. 6 Transection through the second vertebra of a 20-mm. larva. *n.a.*, neural arch; *h.a.*, haemapophysis; *p.a.*, parapophysis; *r.b.p.*, rib-bearer proton.

Fig. 7 Transection through the fourth vertebra of a 23-mm. larva. *a.a.p.*, anterior articular processes; *n.a.*, neural arch; *r.b.*, rib-bearer; *p.a.*, parapophysis.

Fig. 8 Transection through a midtrunk vertebra of a 23-mm. larva. *n.a.*, neural arch; *r.b.p.*, rib-bearer proton; *r.p.*, rib proton; *p.a.*, parapophysis.

Fig. 9 Transection through the sixth vertebra of a 24-mm. larva. *n.a.*, neural arch; *r.b.*, rib-bearer; *r.p.*, rib proton; *p.a.*, parapophysis.

Fig. 10 Transection through a trunk vertebra of a 25-mm. larva. *r.*, rib; *n.a.*, neural arch; *r.b.*, rib-bearer; *p.a.*, parapophysis.

The two processes which were seen just beginning to develop forward from the dorsal portion of the neural arch in the 24-mm. stage in the 26-mm. larva have continued their cephalic growth until they meet the process developing caudad from the preceding vertebra. These will form the articular processes of the definitive vertebra. The transverse process is in about the same condition except that the rib-bearer has developed dorsocaudally over the outer surface of the neural arch. In other words, cartilage is forming in the inner edge of the transverse septum along the line of its attachment to the neural arch.

The cartilage of the rib now extends inward to the distal end of the parapophysis, but procartilage still persists between these two elements. After the fusion of the rib-bearer and the parapophysis, a lateral extension of the transverse process takes place distad of this point. As the animal grows the rib attachment must be shifted laterally to bring it into its final position. This means that procartilage cells must persist between the transverse process and the rib, so that proliferation of cartilage cells may take place and the parapophysis grow laterally. It was in a larva of about this age that Goeppert saw the rib-bearer appearing as a dorsal outgrowth from about the middle of the basal stump. The study of younger stages shows that this is not the case, but that the rib-bearer grows downward and fuses with the distal end of the parapophysis and that the ventral head of the rib is borne upon an extension of the parapophysis which develops laterally after the rib-bearer has united with it.

The rib gives off a dorsal process which becomes the tubercular head. This extends dorsomesally toward the neural arch. In the trunk region it never connects with a corresponding process of the rib-bearer, but in the anterior region this does occur. This will be discussed more in detail later.

DEVELOPMENT OF ANTERIOR TRUNK VERTEBRAE

As before stated, Goeppert's conclusions concerning the dorsal shifting of the rib attachment in urodeles were based upon a comparison of the conditions found in Necturus, Salamandra, and Triton. After the study of a complete series of Necturus larvae,

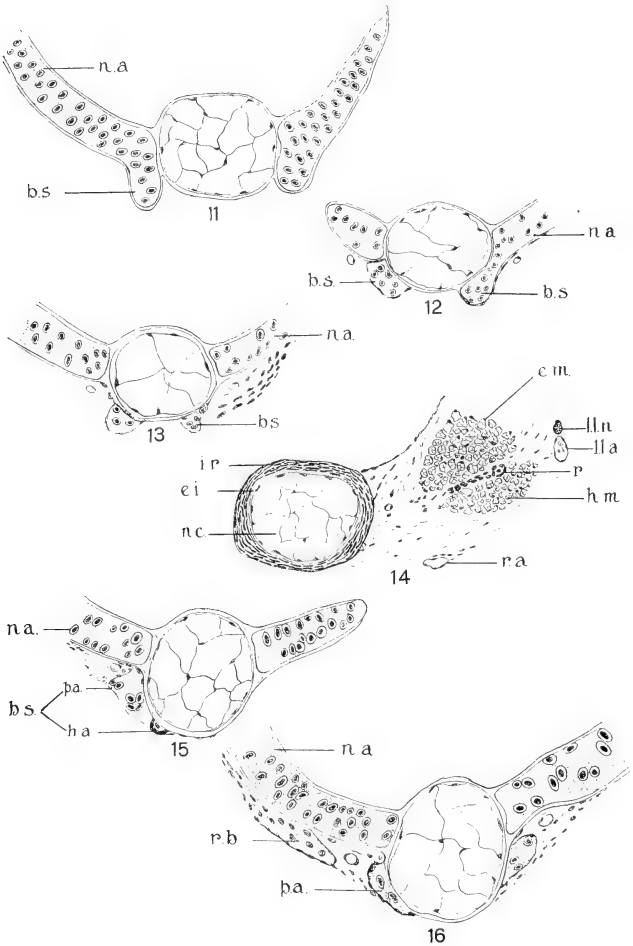
it is believed by the writer that all of the steps in this shifting of attachment can be illustrated by this one form alone. In Necturus the horizontal septum is relatively high in the extreme anterior region of the trunk and is lower posteriorly, and therefore might be looked to with a reasonable amount of assurance, to show how the rib has become attached to the neural arch.

This has already been suggested by Wilder ('03) in his memoir on the skeletal system of Necturus. Wilder makes the following statement in this connection: "It would thus seem, judging from the purely anatomical evidence, that the condition described by Goeppert as characteristic of Necturus, is not a universal one applicable to all the vertebrae, but is restricted to a certain region approximately that of vertebrae 8 to 18."

"It would seem important to investigate the development of the transverse process and rib in certain of the other vertebrae, for example the second and the fourth."

It was found in the present study that Wilder's suggestion was a good one and that the second, third, and fourth vertebrae are important in making intelligible the morphology of these structures.

The first vertebra never bears a definitive rib, although a cartilaginous rib rudiment was found in this vertebra in a 23-mm. larva (fig. 14). The first vertebra has one peculiarity, however, which distinguishes it from all the other vertebrae. The bases of the neural arch of the first vertebra are enlarged and extend much farther ventrad than in others. These enlargements seem to represent basal stumps which have fused with the bases of the neural arch (fig. 11). Continuity of neural arch and basal stump was seen in none of the other vertebrae with a single exception (fig. 12). In a 21-mm. larva in the second vertebra the neural arch extended ventrally exactly as in the first. In following back through the sections, however, it was seen that the ventrally projecting end of the neural arch extended caudad so that a section which passed through the vertebral column just caudad of the neural arch would cut through these caudal projections. Here, in cross-section, they appear as typical basal stumps before the appearance of any lateral cartilage (fig. 13, *b.s.*).


n.a
b.s
11
n a
b.s
b.s
12
n a.
13
b.s
c m.
II n
II a
i r
e i
n c
r
h m
14
r a
n a.
b s
ba
h a
15
n a
r b
ba.
16

Extending downward and inward from the neural arch is the strand of procartilage cells in which will later form the cartilage of the rib-bearer. It is very noticeable here that this condensation of cells weakens markedly and finally disappears in passing toward the notochord. It is further seen that a mesenchymal condensation has appeared all along the inner edge of the transverse septum where it attaches to the neural arch. This represents the dorsal continuation of the rib-bearer proton. Cartilage appears at this stage in the extreme distal portion of the rib.

In the third vertebra a broad cartilage appears on the ventral side of the notochord on one side only, while on neither side is there any suggestion of a lateral cartilage. This cartilage is to be interpreted as basal stump before the development of the lateral cartilage (parapophysis). As in the preceding vertebra, the procartilage cells are seen in the inner edge of the transverse septum, where it is attached to the neural arch and also between the neural arch and notochord. Here again is noticed the decided weakening of this proton mesally. The beginning of the rib proton is on a level with the base of the neural arch.

In the second vertebra of a 22-mm. larva (fig. 15) the basal stump is very broad at the base. The direction of growth is dorsolateral (*p.a.*), this process dominating the development of the ventral projection of the basal stump (haemapophysis) which appears as a single cartilage cell much more advanced than the

Fig. 11 Transection through the first vertebra of a 20-mm. larva. *b.s.*, basal stump; *n.a.*, neural arch.

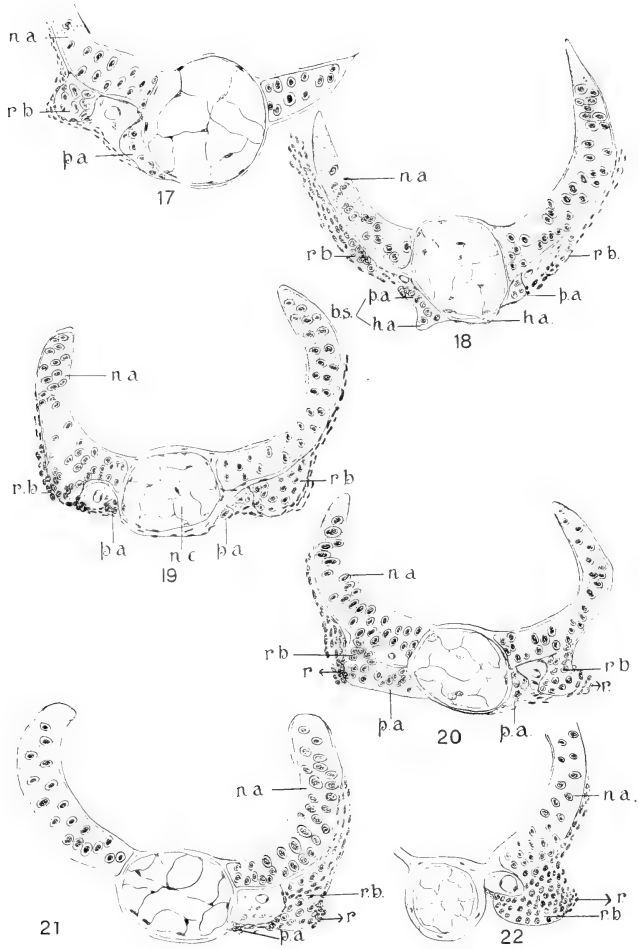
Fig. 12 Transection through the anterior part of the second vertebra of a 21-mm. larva. *n.a.*, neural arch; *b.s.*, basal stump.

Fig. 13 Transection through the posterior part of same vertebra. *n.a.*, neural arch; *b.s.*, basal stump.

Fig. 14 Transection through the vertebral column between the first and second vertebrae. *e.m.*, epaxial muscle mass; *e.i.*, elastica interna; *h.m.*, hypaxial muscle mass; *i.r.*, intervertebral ring; *l.l.a.*, lateral-line artery; *l.l.n*, lateral-line nerve; *n.c.*, notochord; *r.*, rib; *r.a.*, right aorta.

Fig. 15 Transection through the second vertebra of a 22-mm. larva. *n.a.*, neural arch; *p.a.*, parapophysis; *h.a.*, haemapophysis; *b.s.*, basal stump.

Fig. 16 A more caudal transection of the same vertebra. *n.a.*, neural arch; *r.b.*, rib-bearer; *p.a.*, parapophysis.


n a
r b
p a
17
n a
r b
r b.
p a
p a
b s.
h a
h a.
18
n a
r b
r.b
p a
n c
p a
19
n a
r b
r
r b
r.
p a
p.a.
20
n a
r.b.
r
21
n a.
r
r b
22

surrounding cartilage (fig. 15, *h.a.*). Cartilage has developed in the inner edge of the transverse septum along its line of attachment to the neural arch, and this extends downward and inward toward the upwardly developing parapophysis, from which it is separated by mesenchymal cells (fig. 16, *r.b.*).

In the next (third) vertebra, cartilage has not formed all along the line of attachment of the transverse septum to the neural arch, but is found only at its ventral end (fig. 17, *r.b.*). In other words, the rib-bearer appears as a knob of cartilage projecting ventrolaterally from the neural arch, while the parapophysis extends dorsolaterally toward it, the two being separated by a considerable interval. The proton of the rib is continuous with the cartilage attached to the neural arch (rib-bearer) and has no connection with the lateral cartilage (parapophysis) (fig. 17).

It will have been noted by this time that the second and third vertebrae differ in several ways from those farther back in the trunk. These differences can be summed up as follows:

1. Developmental processes seem to be retarded in this region. The parapophyses and the rib-bearer unite much later, the cartilage of the rib develops mesally more slowly, and the haemapophyses persist longer than in the midtrunk region. As before mentioned, this relative slowing up of the development of these structures may be an expression of the reduction of ribs in the cervical region of higher vertebrates.

2. The parapophyses do not lie in a horizontal plane, but project dorsolaterally.

Fig. 17 Transection through the third vertebra of a 22-mm. larva. *n.a.*, neural arch; *r.b.*, rib-bearer; *p.a.*, parapophysis.

Fig. 18 Transection through the second vertebra of a 24-mm. larva. *n.a.*, neural arch; *r.b.*, rib-bearer; *p.a.*, parapophysis; *h.a.*, haemapophysis; *b.s.*, basal stump.

Fig. 19 Transection through the third vertebra of a 24-mm. larva. *n.c.*, notochord; *n.a.*, neural arch; *r.b.*, rib-bearer; *p.a.*, parapophysis.

Fig. 20 Transection through the fourth vertebra of a 24-mm. larva. *n.a.*, neural arch; *r.b.*, rib-bearer; *r.*, rib; *p.a.*, parapophysis.

Fig. 21 Transection through the second vertebra of a 25-mm. larva. *n.a.*, neural arch; *r.b.*, rib-bearer; *r.*, rib; *p.a.*, parapophysis.

Fig. 22 Transection through a trunk vertebra of Amblystoma. *n.a.*, neural arch; *r.*, rib; *r.b.*, rib-bearer.

3. The rib-bearer and the parapophysis approach end to end, while in the trunk region they meet at an angle of 90°.

4. The rib attaches to the rib-bearer and has no connnection with the parapophysis.

On one side of the second vertebra of the 24-mm. larva ventral and lateral cartilages are both present and continuous proximally (fig. 18, *p.a.*, *h.a.*). On the other side lateral cartilage only is present, the haemapophysis (*h.a.*) having disappeared at the onset of bone formation. On the side where both elements are present, the basal stump appears as a forked structure in which growth is taking place in two directions dorsolaterally and ventrally. The lateral outgrowth does not quite meet the cartilage of the rib-bearer, the two being separated by mesenchyme. The rib proton is continuous with the rib-bearer. On the other side the connection between the parapophysis and rib-bearer is almost made, the two being separated by a very narrow zone of procartilage.

In the third vertebra the parapophysis is still unconnected with the rib-bearer, and the rib proton extends laterally from it (fig. 19, *p.a.*). The cartilage of the rib has developed mesally and is separated from the rib-bearer by a limited zone of procartilage cells.

The fourth vertebra of this larva is very important in elucidating the morphology of the rib-bearer, basal stump, and rib (fig. 20). On the left side the basal stump projects laterally from the notochord and is continuous distally with the rib-bearer, as in the stages previously described in the trunk region. The rib is borne, also as in the trunk region, at the point of union of these two elements (*r*). On the right side the parapophysis was tardy in development as compared with the left, and is seen projecting laterally a very short distance and making no connection with the rib-bearer whatever. The rib is borne by the rib-bearer and has no connection with the basal stump.

This condition cannot be explained on the basis of Goeppert's interpretation of the morphology of these structures (fig. 1). Here the development of the parapophysis has lagged behind that of the rib-bearer and the rib has become attached to the

latter before there is the slightest connection between the parapophysis and the rib-bearer. Three points are thereby made clear:

1. The rib-bearer is not an upgrowth of the parapophysis.
2. The distal end of the parapophysis (basal stump of Goeppert) does lose its attachment with the rib and does not become attached to the rib-bearer until after the rib has connected with the same structure.
3. The rib simply shifts its attachment from the basal stump to the rib-bearer as the horizontal septum moves upward.

After the attachment of the rib to the rib-bearer the parapophysis may develop dorsolaterally and fuse with the rib-bearer secondarily. This connection may again be lost after ossification sets in. This will be discussed more fully later.

Another possibility is that the parapophysis may be suppressed altogether. This is seen to be the case in the third vertebra of a 25-mm. larva (fig. 21). Here haemapophyses do not appear at all and the presence of one or two cartilage cells next to the notochordal sheath is all that marks the position of the parapophysis. The ventral end of the rib-bearer projects downward and inward toward the notochord, terminating in a weak strand of cells which extends mesally to the vestige of the parapophysis. The rib is directly continuous with the ventral end of the rib-bearer (fig. 21, *r.*).

If this vertebra be compared with that of a similar stage in Amblystoma (fig. 22), it will be seen to be strikingly similar. The chief difference is that the rib-bearer of the latter is continuous with the cartilage of the neural arch. However, many instances of a direct connection between these two elements were found in Necturus, although they are usually separated. (Compare rib-bearer of opposite sides in fig. 19.) Furthermore, in place of the weak strand of cells between the rib-bearer and notochord found in Necturus, in Amblystoma there is a thin bar of bone.

DEFINITIVE ESTATE

While the body of the animal is growing the ribs must shift laterally to come into their final position, thus necessitating the elongation of the transverse processes. This is interfered with by the formation of bone around these structures. Hard skeletal parts always interfere with the growth of softer parts, and provision must always be made for it. In higher animals bone growth and absorption go hand in hand. In Necturus, however, no resorption of bone deposited around the transverse process and rib was observed. Growth is provided for by the failure of bone to form over the point of union between the proximal end of the rib and the distal end of the parapophysis. Furthermore, between these two elements is found procartilage cells which by proliferation bring about a lengthening of the parapophysis. In principle it is similar to the lengthening of the long bones through the proliferation of cells in the diapophysial plate. This point of growth together with the one at the distal end of the rib makes possible the accommodation of these structures to the growth of the body.

As before mentioned, the dorsal head of the rib develops as a dorsal outgrowth from the rib itself. In the trunk this cartilaginous process does not reach the vertebra, connection being made by a strand of connective tissue. In the second, third, and fourth vertebrae the tubercular head of the rib meets a corresponding outgrowth from the rib-bearer. Between the two, procartilage is found and no bone develops over the articulation. This again must be interpreted as a provision for growth, for as the parapophysis grows outward the dorsal part of the transverse process must keep pace with it. The transverse processes of the second, third and fourth vertebrae therefore possess both a ventral and a dorsal cartilaginous rod surrounded by a sheath of bone. Between the dorsal and the ventral rod a thin layer of bone develops which Wilder ('03) calls the vertical lamina.

Goeppert, in describing the transverse process of Necturus, states that the dorsal part does not appear to be so clearly a rod as it does in Salamandra, and that it lacks the distal excavation seen in Salamandra, the tubercular head of the rib not attaching directly to it.

As Wilder pointed out, the distal cavity which contains the dorsal cartilaginous process of the rib-bearer in the living state is present in the second, third, and fourth vertebrae in Necturus, and this connects with the dorsal head of the rib.

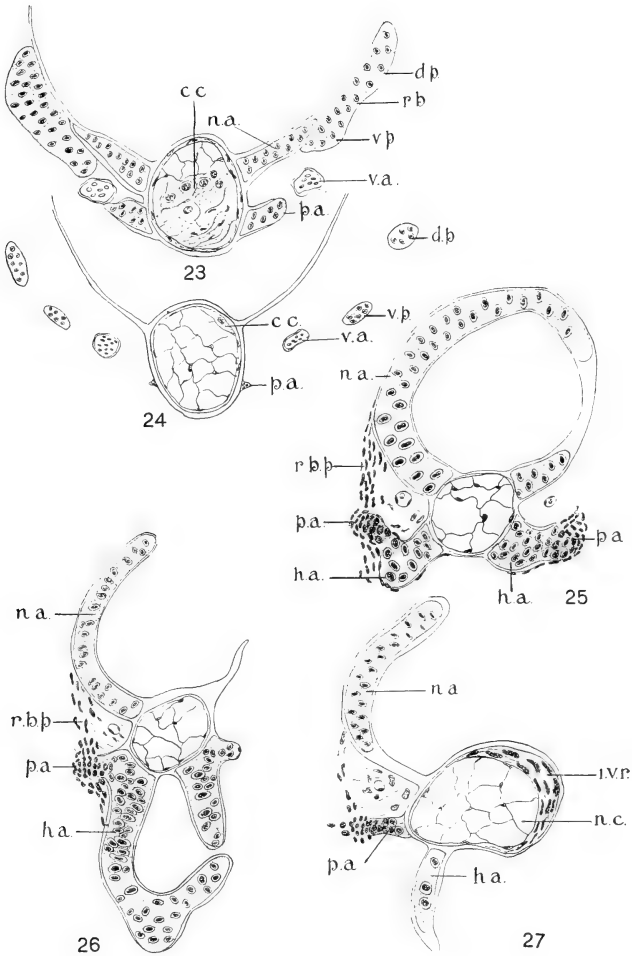
In studying cross-sections of the second vertebrae of a 70-mm. larva, it was observed that the ventral cartilaginous rod of the transverse process is located dorsad of the vertebral artery. In the trunk vertebrae this element is ventrad of the same artery. Figures 23 and 24 illustrate the morphology of this vertebrae. The parapophysis (fig. 23, *p.a.*) extends a short distance laterad from the notochord, but makes no connection with the rib-bearer (*r.b.*)

Two processes are seen developing caudolaterally from the rib-bearer (figs. 23 and 24, *d.p.*, *v.p.*), and these connect with the heads of the rib. This means that the capitular and tubercular heads of the rib of the second vertebra are attached to outgrowths of the rib-bearer and the parapophysis takes no part in this connection.

This failure of the parapophyses to connect with the rib-bearer may or may not be secondary. As was seen by the study of younger stages, the parapophysis may be entirely suppressed. In other cases it may be poorly developed and make no connection with the rib-bearer or it may connect only to be again separated when bone begins to form. However, whether or not the connection between these two elements is made, the rib is borne upon outgrowths of the rib-bearer.

If the macerated vertebral column of an adult Necturus be examined (fig. 31), it will be observed that the transverse processes of the second and third vertebrae are higher than those farther caudad. In tracing the ventral rod of the transverse process of the second vertebra (*v.p.*) proximad, it will be seen to extend dorsad of the foramen for the vertebral artery. In the third vertebra it appears to be on the same level with this foramen, while in the fourth it extends below it.

Although in the third vertebra the parapophysis in a greater number of cases connects with the rib-bearer, here also the rib is attached to outgrowths of the rib-bearer. In the fourth vertebra


c c
n.a.
d.p
r.b
v.p
v.a.
p.a
d.b
23
c c
v.p
v.a.
p.a.
24
n.a.
r.b.p
p.a.
h.a.
p.a
h.a.
25
n.a.
r.b.p
p.a
h.a.
26
n.a
I.V.P.
n.c.
p.a
h.a.
27

the capitular head is borne upon an extension of the parapophysis, while the tubercular head is attached to a process of the rib-bearer.

It is of interest to note that the higher position of the first three ribs is in some way correlated with the formation of a direct connection between the tubercular head of the rib and the dorsal process of the rib-bearer. It is also important to note that the relation of the rib to the rib-bearer in these anterior vertebrae is almost exactly similar to the relations of the same structures throughout the trunk region of Amblystoma and Salamandra. The one minor difference between them is that in Necturus the rib-bearer is generally separated from the cartilage of the neural arch by connective tissue, while in Amblystoma and Salamandra the two are continuous. As shown in figure 19, however, this continuity often obtains in Necturus.

DEVELOPMENT OF VERTEBRAE IN THE TAIL-TRUNK TRANSITIONAL REGION

In the trunk region of the younger larvae (21 to 22 mm.) it has been seen that distinct ventral cartilages or haemapophyses often appear. In some vertebrae these are independent elements and in others are fused with the bases of the parapophyses and appear as ventral projections of the same. In several cases the basal stump appears to be growing in two directions, laterally and ventrally, although the lateral element becomes dominant and the ventral element in time disappears.

Fig. 23 Transection through a second vertebra of a 70-mm. larva. *c.c.*, chordal cartilage; *d.p.*, dorsal process of rib-bearer; *v.p.*, ventral process of rib-bearer; *n.a.*, neural arch; *v.a.*, vertebral artery; *r.b.*, rib-bearer; *p.a.*, parapophysis.

Fig. 24 More caudal transection of the same vertebra. *d.p.*, dorsal process of rib-bearer; *c.c.*, chordal cartilage; *v.p.*, ventral process of rib-bearer; *v.a.*, vertebral artery; *p.a.*, parapophysis.

Fig. 25 Transection through the last trunk vertebra of a 25-mm. larva. *n.a.*, neural arch; *r.b.p.*, rib-bearer proton; *p.a.*, parapophysis; *h.a.*, haemapophysis.

Fig. 26 Transection through the first tail vertebra of a 25-mm. larva. *n.a.*, neural arch; *r.b.p.*, rib-bearer proton; *p.a.*, parapophysis; *h.a.*, haemapophysis.

Fig. 27 Transection through the second tail vertebra of a 26-mm. larva. *i.v.r.*, intervertebral ring; *n.c.*, notochord; *h.a.*, haemapophysis; *p.a.*, parapophysis; *n.a.*, neural arch.

In the transitional region between the trunk and tail no separate haemapophyses were found in any of the larvae studied. The haemal arch is formed by the downgrowth of the bases of the parapophysis and not by the elongation of distinct haemapophyses, as in Salamandra and Amblystoma.

In the 25-mm. larva (fig. 25) in the last trunk vertebra the proximal portion of the basal stump shows a tendency to bulge downward. On one side this is very noticeable, while on the other the process is in an incipient stage. On the left the proximal portion of the basal stump bends downward considerably below the horizontal septum. A short distance from the notochord, growth apparently is taking place in two directions, laterally to form the lateral process and ventrally to form the haemal-arch element. The more ventral outgrowth becomes dominant over the lateral one and leaves it behind, so that it appears as a lateral process of the haemapophysis. On the other side the ventral outgrowth has not yet become dominant over the lateral and appears here only as a rounded knob projecting slightly ventrad from the base of the parapophysis (fig. 25).

In the first tail vertebra of the same larva (fig. 26) both haemapophyses have developed ventrally and completed the haemal arch below. The parapophysis appears as a small lateral process of the haemal arch. In the second tail vertebra this lateral process has lost its connection with the haemal arch and exists as an independent element (fig. 27, *h.a.*).

It is seen, therefore, that the basal stump of Necturus shows a tendency to fork not only in this region, but also farther forward in the trunk of younger larvae. Anteriorly the lateral division becomes dominant as the parapophysis and the lateral disappears, while posteriorly the ventral one becomes dominant as the haemapophysis and the ventral disappears.

These two divisions of the basal stump, however, tend to separate secondarily not only in the anterior tail region, but also in the trunk. This separation is permanent in other salamanders and in Polypterus, but, as Goeppert states, such cases in all probability represent a secondary separation.

SUMMARY

1. The protons of the rib, rib-bearer, and parapophysis are all condensations in the muscle septa, and are therefore continuous at this stage. The proton of the rib and rib-bearer is made up of a greater number of cells, which is in sharp contrast to the proton of the parapophysis in this respect. The parapophysis is made up of a few cells more or less regularly lined up between the notochord and the lateral muscle mass (fig. 2).

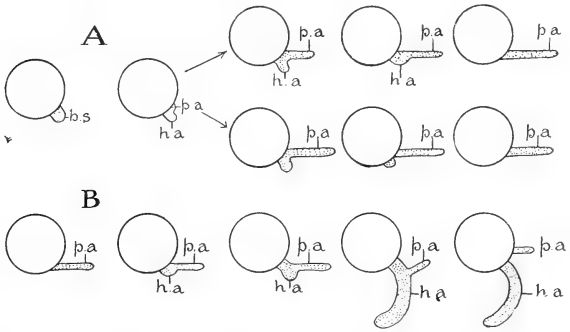


Fig. 28 Series of diagrams to illustrate the development of the parapophysis and haemapophysis in Necturus. A, trunk region; B, tail-trunk transection region.

2. The first cartilage to form is that of the basal stump which appears as a knob projecting from the notochordal sheath in a lateroventral direction (fig. 28A, *b.s.*). Later a laterodorsal outgrowth from this becomes the parapophysis (fig. 28A, *p.a.*).

3. The first cartilage of the rib appears distally, and this later develops mesally (figs. 29 and 30, A and B).

4. The first cartilage of the rib-bearer appears next to the neural arch (fig. 29A). Later this develops ventrally and fuses with the distal end of the parapophysis, and also develops dorsocaudally over the outer surface of the neural arch (fig. 29B).

5. The proximal end of the rib is relatively high in the second and third vertebrae; i.e., it is on a level with the base of the neural

arch (fig. 30A). Posteriorly the rib is on a level with the middle of the centrum (fig. 29A).

6. The parapophyses of the vertebrae in which the ribs are high do not lie in a horizontal plane, as they do farther back in the trunk, but extend dorsolaterally and approach the rib-bearer

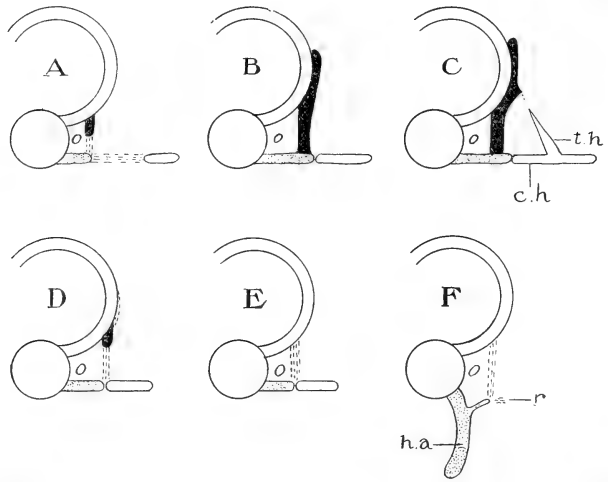
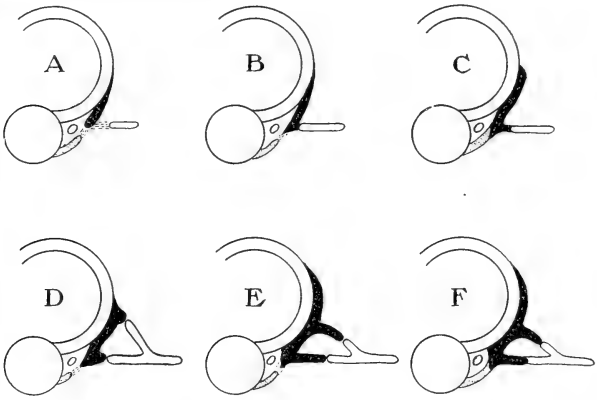


Fig. 29 Series of diagrams to illustrate the morphology of the ribs and transverse processes in the trunk region of Necturus. *c.h.*, capitular head of rib; *t.h.*, tubercular head of rib; black, rib-bearer; stippled, parapophysis and haemapophysis; white, rib. A, B, and C represent successive stages in the development of these structures. D, E, and F show the successively less differentiated condition encountered in passing toward the tail in a larva in which the transverse processes of the anterior vertebrae are at a stage similar to that represented by B.

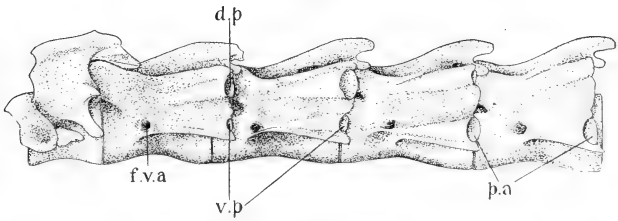
end to end (fig. 30A). In this anterior region the rib-bearer and parapophysis do not fuse until relatively late, while the rib becomes attached to the rib-bearer before rib-bearer and parapophysis come together (fig. 30 A, B, C).

7. In the second and third vertebrae the capitular as well as the tubercular heads of the rib attach to corresponding processes of the rib-bearer. In these vertebrae the parapophysis

takes no direct part in the formation of the rib-attachment apparatus (fig. 30 E and F).



30



31

Fig. 30 A series of diagrams to illustrate the development of the transverse process in the anterior trunk region. Development may proceed in either of the following ways: A B C F or A B D E.

Fig. 31 Drawing of the lateral aspect of the first five vertebrae of an adult Necturus, *f.v.a.*, foramen for vertebral artery; *d.p.*, dorsal process of rib-bearer; *v.p.*, ventral process of rib-bearer; *p.a.*, parapophysis.

8. In the trunk region the capitular head of the rib attaches to the parapophysis and the tubercular head makes no connection with a process of the rib-bearer (fig. 29 B and C).

9. In the trunk the rib-bearer fuses with the distal end of the parapophysis, and as growth takes place the distal end of

the parapophysis extends laterally past this point of union (fig. 29 A, B, C).

10. Between the dorsal and ventral cartilaginous rods of the transverse processes and the dorsal and ventral rib heads in the second and third vertebrae, procartilage cells persist which by proliferation bring about the elongation of the transverse process. In the trunk the tubercular head of the rib has no cartilaginous connection with the rib-bearer, so this provision is necessary only in the case of the parapophysis.

11. The haemal-arch element (haemapophysis) appears as a downgrowth from the base of the parapophysis. This growth becomes dominant over the parapophysis which in the first tail vertebra appears as a lateral process of the haemal arch (haemapophysis). It loses its connection with the haemal arch in the second tail vertebra and appears as a lateral projection from the notochord (fig. 28B).

CONCLUSIONS

1. The dorsal shifting of the horizontal septum is the direct cause of the change in rib attachment from the centrum to the neural arch.

2. That the rib is an independent element is indicated by:

a. The apparent ease with which it loses its connection with the parapophysis and becomes attached to the rib-bearer.

b. In the very earliest stages the rib proton may appear independent of any vertebral element.

3. Goeppert's belief that the rib never actually loses its attachment to the basal stump is incorrect. In the second and third vertebrae it has been seen that the rib connects with the rib-bearer before this structure and the parapophysis (Goeppert's basal stump) unite.

4. The rib-bearer is not a dorsal upgrowth from the middle of the basal stump, as Goeppert maintains, but is a chondrification in the inner edge of the transverse septum which fuses with the distal end of the parapophysis. As growth of the body takes place, the distal end of the parapophysis elongates laterally past this point of union.

5. The distal excavation which Goeppert did not observe in the dorsal part of the transverse process of Necturus is present in the second, third, and fourth vertebrae. This has already been pointed out by Wilder. Therefore, in these vertebrae the cartilaginous tubercular head of the rib is attached to an outgrowth of the rib-bearer. In the remaining trunk vertebrae this connection is not made.

6. The ventral cartilaginous rods of the transverse processes in the second and third vertebrae are not homologous with those of the other trunk vertebrae. This ventral rod in these two vertebrae is an outgrowth of the rib-bearer, dorsad of the vertebral artery and bears the capitular head of the rib. The ventral rod of the transverse process in the remaining trunk vertebrae represents an elongation of the parapophysis which passes ventrad of the vertebral artery.

7. The relation of the transverse process and rib heads of the second and third trunk vertebrae in Necturus is exactly similar to the condition throughout the trunk in Amblystoma and Salamandra.

8. The primitive basal stump (Gadow's basiventral element) develops a lateral process, the parapophysis, and a ventral, the haemapophysis. In the trunk region of a younger stage, both are present. In some vertebrae these are joined and in others are separated. This separation is secondary. In the trunk the parapophyses persist and are connected with the ventral head of the rib. The haemapophyses here disappear, while in the tail region they elongate to form the haemal arch and the parapophyses disappear.

9. In conclusion, therefore, it may be said that the change in attachment of the rib, from the centrum to the neural arch in urodeles, is not the complicated process that Goeppert thought. The capitular head of the rib does not remain attached to the basal stump or vestige of it, but loses its connection with the parapophysis and joins with the rib-bearer (compare figs. 29 and 30). This change in rib attachment is correlated with the dorsal shifting of the horizontal septum.

LITERATURE CITED

BUDGETT, JOHN SAMUEL 1901 On the structure of the larval Polypterus. Trans. of Zoöl. Soc. London, vol. 16, part 7, Oct., 1902; or, Budgett Memorial Volume, 1907, pp. 154.

GADOW, H., AND MISS E. C. ABBOTT 1896 On the evolution of the vertebral column in fishes. Phil. Trans. Roy. Soc. London, vol. 186.

1896 On the evolution of the vertebral column in Amphibia and Amniota. Phil. Trans. Roy. Soc. London, vol. 187.

GOEPPERT, E. 1895 Zur Kenntnis der Amphibienrippen. Morph. Jahrb., Bd. 22.

1895 Untersuchungen zur Morphologie der Fischrippen. Morph. Jahrb., Bd. 23.

1896 Die Morphologie der Amphibienrippen. Festschrift für Gegenbaur, 1.

1897 Bemerkungen zur Auffassung der Morphologie der Rippen in Rabl's Theorie des Mesoderms. Morph. Jahrb., Bd. 25.

1898 Erläuternde Bemerkungen zur Demonstration von Präparaten über die Amphibienrippen. Verhandl. Deutsch. Zoöl. Gesellsch. Leipzig.

GOETTE, A. 1878 Beiträge zur vergleichenden Morphologie des Skelettsystems der Wirbelthiere. II. Die Wirbelsäule und ihre Anhänge. Arch. f. mikro. Anat., Bd. 15.

1879 Same. Bd. 16.

RABL, C. 1892 Theorie des Mesoderms. Morph. Jahrb., Bd. 19.

1897 Theorie des Mesoderms. 1. Teil, Vorwort. Leipzig.

SCHAUINSLAND, H. 1901–06 Die Entwickelung der Wirbelsäule nebst Rippen und Brustbein. In Hertwig's Handbook. 3. II. III. Bibliography.

WILDER, HARRIS HAWTHORNE 1903 The skeletal system of Necturus maculatus, Rafinesque. Memoirs of the Boston Society of Natural History, vol. 5, no. 9.

Resumen por el autor, G. H. Bishop.

El metabolismo celular en el cuerpo adiposo de los insectos.

I. Los cambios citológicos que acompañan al crecimiento y la histolisis del cuerpo adiposo de Apis mellifica.

En las células del tejido adiposo de la larva de la abeja al principio de la ninfosis, algunos de los gránulos nucleares están dispersos por el citoplasma, para funcionar como cromidios en el desarrollo de los glóbulos de reservas albuminoides. Las vacuolas grasas que pasan hacia el centro indentan al núcleo alargado; la pared nuclear desaparece, el nucleoplasma y el citoplasma se mezclan parcialmente y este material se extiende periféricamente como trabéculas desde la región nuclear hasta cerca de la membrana celular. Los gránulos nucleares pasan depués á través de los intersticios de las vacuolas, y pasan hacia el exterior en las trabéculas. La membrana nuclear se regenera alrededor de la vesícula muy deformada, las trabéculas que se extienden desde el núcleo se desintegran, y los gránulos cromidiales se desarrollan á expensas del citoplasma y las vacuolas grasas que contiene en glóbulos que acaban por llenar la célula. Estos glóbulos parecen poseer al principio una estructura uniforme, pero más tarde adquieren vacuolas centrales, con una corteza periférica de material basófilo, que finalmente se divide en finas partículas en la superficie de los glóbulos. Al disolverse la membrana celular los glóbulos quedan libres, disolviéndose en la sangre para ser utilizados como alimentos para el crecimiento del tejido imaginal. Si se considera al crecimiento del tejido adiposo como un proceso anabólico de acumulación de alimentos para el desarrollo imaginal y la disolución de los glóbulos albuminoides como el correspondiente proceso catabólico, la transformación de las substancias celulares—la grasa de las vacuolas, la matríz citoplásmica y los gránulos nucleares—en la forma común de glóbulos albuminoides tiene lugar como un éstado intermediario de metabolismo intracelular, siendo fisiológicamente cada célula en cierto grado un 'sistema cerrado.'

Translation by José F. Nonidez
Cornell Medical College, New York

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 17

CELL METABOLISM IN THE INSECT FAT-BODY

I. CYTOLOGICAL CHANGES ACCOMPANYING GROWTH AND HISTOLYSIS OF THE FAT-BODY OF APIS MELLIFICA

GEORGE H. BISHOP

Zoological Laboratories of the University of Wisconsin

SIX TEXT FIGURES AND THREE PLATES (THIRTY-SIX FIGURES)

CONTENTS

INTRODUCTORY

The honey-bee larva, just at the beginning of pupation, exhibits in the cell of its fat-tissue an abrupt and striking cytological transformation. The nuclear wall disappears, and the peripheral fat-vacuoles of the cell approach the nucleus; through the interstices between these, granules of basophile material, presumably of the nature of nucleoli, pass from the nuclear area and invade the cytoplasm as chondriosomes. By progressive absorption, both of the surrounding cytoplasmic matrix and of its fat-vacuoles, these granules finally develop into globules containing albuminoid material, which are discharged into the blood on dissolution of the cell wall. The nuclear membrane is meanwhile reformed, without nuclear division, and may persist until after disintegration of the cell wall and discharge of its contained globules.

The cytological structure of these fat-body cells, or trophocytes, will be described in the following account, and especially the cytolytic changes they undergo during larval pupation. It has seemed advisable to enter in the second part of this paper, into certain rather speculative considerations which, based on the work so far accomplished, form the tentative framework for further research; but no attempt is made at this stage of the investigation to propound the ultimate analysis of cell metabolism in the insect fat-body.

The study of somatic cell metabolism has led to a wide diversity of interpretation, depending on the point of view of the special research dealing with it. The result has been a 'chemical' theory of function, or a 'physical theory,' or a 'genetical' theory, when an adequate biological interpretation must involve all of these. Especially in general physiology and physiological chemistry does a large and rapidly augmenting body of data, specifically applicable to the functioning of the somatic cell, invite a reconsideration of normal 'resting' cell structure; it demands, in fact, a more energetic name for the 'resting' cell itself. As a method of coordinating these specialized studies, it should be profitable to subject some one tissue to as many different techniques as conditions admit of, and the bee fat-body

tissue seems well adapted for this. The present paper aims to lay down the anatomical basis for such a series of experiments. In part I the rôle of the fat-body is defined in relation to general larval activity, and the structure of its cells as functional elements is described and in part II is discussed the functional significance of these cell elements in metabolism.

Acknowledgments are due especially to Dr. M. F. Guyer, of the University of Wisconsin zoological laboratories, for much valuable advice, and to Dr. Wm. S. Marshall, of the same institution, for literature references and suggestions as to insect metabolism.

THE RÔLE OF THE FAT-BODY IN LARVAL METABOLISM

1. Larval development

The larvae of the honey-bee are available in quantities and lend themselves readily to investigation. From the laying of the egg to emergence of the imago, a larva passes its existence in a cell approximately 4 mm. across by 9 deep. For the first part of its life it is half immersed in the partially digested food administered to it, the food being later disgorged by the worker bees directly into its mouth. It lies in the position in which it was first hatched until the confines of the cell containing it compress its flabby bulk into a flattened ring, whereupon it straightens itself out and spins a cocoon. The larva then pupates and is quiescent until it quickens as a mature insect.

From the standpoint of metabolism, the bee larva approaches what might be called a closed system. During a developmental period of twenty-one days it passes no excreta. The content of the malpighian tubules and a few faeces collect in the hind intestine, which, however, does not make functional connection with the stomach until late in development; the stomach contents are regurgitated at the beginning of pupation. The food taken in is of determinable and relatively unvarying composition. The developmental period is definite in time, and the temperature of the bee cluster is maintained with remarkable constancy. Thus the natural environment of the larva furnishes controlled experi-

mental conditions difficult to duplicate for animals undergoing a less monotonous adolescence.

Internally the bee is no less adapted to experimental investigation. Its response to a life of sequestered inaction has been a repression or a rudimentary development of many of the larval organs that would be required to adapt a larva to an active and independent mode of life. Locomotor muscles, elaborate modifications of the digestive system, complications of chitinous hypodermis for protection or aggression, are little developed or are lacking entirely. The larval life is given over to one function predominantly—the storage of nutriment—and this stored-up nutriment, as the fat-body, comprises at the time of metamorphosis three-fourths of the body tissue (blood excepted).

After hatching, for five and a half days the chief activity of the bee larva consists in the storage of food as fat-body tissue. During the first four days of pupal existence this fat-body is almost entirely histolyzed to furnish nutriment for the growth of imaginal tissues. Hence, in the building up of this food into fat-body cells, and the breaking down of these cells into tissue nutriment again, one is justified in looking for a highly specialized and, in a sense, an isolated process of nutritive metabolism.

2. Histology of the fat-body

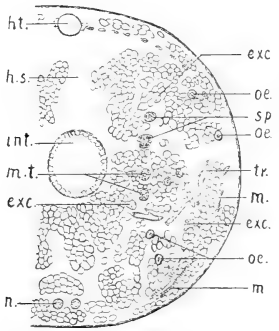
Histogenesis. The fat-body in insects is a tissue of mesodermal origin, the cells of which originate in the embryo by division and segregation from the inner surface of the mesodermal tubes along what is destined to be the ventrolateral aspect of the body-space. These cells form a mass of tissue running along the body between the intestine and the ventral body wall, attached to each. Typically, during embryonic or early larval life, the cells multiply to the full number to which the larval fat-body will attain. Thereafter, the growth of this tissue consists in enlargement of its cells, and not in their numerical increase. The bulk of the tissue relative to the size of the body varies in larvae of different insects from a narrow band on either side of the nerve chain in the embryonic position to a massive growth which comprises the greater part of the bulk of the larva. In the bee

embryo (Nelson, '12) this tissue develops chiefly from the splanchnic, instead of the parietal mesoderm as is usual in insects. This gives rise to two groups of cells on each side of the nerve chain, to which are added third groups from the parietal layer near the heart. In the larva it comes to extend dorsalward in two layers—splanchnic, enveloping the intestine, and parietal, lining the body wall. At metamorphosis, this tissue is disintegrated with the other larval tissues, and is replaced by an imaginal tissue, generally of much smaller bulk and more peripheral location.

Differentiation. Starting as a tissue of homogeneous composition in the bee, the fat-body attains complexity by reason of two processes. Certain cells become differentiated from the fat-tissue itself, and other elements invade it from the outside. During larval life, scattering cells have been described that either lose their fat-globule content or never acquire any, and, as larval life proceeds, develop characteristic granules of sodium urate. These have been termed excretory cells. From outside the fat-body altogether the tissue is invaded by large amoeboid cells originating segmentally from the margins of the spiracles, which from their wine-red color in certain insects are termed oenocytes, and to which are ascribed a secretory, and by some, a respiratory function. Leucocytes are also found wandering through the fat-tissue, presumably from the blood. After metamorphosis, pericardial cells grow down in strings from the heart region and become associated with the fat-body, as do also the imaginal oenocytes. The fat-cells themselves acquire during imaginal life granules of sodium urate. The functions of these various elements are even more obscure in the imaginal than in the larval economy.

Anatomy and function. Ignoring for the time being the conflicting interpretations that have been placed upon the fat-body function, we note that the mass of this tissue in the bee, aside from any other consideration, suggests a food reservoir. In larvae a few days old the fat-body envelops most of the other organs. It extends from the head region through the extreme caudal segments, and, except for blood spaces, fills all the region

from the intestine to the nerve cord. From here it extends dorsally in two chief layers, the intestinal layer just failing to close over the gut dorsally, and the margins of the parietal layer adjoining on either side the dorsal blood vessel. Through the transparent chitin of the grown larvae may thus be seen a clear



Text fig. A One half of a cross-section of a mature bee larva. *ht.*, heart; *h.s.*, haemal space; *int.*, mid-intestine; *n.*, ventral nerve cords; *sp.*, spinning glands; *m.t.*, malpighian tubules; *m.*, muscles; *exc.*, excretory cells of the fat-tissue; *oe.*, oenocytes. The remainder of the cells, not shaded, are the fat-cells or trophocytes. The cells of this tissue are arranged in folds or laminae, each two to five cells thick; the lamina run in general longitudinally in the body and are separated by blood spaces, which also surround all the organs. The oenocytes and excretory cells, both amoeboid, lie sometimes completely embedded in the fat-tissue, sometimes along the margins of the blood spaces. Roughly, three regions may be distinguished: a visceral or splanchnic layer of the tissue ventral and lateral to the intestine; a parietal layer along the body wall, ventral and lateral; and a cardiac layer, extending from the heart region lateroventrally on either side the dorsal haemal space. These layers correspond to the three mesoblastic anlages of this tissue in the embryo. Camera-lucida drawing.

blood space below the dorsal heart and pericardium, and through this again is visible the yellowish content of the mid-intestine, flanked on either side by white fat-tissue (text fig. A).

In cross-section the fat-body is seen to be longitudinally laminated, each lamina two to five cells thick, and extending from the central or peripheral layer into the blood space. A large proportion of the cells thus lie in immediate contact with the blood.

There are no trachea extending into the laminae. In the deeper layers are embedded the malpighian tubules and the salivary and spinning glands. The hind-intestine, unlike the mid-intestine, is entirely enveloped.

3. Relation to larval activity

This tissue occupies a space so prominent and so extensive in the larval anatomy and forms so large a proportion of its mass that the larva seems to be merely a mechanism for nourishing the fat-body, rather than the fat-body an organ of the larva. This illusion is supported by the low degree of development of any organs not concerned with the assimilation of food; i.e., the fat-body itself, the digestive apparatus, including the salivary and spinning glands, and the excretory system. The physiological assimilation of most of the food the larva takes goes on in the fat-body, the functional unit of which is the individual fat-cell.

As in all the Hymenoptera, the metamorphosis is complete. It involves the destruction of all the typically larval organs and the formation of a full set of imaginal tissues. This degenerate and highly specialized larva, consisting of a living sac full of stored-up nutriment, thus transforms into one of the most elaborately and highly specialized of mature insects. The transformation is so extreme and so abrupt that it demands the interruption of even such activity as the bee larva exhibits, and the pupal stage is entirely inert.

4. Nutrition and metamorphosis

The fat-body of the bee, compensating as it does for the large masses of muscle, etc., developed by more active larvae, is also presumably a more efficient mechanism for food storage, by reason of its high fat content, than the usual larval tissue. The histolysis during larval metamorphosis, therefore, is to be considered as more than a mere removal of a no longer useful larval organ; an intracellular digestion goes on by which a large amount of reserve tissue nutriment is elaborated with very little waste. Its cells may be interpreted as having two methods of functioning,

on the one hand, storage of food materials especially adapted to tissue growth and, on the other, a mechanism by which, during pupation, this reserve is further modified into food constituents suitable for immediate utilization by imaginal tissues. With a study of these two phases of the activity of the cells the following discussion concerns itself.

DEVELOPMENT AND TRANSFORMATIONS IN STRUCTURE OF THE FAT BODY CELL OF THE BEE LARVA

1. Historical

Except for quite recent papers, the literature on the insect fat-body has been reviewed so thoroughly that repetition is superfluous (Anglas, '00; Perez, '02, '11). In general, three lines of attack have been made on the question; 1st, the investigation of the larval fat-body as the precursor of the imaginal fat-body; 2nd, the study of the mechanism by which the larval fat-tissue is histolyzed (autolysis of phagocytosis), and, 3rd, consideration of the fat-body as a larval food storage reservoir. The elements concerned are the fat-tissue cells with their fat-globules and albuminoid granules, the oenocytes, the phagocytes, and leucocytes.

a. Metamorphosis to imaginal fat-body. Four methods of origin have been assigned to the imaginal fat-body: 1) from embryonic cells developed from fragments of the fat-cells (Auerbach, '74); 2) from embryonic cells developed from degenerating muscle cells (Anglas, '00); 3) by reformation of dispersed fat-cell fragments about the old cell nucleus (Koschevnikow, '00); 4) by persistence of certain larval fat-cells to form the imaginal tissue (Ganin, '75; de Bruyne, '98; Berleze, '01; Perez, '02, '11). The last interpretation seems best established.

b. Destruction of larval fat-body; autolysis and phagocytosis. The discussion of the release of the food materials of the larval fat-body cells has taken to some extent the form of a debate as to the respective merits and relative prevalance of autolysis, i.e., histolysis without attack by phagocytes, and phagocytosis or destruction by leucocytes or other wandering cells. Five interpretations have been placed upon the facts: 1) The development

of 'Körnchenkugeln' from fat-cell débris, with aquisition of a nucleus (Weissmann, '64). 2) The attack of fat-cells by 'excretory-secretory cells' (Anglas, '00). 3) Lyocytosis, or dissolution of the cells by enzymes from the imaginal tissue cells developing (Anglas, '02). 4) Autolysis (Terre, '99; Perez, '02, '11). 5) Phagocytosis (Kowalewski, '85; van Rees, '87; deBruyne, '98; Karaweiew, '98; Anglas, '00; Perez, '02, '11). The evidence seems best for the processes of autolysis, with or without leucocytic absorption of the débris, and phagocytosis by the leucocytes themselves where a precocious breaking down of the trophocytes is necessary.

c. Metabolism in the larval fat-body. After considerable speculation on the possibility of excretory and other manners of functioning of this tissue, attention has turned to the process by which food material is released from the fat-cells and prepared for tissue nourishment. Storage of nutriment in the fat-body was first pointed out in 1875 by Kunckel d'Herculais.

Berlese, '99, (on the Diptera), believed that material from the histolyzing intestine passed through the blood to the fat-cells, forming the albuminoid granules. He named the fat-cells 'trophocytes' from this activity. Action on the granules by nuclear enzymes was thought to render them basophilic, in digesting them for tissue nourishment.

Anglas, '00, (wasp and bee), observed that in the wasp certain cells undergo a marked transformation, assigned to attack by excretory-secretory cells. The nuclear membrane disappears, nuclear and cytoplasmic materials mingle, and the nucleus can finally no longer be discerned. This process of phagocytosis was not described specifically, for the bee, though the author states that the two forms are closely similar. The transformations described were not noted in other than those cells attacked by the phagocytes.

Perez, '02, (Formica), and '11, (Polistes), describes the growth of fine granules formed in the region around the nucleus, to albuminoid globules, and a modification of the nucleus, without attack of phagocytes. The fat-globules disappear presumably by digestion, with the growth of the albuminoid globules. The

nucleoli increase in number to this stage (beginning of pupation), and the nucleus as a whole decreases in size with the formation of the granules. The globules developed somewhat differently in the different forms studied.

Hufnagel, '11 (Hyponomeuta), described an 'epuration' of chromatin from the nucleus of the cell. This process was observed to commence in the larva, but persisted throughout pupation to the formation of the imaginal fat-body. Granules of chromatic material formed within the nucleus passed into the cytoplasm and became enclosed by a chromatic portion of cytoplasm. Different stages of 'condensation' of the chromatic substance were observed in the same cells, due to the fact that the granules were developed, not all at once, but in a successive order. The globules were finally expelled from the cell and engulfed by phagocytes. The fat-cells persisted after this expurgation of nuclear material to form the imaginal fat-body.

Hollande, '14 (Vanessa), conducted a chemical investigation of the contents, especially of the albuminoid reserve globules, of the fat-cells. He found that the globules in this form developed as in other larvae from granules formed close about the nucleus; that granules of sodium urate were also formed here, and he concluded that the process represented an expulsion from the nucleus, and consequent digestion, of nucleic acid containing material, from which sodium urate was split off almost immediately by enzyme action as waste material. This was demonstrated to be formed endogenously, not acquired from without in the functioning of the fat-body as an excretory organ. The development of the granules from feebly basophile particles to globules with acidophile margins, and finally to hyperacidophile bodies, apparently by attack of enzymes, was checked by microchemical tests which showed a transformation from nucleoproteids to albuminoids, and finally to biurette polypeptids and crystalloids. The possibility is considered that part of the fat is transferred to albuminoid (the reverse of the process reported by Weinland ('08) of transformation of albuminoid to fat by fly larvae), but no evidence was offered for such a process. Crystalloids were observed in the center of fat-globules.

Nakahara (17), in a research directed primarily to the functioning of amitosis in the insect fat-body, reported incidentally on the larval development of the fat-cell in Pieris. In 'second-stage' larvae, apparently still very young, nuclear ramification was observed, and in the 'third' stage spherical albuminoid globules in the cytoplasm; amitotic division of the nucleus occurred with the production of as many as five nuclei to a cell. At a late larval stage some of the albuminoid globules "begin to show dark dots, taking basic stains, indicating that the transformation of albuminoid substance into urates is beginning to take place" (on the assumption that the basophile stain indicates the degeneration of albuminous material to purine bodies, rather than the acquisition of nuclear substance). "This possibly may be regarded as one of the first signs of a histolytic process. Soon afterwards, just before the larva enters the prepupal stage, the nucleus loses its membrane and its structure becomes more or less indistinct. This is, I believe, the sign of a karyolytic process, which concludes the activity of the larval adipose cells." The further progress of these structures is not followed. Amitosis in the fat-cells is inferred to result in the increase of surface advantageous to active nuclear functioning.

From these accounts may be derived a general description of the fat-cell changes during growth and metamorphosis. The conflicting details of various authors, while perhaps due in part to faulty or incomplete observations of fact, are probably more largely due to the actual differences in the details of the process in the different orders of insects worked upon. It will be difficult to compare these details critically until a more accurate knowledge can be obtained of their physiological significance.

2. Anatomical data. Structural changes accompanying cell metabolism in the fat-body of Apis mellifica

a. Material. Material was procured at closely timed stages and examined consecutively with various stains. It was found convenient to divide the larval and pupal development more or less arbitrarily into periods according to the cell changes, as follows:

A. Embryonic development, in egg, two days; and multiplication stage in fat-body, egg, and young larva, one tot wo days.

B. Growth period. Characterized by one large fat-globule in cell, and irregular cell shape. One to two days, text figure B and plate 1, figure 1.

C. Fat storage. Development of peripheral ring of globules, with further increase of size. Three days. Text figure C and plate 1, figures 2 and 3.

D. Nuclear transformation. Cessation of larval feeding, spinning of cocoon, quiescence. Few hours, of sixth day of larval life. Text figure D and plate 1, figures 4, 5, 8.

E. Development of albuminoid globules. Head of imago forms, prepupa stage. Two to three days. Text figures E and F and plate 2, figures 11, 12, 13.

F. Globules released. Imaginal form assumed. About six days. Plate 2, figures 14 and 16.

G. Imaginal fat-body formed, young bee emerges. Last two days of pupation.

It will be noted that these periods represent not equal periods of time, nor changes of larvae form, but are based on changes within the cells themselves. From three to five substages were examined in the critical periods, D, E, and F.

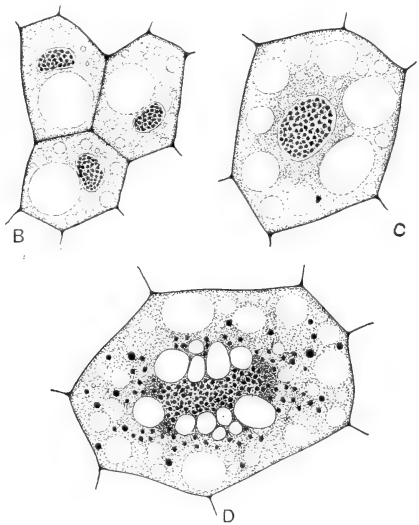
b. Technique. The technique employed in the microscopic work was the conventional cytological technique for material to be sectioned. The typical method was as follows:

A little fixative was injected into the blood space of a larva or pupa with a capillary pipette, to harden the tissues sufficiently for further cutting. The larva was then placed under fixative and slit open along the back with fine curved-handled scissors, since the fixatives would not readily penetrate the impervious chitin. Under this treatment the larval stages showed slight distortion in the shape of the cells, due to contraction of body-wall muscles which drew the slit-open larva into a bowed position, with a consequent stretching of the intestinal layers of fat-body cells. The intracellular structure, as checked by larvae killed in hot water, was not materially distorted. Hot water, however, seemed to leave the fat-globules less accurately defined.

The material when fixed was slit sagittally, and sections of one half the body cut transversely, 3 or 4 mm. thick. These were carried up to 85 per cent alcohol by means of an apparatus designed to produce a very gradual dehydration without shrinkage (Bishop, 17). The pieces of tissue were then cleared in redistilled anilin oil, passed through xylol and paraffin, changes being made by small degrees, and infiltrated for two to four hours at 56 to 58°. Sections were cut 4 to 10 μ. The standard fixative used was Allen's B15 formula, with or without urea, although preparations were made with picro-formol, formol-acetic, Flemming's, Gilson's, hot water, etc. The stains used most successfully were Heidenhain's iron alum haematoxylin, safranin-gentian violet mordanted with Gram's solution, and Delafield's haematoxylin, for chromatic structures; polychrome methylene blue proved a very delicate stain for the nuclear membrane; for cytoplasmic structures, the albuminoid globules, etc., eosin, aurantia, acid fuchsin, licht grün, orange G, and Congo red. Eosin and aurantia gave good differentiation of the globules. Fat was stained with sudan III and osmic acid. An old much-used bottle of Mallory's phosphotungstic haematoxylin, diluted with equal parts of distilled water, gave beautiful preparations showing all structures, nuclear, cytoplasmic, and nuclear membrane, but stained so generally that it masked all counterstains used for qualitative differentiation in cytoplasmic structures, such as the albuminoid globules.

c. Microscopic anatomy. The first stage to which close study has been directed is that designated B, in which the fat-cells, or trophocytes, having ceased to divide, are laying up appreciable stores of globular fat. Before this stage, during what may be designated rather loosely as a multiplication period, this tissue is characterized rather by the irregular shape of the cells than by their visible fat-content. From this period forward, the deposit and the metabolism of fat appear under the microscope as the most striking and characteristic activities of the tissue. An analysis of text figures B to F will comprise a presentation of the chief anatomical findings of this paper.

Stage B-C. In figure B, the distinctive elements brought out by staining are as follows: in the cytoplasm typically one large fat-globule, and generally several smaller ones, showing after alcoholic extraction as clear spaces in a homogeneous, non-



Text fig. B Larval fat-tissue cell, stage B, shortly after hatching, characterized by one large fat-vacuole, which may distort the nucleus.

Text fig. C Larval fat-tissue cell, stage C—rapid cell-growth period, the usual larval appearance—characterized by a peripheral ring of fat-vacuoles, central densely staining cytoplasmic area, and densely granular nucleus.

Text fig. D Larval fat-tissue cell, stage D—larva just becoming quiescent, dispersion of nuclear granules into the cytoplasm—characterized by a central ring of fat-vacuoles indenting the nuclear vesicle, by loss of the nuclear membrane, and by dispersal of the nuclear granules.

granular, or finely granular, rather heavily staining matrix; and in the nucleus, many large chromatic granules in a clear, lightly staining nuclear sap, with smaller granules and a very lightly staining linin network between them, the whole enclosed by a

faintly basophilic nuclear membrane. The amount of chromatic nuclear material and the density of its staining reaction suggest pronounced nuclear participation in the cell's metabolism. The nucleus is as a rule pushed to one side and distorted, even to the extent of being indented, by the one large fat-globule.

This stage of the fat-cell is not limited by definite or abrupt changes either at its inception or at its conclusion. The fat-globule increases in diameter, at first more rapidly than the cell containing it, compressing the nucleus as it enlarges. Later it grows more slowly (relatively) until the nucleus relaxes to the smooth oval shape of the later stage.

Stage C. Text figure C presents a section of a larger fat-cell, the difference in structure of which, from that of figure B, appears to be due less to a difference of physiological functioning than to mere increase in size and content. The cell comes to present an appearance strikingly different from the first figure, by the mere mechanical rearrangement of elements whose individual aspects are precisely the same as in the former stage. The nucleus is surrounded by a homogeneous mass of cytoplasm extending uniformly from nuclear membrane to cell wall, except where displaced by fat-globules. A peripheral ring of these globules, the largest of which approximate the size of the prominent globule of the former stage, and the smallest of which surpass the limits of microscopic vision, displace most of the readily staining cytoplasm from among them, and give the appearance of lighter staining in this region. The globules are often so numerous and so massed that they distort each other from the characteristic spherical form. This accretion of peripheral fat continues with a progressively increasing number of vacuoles, until just after the sealing over of the larva and the cessation of the nutritive supply.[1]

[1] Some light is thrown on the mechanics of the change in fat disposal from stage B to C (text figs. B and C) by a consideration of the surface: volume ratio. The volume of a sphere increases as the cube of its radius; the larger the cell diameter relative to the diameters of the contained fat-vacuoles, the larger will be the radius of that portion of the cell's volume into which the non-fatty material gathers centrally. For instance, one-half the volume of a sphere whose radius is 1 is contained in a sphere at its center whose radius is 0.79, the other half in a peripheral shell of 0.21 thickness. If, say, 50 per cent of the cell were fat, it could

Stage D. In figure D is pictured a stage which differs from the two former stages figured not only in the mechanical arrangement of the cytological elements, but evidently in the nature and condition of the substances present. Both nucleus and cytoplasm show marked changes from the previous stage. In the cytoplasm the peripheral vacuoles have decreased in size and number, while a layer of them has appeared centrally along the sides of the oval nucleus. Early in this stage the nuclear membrane has either disintegrated or lost its precise staining capacity, and the nuclear granules have become more scattered than previously throughout an area more elongated than the oval of the former nucleus. Finally, in the cytoplasm, and especially out from the

be gathered into one layer at the cell's periphery whose thickness would be about one-fifth the radius of the cell. But gathered into one vacuole (as in fig. 1), the globule's diameter would be nearly four-fifths that of the cell, and must push the nucleus to one side. With the fat in smaller vacuoles relative to the size of the cell, the center of the cell may be free from fat (fig. C.)

The vacuoles of the larger cells are not much larger than the prominent ones of the earlier stage. The reason for the limit to the size of the vacuoles, which seems to be the factor causing the difference of appearance between them, may be deduced tentatively from a consideration of the relation of the volume of a sphere to its absorptive surface. Considering the fat-cell as a chemical plant for the metabolism of fat as well as a storehouse for the product, the chemical activity must take place somewhere between the cell wall and the vacuole where it is deposited. The fat is presumably condensed into vaculoes from the emulsoid form, from the peripheral cytoplasm where it is elaborated. In a small cell the distance is small through which material must be transported from any part of the surface to the one large vacuole, but with growth of the cell the distance increases, and, moreover, the volume of the cell and presumably, the rate of fat metabolism, increase even faster—as the cube of the linear distance. A number of relatively small vacuoles dispersed through the cell's substance, and especially near its surface, where the material for fat production must be received from the blood, will furnish more surface and distribute this surface more effectively for the accretion of fat than one large one, and as the rate of fat production is increased small vacuoles will be condensed before the material can be transferred to the original large one. Moreover, the ratio of surface to volume would be greater in many small vacuoles then in one large one, and the small vacuoles would consequently 'grow' faster, which would make for uniformity of size. The anatomical developments satisfy this hypothesis, without demonstrating its finality. As the cell diameter increases, the fat tends to be deposited in smaller and more peripheral vaculoes, and especially in the queen larva, where the development is most rapid and the cells are largest, the fat-vacuoles are both relatively and actually smaller than in the worker. Differences in consistence of the cytoplasm may also effect the size of vacuoles.

ends of this elongated nuclear area, appear dark staining granules identical in size and shape, at first at least, with the nuclear granules, and exhibiting certain of the staining reactions of these. An interpretation may here be anticipated; that these bodies are identical with the larger chromatic or basophile granules of the nucleus; that they leave the nucleus and invade the cytoplasm when the nuclear wall disintegrates, or becomes permeable to them; that their subsequent activity may be in part at least an enzymatic one, and is certainly concerned with the further metabolism of the stored fat of the cell.

The detailed anatomy of this stage merits closer scrutiny (pl. 1, figs. 5, 6, 7). Several facts are apparent. First, vacuoles from the peripheral ring may be traced passing in toward the nucleus, through the densely staining cytoplasm surrounding it; and the appearance of basophile granules in the cytoplasm coincides with the disappearance of the distinct outline of the nuclear vesicle—may, in fact, shortly precede the indentation of the nucleus by fat-vacuoles. This picture is so constant and so characteristic of whole sections, when it occurs at all, that it evidently signalizes an important crisis of the metabolic activity of the larva itself. The series of changes follows the cessation of feeding, and precedes the transformation of larva to pupa, so immediately, that their interpretation must be correlated with the process of metamorphosis as a whole. If other forms which have been worked upon exhibit the same phenomena, the failure of the workers handling them to demonstrate this change may be accounted for by the abruptness and rapidity with which the cell is transformed from one relatively permanent state to another.

The first sign of the transformation is the diffusion of the nuclear wall and the elongation of the nucleus in the long axis of the oval. The line of demarcation between nucleus and cytoplasm does not at once vanish, but gradually blurs, as if the substance of the membrane were partially dissolved by the material on either side of it, or as if a membrane, formed by surface tension between two non-soluble substances were obliterated by their becoming soluble. This blurring is most pronounced at the ends of the nucleus, and here a little later the transition

from nucleus to cytoplasm becomes least abrupt. The margins of the central vacuoles take the stain more sharply than the adjacent cytoplasm—a conditon not obtaining for the periphral vacuoles—and they lie so close to one another as virtually to form a reënforcement to the diffuse nuclear wall, which appears to follow their contour and fill their interstices. The heavier stain in their margins may be due to the presence of the substance of the nuclear vesicle in their surface films. They appear to compress the nucleus, which elongates to two or three transverse diameters. The cytoplasm of the central portion, surrounding these central vacuoles, takes the stain more heavily than that surrounding what peripheral vacuoles still persist.[2]

The cell is now in the condition represented by figure D. Material at this stage shows the basophile granules not only just outside the nucleus, but precisely in the areas at the ends of the nucleus from which the nuclear wall has disappeared (pl. 1, figs. 8, 9). The diffuse structure and pronounced staining reaction of the region renders difficult the exact location of these granules with respect to the blurred residuum of the nuclear wall. The appearance occasionally is that of a gap pushed outward through the wall between nucleus and cytoplasm, flanked on either side by the fat-globules; the granules contained in the nucleus are escaping through the opening.

The granules in the cytoplasm increase in number and become evenly dispersed from nucleus to cell-wall. They are not confined to the regions of the cells near the ends of the nucleus where they first appear. They seem also to have passed out laterally between the vacuoles in considerable number. These granules enlarge to spherical globules, and at the first the largest lie well

[2] What the difference is between the central and peripheral cytoplasm is not clear, but the different aspect seems to be due to the different distribution of globular or finely emulsified fat. If it were demonstrated that the fat were synthesized in this region next the cell wall, and deposited in the cytoplasm in the form of an emulsion, from which it condensed as the fat-content increased to droplets which grew by accretion into vacuoles, then the lighter staining reaction might be assigned to the fine dispersal of fat in the cytoplasm peripherally. This may be a partial explanation, but, as will appear from later consideration of nuclear activity, this central cytoplasm seems to be influenced also by the nucleus more pronouncedly than the peripheral.

toward the periphery of the cell, where one might expect to find those which had earliest left the nuclear region. The central fat-vacuoles also recede from the nucleus, and become dispersed among the enlarging granules. The nuclear membrane gradually reappears, as the vacuoles leave the region of the nucleus; but the latter does not reassume its former oval shape; it becomes even more attenuated, sometimes so extended that if straightened, it might touch the opposite sides of the cell. The ends of the nucleus are the last regions to be enclosed. Often these may be seen open in a nucleus of a much later stage of the cell, with small dark-staining granules near the aperture (text fig. E). Cells may be observed in which, instead of two ends, three or more areas of a nucleus appear to have opened out; and a plane section would fail to reveal the extent of this radiate condition of the nucleus in a large proportion of the times it might exist. In the queen pupa, a multipolar extravasation of the nuclear granules is the rule.

Stage E. The result of these changes is seen in figure 5. After the previous stage the fat cell does not increase in size. Once the nuclear wall is reformed there is no further visible evidence of activity within the nucleus. The wall stains much more sharply than before its dissolution. The large basophile granules are still present in considerable numbers, but the extreme distortion of the nucleus makes difficult a comparison with previous stages as to its size or content. The finer basophile granules are more numerous, relatively to the number of the larger ones which remain in the nucleus, than before the transformation.

Outside the nucleus, however, the granules undergo a definite development. They enlarge, staining less deeply with basic dyes as size increases, and finally taking an acid stain, until, with the same staining technique as before, the cytoplasmic dye absorbed is often more prominent than the nuclear. They finally become (typically) vacuolated spheres, the granular peripheral shells of which stain slightly darker than the cytoplasm of cells in previous stages, and the centers of which often appear to be dissolved out in preparation much as the fat-globules are. In the meantime both the cytoplasm and most of the vacuoles

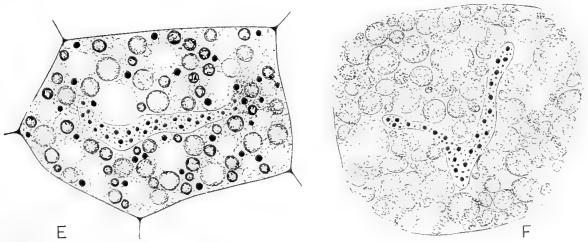
contained in it disappear. Since the spheres which develop from the basophile granules fill the cell, the unavoidable conclusion is that both cytoplasm and cytoplasmic fat-vacuoles are absorbed as material for the growth of the spheres (pl. 2, figs. 14, 16). Further evidence is adduced from the cytoplasmic staining reaction of these spheres, and, finally, the peripheral shell, and especially the inside margin of it, is blackened by osmic acid, indicating the presence of fat.

Stage E late. Figure F shows the later stage of the process which brought about the condition pictured in figure E. A few fat-globules are still present, and a few basophile granules are still in early stages of development. The nucleus has the same aspect as before, except that while earlier stages often show the ends of the nucleus open, in this later stage the nuclear membrane is always intact. The rest of the cell is occupied by the spheres developed from the basophile granules, the interstices of which are filled by a very light-staining cytoplasmic matrix (pl. 2, figs. 14 to 16).

As the pupa takes on the form of the imago and its tissues demand food material for imaginal development, the trophocytes proceed to the final stages of development and disintegration. The cytoplasmic matrix stains less and less densely, and is replaced by the growing spheres, until only a clear plasma remains between the latter. The cells become loosened from each other, and round up from a polyhedral to a spherical form, while the interstices so formed fill with lymph from the body fluid. The cells subsequently float free in the body cavity. Finally the cell wall itself dissolves or disintegrates (pl. 2, fig. 14), and the spherical globules are released, to dissolve eventually and lose their integrity in the body fluid.

The nuclear membrane. The behavior of the nuclear membrane in this process is particularly striking. The nuclear plasma in these fat-body cells appears to become fixed into an exceedingly fine coagulum, so fine that its aggregations cannot be distinguished under the microscope. It thus gives the appearance of a lightly staining, but entirely homogeneous mass, which tends to take a basic stain as the change in form comes on. The

peripheral cytoplasmic mass (in the interstices of the fat-vacuoles) precipitates upon fixation to a finely reticulate network, the clear interstices of which are large enough to be distinguished under the high power (pl. 1, fig. 3). The central or perinuclear cytoplasm fixes to a finer granulation, not so fine as the nucleoplasm, but more homogeneous than the peripheral, and considerably



Text fig. E Fat-tissue cell, stage E—early pupa—characterized by the reformation of the nuclear membrane, growth of the nuclear granules to albuminoid globules, disappearance of the fat-vacuoles, and resolution of the cytoplasmic matrix.

Text fig. F Fat-tissue cell, stage F—cells ready to disintegrate, medium-stage pupa; albuminoid globules matured, centers of globules acidophile, peripheral granules in their walls feebly basophile, most of the cell cytoplasm absorbed by the globules.

more densely staining. Some of the peripheral cytoplasm may be carried centrally as the fat-globules approach the nucleus, especially in cells of the queen larva (pl. 1, figs. 4, 8). With delicate staining the membrane separating these two masses, of nucleus and cytoplasm, has no discernible structure or organization, but appears to be merely a phase border between two immiscible fluids. The nucleus maintains the globular shape

characteristic of such a condition as long as this state of the membrane obtains: The first evidence of a change in the nucleus appears as a slight modification in the condition of this phase difference—a modification which is noticeable microscopically as a slight thickening, a more cloudy staining, and less sharp definition. This takes place even before the fat-globules reach the nuclear region (stage of fig. 3, pl. 1). As the process goes further, the membrane may finally be completely dissipated, and no residue left that can be distinguished as the material of which it was composed. The border-ground between nucleus and cytoplasm then grades imperceptibly from one to the other, staining only a little more densely where the two materials appear to diffuse; as if each retained its intrinsic staining capacity, and the resulting stain was an additive effect of the characterisic staining of both. This border-ground now appears to offer no resistance to the passage of the basophile granules, which are found indiscriminately on either side and within the region where the two materials are diffused (pl. 1, figs. 5, 6, 7).

Along the sides of the nucleus it is exceedingly difficult to discern just what the state of the border is, for here the central fat vacuoles which indent the nucleus complicate the picture. The surfaces of these vacuoles (or more accurately, the surface of the material surrounding the vacuoles) stain more sharply and more densely than the surfaces of vacuoles situated more peripherally in the cells; and since these surfaces lose their sharp staining capacity as the vacuoles disperse throughout the cytoplasm, it may be inferred that they are enveloped by a film consisting of a mixture of the nuclear sap and cytoplasm, and that the staining of this film is in reality a stain of the same nucleus-cytoplasm complex as was the staining of the nuclear membrane itself.[3] The conditions causing a phase membrane to disintegrate from the nucleus-cytoplasm surface might affect the surface of the fat-vacuoles more tardily, and these might still

[3] This supposition is of course somewhat hypothetical. Different proteins, in colloid form, are affected differently by changes of acidity, some becoming more and some less hydrophilic. A change in acidity of one or another in turn would cause a change in the respective viscosities, which might be conceived to result in membrane formation and other complicated physical phenomena, even such as

give evidence of the membrane after the nuclear membrane in their interstices had become pervious to the basophile granules. But microscopically this condition cannot be demonstrated, except by inference from the presence of granules immediately outside the layer of fat-vacuoles.

Conditions in the metabolic activity of the cell may be pictured, e.g., cutting off of the nutrient supply to the cell with cessation of larval nutrition, or possibly some regulatory mechanism in the cell itself, which would at this specific stage of development cause the constituents of nucleus and cytoplasm to approach such a degree of acidity, say, as would cause them to approach each other in fluidity. A membrane formed due to their previous difference in consistency would then be destroyed, and the two colloids might diffuse in the region of this surface.[3] The same force which in the intact condition of the nuclear wall prevents the nuclear granules from fusing or agglutinating—apparently some repellent force, such as like electrical charge—would upon equalization of the physical conditions within and without the nucleus cause a dispersal of the granules throughout the cell. This dispersal would go only so far as to render the granules equally numerous per volume within and without the nuclear area; that is, not all the granules would pass into the cytoplasm, but the process would cease when the granules were about equally dispersed throughout the cell. It is worth noting that this is approximately the case; moreover, that the granules tend to remain for a time within the more central densely staining cytoplasm—although the immediate change in the character of the granules as they leave the nuclear area and commence to absorb cytoplasmic substances renders precarious too strict an interpretation of the appearances.

d. Variations from type. The above description of the fat-body-cell development is based upon a study of, and applies most accurately to, the cells of the abdominal region of the worker

may be thought of as taking place between nucleus and cytoplasm. In the case of the fat vacuoles this explanation does not call for a destruction of their surface membranes, for the fat does not become more soluble in the cell protoplasm than it was before. A condensation of material at the surface of the fat-vacuole might account for its heavy staining capacity.

larva and pupa. This cell is chosen as a type because its development not only includes all the fundamental phenomena of the other forms, but also because most of these phenomena are here displayed in an orderly fashion. The process may, in fact however, be modified in three respects, depending apparently, 1st) on the rapidity with which the change from larva to pupa and from pupa to imago takes place; 2nd) on the sex of the larva, and, 3rd) on the locus, in the body, of the cell under consideration. Perhaps these divergencies from what have been described as the typical process may be correlated to a considerable extent with the nature of the food supply of the respective larva on the one hand, and on the other, with the demand for tissue-building materials, made by the imaginal tissues on the larval fat-body. More specifically, the difference, 1st) in the rate of the change which takes place in the fat-body cells, at the time of pupation, of worker and queen larva, respectively, seems to be correlated with the difference in the total time of development required by these forms (seventeen days for the queen, twenty-one for the worker), which is again usually assigned to a difference in larval feeding; the difference, 2nd) in the aspects of larval cells in the fat-bodies of the different sexes (male and female), seems to be due chiefly to a difference in the proportion of fat stored in them, which again is probably correlated with the difference in the food supplied to the larvae of the different sexes; while, 3rd), the 'precocious degeneration,' noted by Perez, of the cells of the thoracic region, which go to pieces before those of the abdominal region, and before all the albuminoid globules contained in them are fully developed, might reasonably be assigned, in the light of the theory of reversible enzyme activity in cell metabolism, to the earlier and more rapid exhaustion of the end-products of katabolic enzyme activity in the fat-cells, by the earlier development of the bulky thoracic muscle masses. (This conception approaches, but is not identical with the 'lyocytosis' of Anglas.) The nature of these three modifications of the typical process will be described.

Difference between queen and worker. In the queen larva the disintegration of the nucleus of the fat-body cell and the dispersal

of the basophile granules into the cytoplasm is considerably more striking, more abrupt, and in a sense more violent than in the worker. The nucleus, instead of being enclosed by fat-vacuoles in all but two, or a few regions literally sprawls all over the cell, and sends out what might be described as trabecular processes in every direction even as far as the periphery. The fat-vacuoles are also smaller, both relatively and actually, than in the worker. The nuclear granules are carried with these trabeculae pretty evenly throughout the cell, not only in a few directions or from a few poles of the nucleus as in the cell of the worker larva.[4] (pl. 3). The appearance is almost that of an explosive phenomenon, and the result is that the granules are not only more immediately dispersed through the cytoplasm, but all begin their development at about the same time (pl. 1, fig. 8), and they tend to remain more nearly the same size throughout the cell's existence. The diffusion of cytoplasmic and nuclear material is also more extensive in the queen larva. Instead of three quite clearly defined zones, consisting of peripheral cytoplasm lightly staining and interspersed with fat-globules, central cytoplasm more densely staining, and nuclear sap, there are now two regions irregularly disposed, consisting, respectively, of the peripheral cytoplasm and its fat-globules and the central cytoplasm and nuclear sap interdiffused.[5] The nuclear granules do not remain in the denser region; the two cytoplasmic areas gradually diffuse. The nucleus reëstablishes itself out of the diffused mass into an irregular many-processed body containing the typical chromatic material in a clear lightly staining medium. The later stages approach very closely the later stages of the worker pupae[6] (pl. 2, figs. 15, 16).

[4] Plate 3 shows twenty cells from one-half of a single cross-section of a queen larva, just in the stage of nuclear dispersion. The nuclear area, shaded black, can here be distinguished by the methylene-blue stain, though eosin stains nucleus and cytoplasm both.

[5] The granules of the peripheral trabeculae, outside the nucleus proper, stain less densely with nuclear stains (pl. 3).

[6] The exact nature of the difference in food which might occasion this difference in metabolic rate has not been investigated in detail. It is known that the queen larva is fed during its whole feeding period upon the so-called 'royal jelly,' a partially digested compound of fairly constant proportions of fat, carbohydrate,

Difference in queen and drone. The difference in the aspects of the fat-body of queen and drone is exhibited chiefly in a lesser portion of visible fat-content in the male. This may be a result of a difference in food. The drone, like the worker larva, is fed at first on partially digested food, but later receives considerable crude pollen. A high percentage of protein, and particularly of nuclein-forming materials, such as pollen yields, may be considered necessary in the drone's diet to provide material for building up the testes, which shortly before emergence of the drone have displaced the fat-body, and nearly fill the large abdominal cavity. At the termination of the larval ingestion of food these organs are present, but slightly developed. Their growth during pupation must be at the expense of the fat-body, which tissue may be expected to have stored up the proper nutrient elements in the proper proportions for that development. The testes, large as they are, must demand a higher proportion of nuclein-forming materials for the development of their sperms than any comparable organs in the worker or queen pupae.

Difference in different body regions. No significant difference in the development of the fat-body cells of thorax and abdomen is discernible until after the disintegration and reforming of the nucleus, and the partial development of the albuminoid globules. At a relatively late period in this development, but before all the globules of the cells concerned have attained the final structure and staining capacity of the typical cell contents, scattering

and protein, prepared in the midintestine of the young workers or nurse bees. The worker larvae are fed this material the first three days of their life, after which considerable undigested pollen and honey is added. Investigators have been unable so far to assign any other difference in treatment as a necessary cause of the different development of the worker, and this cause seems to be a sufficient one. The question remains whether this difference in the effect of the different foods is due to partial digestion merely or to the extraction or modification of some constituent of the crude pollen, or honey, which, when fed the worker larva without modification, retards or modifies its development. Considering the fact that pollen,the chief protein-containing constituent of the larval food, contains a high percentage of nuclein, it seems possible that some constituent of nucleic acid, such as purines, may be modified or extracted from the queen's food, and left in the larval metabolism of the worker to modify the development of the imago.

cells, more numerous in the thoracic region where they are destined to be replaced by the thoracic muscles, undergo a 'precocious degeneration,' as stated by Perez. This change comprises in the bee larva the dissolution of the cell wall and the release of the cell contents into the blood space. Those globules which appear not fully developed still stain with the nuclear dyes, and especially with Heidenhain's haematoxylin; they are still small in size and compact in structure, without the vacuolated center which seems to be characteristic of a late stage of the normal development. This precocious change is apparently not associated in the bee with the presence of any unusual cellular element such as the leucocyte, nor of any condition other than the early development of large masses of tissue in this region.

3. The metabolic significance of the changes in structure

Tracing the fate of the larval food through the nutritive mechanism, the following résumé may serve to correlate the nutritive process with the cellular metamorphosis.

The partially digested food of the early larva, the 'royal jelly' elaborated by the worker bees, contains carbohydrate, fat, and protein. This special food the queen larva receives all during larval growth; after the third day the worker is fed considerable amounts of honey and undigested pollen, and the male still larger proportions of pollen. The fat and practically all of the carbohydrates taken up by the fat-body are stored as fat-droplets[7] until, at the beginning of metamorphosis, these droplets are worked over into the so-called albuminoid globules developed from granules arising from the nucleus.

Since there is very little protein in honey, the bulk of the nitrogeneous food comes from pollen, chiefly in the form of nucleoproteids. These are presumably stored up as nucleoproteids in nuclear chromatin and the chromatoid granules, and as more simple proteins in the acidophile cytoplasmic matrix. In the

[7] Nakahara reports glycogen in the developing fat-cells of Pieris demonstrated by Gage's methods. Glycogen could not be demonstrated in vitro in these tissues by the ordinary chemical test of caustic hydrolysis and treatment with iodine.

development of the albuminoid granules the chromatoid granules of the nucleus, the protein of the cytoplasm, and the fat of the vacuoles are all utilized, and merged into a form where the different constituents are not only different from the former cell constituents, but also can no longer be distinguished from each other by staining reactions. The conclusion can be drawn, however, that these albuminoid globules represent the cell element in which the chemical transformations take place by which all the cell constituents (except possibly the residual nucleus) undergo chemical reorganization in preparation for tissue use.

In the following section of this paper an interpretation of the changes in structure and in staining reaction of the cell elements will be undertaken. A bibliography there included will also cover the first section.

PLATES

PLATE 1

EXPLANATION OF FIGURES

Fat-tissue cells from larvae of the honey-bee. × 330

Figures 7 and 9, × 1000

Figures 1, 2, 3, 6, and 7, iron-alum haematoxylin; figure 4, safranin-gentian violet mordanted with Gram's solution; figure 5, iron-alum haematoxylin and eosin; figures 8 and 9, iron-alum haematoxylin and safranin.

1 Worker bee larva fat-tissue cell, early larval stage B, showing nucleus pushed aside by one large fat-vacuole, with others forming.

2 Same as above, stage later, C, peripheral ring of fat-vacuoles forming, nucleus in center undistorted.

3 Queen bee larva fat-tissue cell, late larva, stage C; pressed out of shape at the edge of a mass of cells, cut a little at one side of center. A precocious scattering of nuclear granules is taking place, before the fat-vacuoles have reached the central nuclear area.

4 An attenuated cell of early prepupal stage, D, queen larva, showing nuclear dispersion of granules, and their developemnt into globules. In other sections the granules may be seen in passage from the sides of the nucleus as well as from the ends. The nuclear vesicle is apparently beginning to reform here along the sides.

5 Slightly earlier phase than above of stage D, transforming queen larva, nucleus actively dispersing granules of basophile material, ring of fat-vacuoles pressing it centrally. A more spherical cell would show trabeculae of densely staining material at other regions than the ends of the cell nuclei. Long and narrow cells are chosen here for simplicity and definition of the cell conditions.

6 and 7 early prepupal stage D, worker larva, cross-section of a cell of the shape shown in figure 5, cut through one end of the nucleus, and an enlarged drawing of the central region of the same. Centrally, the nuclear area still contains large granules, interspersed with many smaller ones, and the whole is surrounded by a characteristic ring of fat-vacuoles, with very sharply defined walls. Through the interstices of these vacuoles nuclear granules are still passing. Peripherally to this again, the central cytoplasm extends out into many small trabeculae through the peripheral lightly staining cytoplasm, to the marginal region of the cell.

8 Cell from a queen larva, same stage as above, longitudinal section, and figure 9, a higher magnification of one end of its nuclear region. The typical condition of smaller fat-vacuoles and a more violent dispersion of granules, with more attenuated and numerous trabeculae, is characteristic of the queen larva as compared with the worker. Most of the cells of the queen larva are even more complex. Here again, as above, an elongated bi-polar cell was chosen, for comparison with the typical bipolar cell of the worker. Extreme destaining has obliterated the finer nuclear structures.

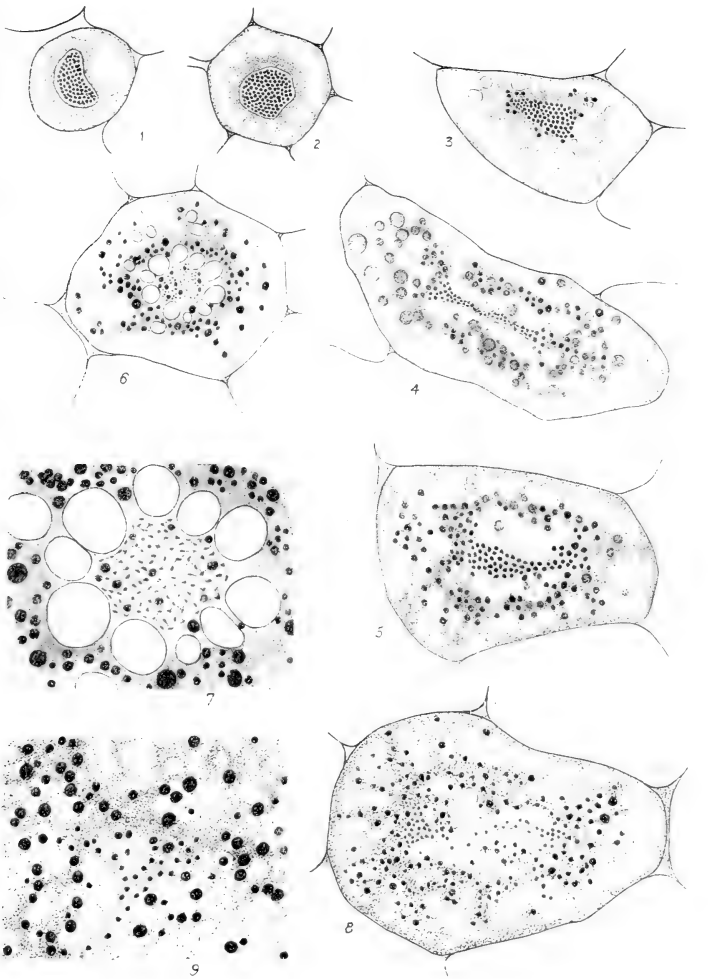

1
2
3
6
4
7
5
9
8

PLATE 2

EXPLANATION OF FIGURES

Fat-tissue cells from pupae of the honey-bee. × 330

Figures 11, 13, and 15, safranin-gentian violet with Gram's solution; figure 10, polychrome methylene blue and eosin; figures 12, 14, and 16, iron-alum haematoxylin and eosin.

10 Late stage D, worker prepupa, nuclear vesicle reforming. The nuclear wall stains sharply with methylene blue; granules and globules of nuclear origin in the cytoplasm fix eosin after they pass the nuclear border, and methylene blue as long as they are within it. The two cells show the two most typical shapes of cell and nucleus of the worker larvae, i.e., bipolar and tripolar.

11 Stage E, early queen pupa, multipolar nucleus, with reformed wall, albuminoid globules all in about the same stage of development though of different sizes.

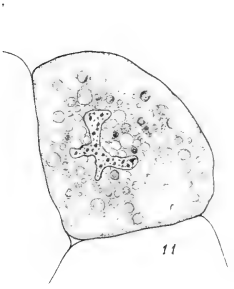
12 Intermediate stage of albuminoid globule formation, stage E, early worker pupa. Granules of different sizes and different stages of development in the same cell, presumably due to successive emission from the nucleus.

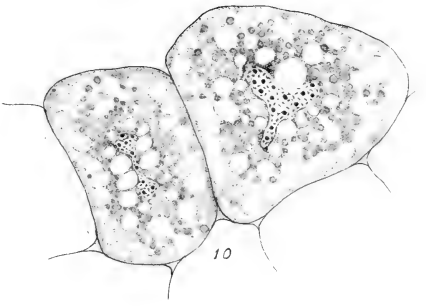
13 Same as figure 12, different stain.

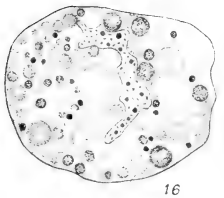
14 Unusually large cell from a worker pupa, stage E to F, undergoing dissolution of the cell wall—at upper left hand—before complete elaboration of its albuminoid reserves.

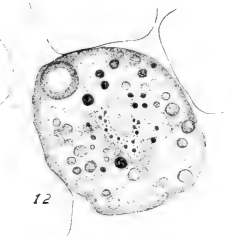
15 Another cell from the same slide as figure 14, less than 2 mm. away, on opposite side of the intestine; both are of the same size and the same distance from the surface of the tissue as fixed, and both are in the layer of cells next the intestine. The difference is a metabolic one, of unknown causation. In figure 15 may be seen two leucocytes, but no actual leucocytic adherence to or attack on fat-cells could be discerned here. The fat-cells had become loosened from each other and had rounded up, and were nearly surrounded by the body fluids.

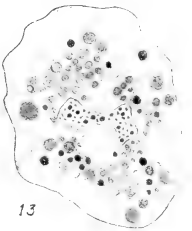
16 One of the smaller cells from a queen pupa, showing conditions more typical of the worker pupa (figs. 12 and 13) in greater degree of diversity of development of globules, fewer number of them, etc., than the larger cells of the worker pupa tissue itself exhibits (figs. 14 and 15). The probable explanation of this is that the difference in development depends on metabolic rate of nutrition, higher in general in the queen larva, and that the smaller cells from the queen larva or pupa developed more slowly, due to isolation from nutriment, etc., than the best nourished of the worker larva's cells.

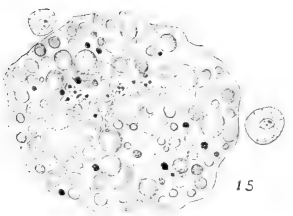

11


10


16


12


13


15

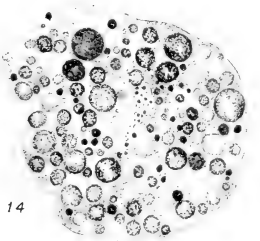

14

PLATE 3

EXPLANATION OF FIGURES

Cell shape and nuclear transformations

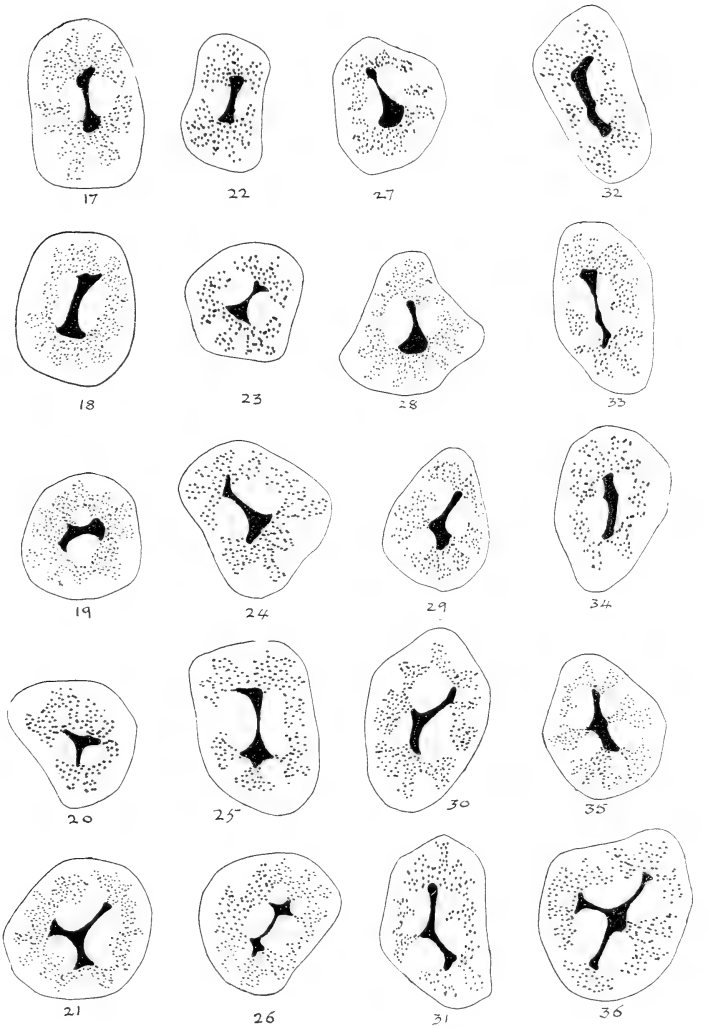
Figures 17–36, × 165. Stain, methylene blue and eosin.

All these drawings were taken from one-half of one-cross section of a queen larva whose fat-cell nuclei were just in the act of dispersing their nuclear granules into the cytoplasm. The black areas are those in which the nuclear granules were stained by methylene blue, the stippled regions are the areas of dense nucleocytoplasmic trabeculae through which the granules are dispersing from the nuclei; this stains deeply red with eosin. The clear areas are the regions of lighter stained cytoplasm containing fat-vacuoles.

This plate is designed to show the relations of these three regions in the cells of this stage; to demonstrate the relation between their disposition and the cells' shape, and to present evidence bearing on the mechanics of the process of nuclear extravasation and dispersal of basophile granules. Only those cells were drawn which appeared to be cut almost exactly through the median plane of the nucleus, and in the plane of greatest nuclear extravasation, through other trabeculae than those figured of course extended above and below the plane of the section.

In figures 17–21 are indicated variations from the bipolar to the tripolar type of cell; in figures 22–25 from bipolar to hexapolar; in figures 27–31, from bipolar to asymmetrical tripolar, or possibly multipolar, and in figures 32–36 variations from bipolar to tetrapolar cells. Each cell exhibits that type of nuclear distortion which will most effectively distribute the nuclear granules throughout the cytoplasm of a cell of that particular shape.

Comparing figure 3, plate 1, which is of a cell before this stage, but shows the beginnings of it, it is apparent that the central cytoplasm and nucleus both assume approximately the shape of the cell outline even before the fat-vacuoles approach the nucleus. This may be considered a predisposing factor in directing the trabeculae later, and may be taken to indicate incidentally that nucleoplasm and cytoplasm are so little different in density or consistency that the distortion by fat-vacuoles affects the former against whatever surface tension the nuclear membrane may exhibit, tending to form the nucleus into a sphere. When the fat-vacuoles later move toward the nucleus, and the larger of these press in through the central cytoplasm toward the vesicle, they may be the active agents in pressing the nuclear mass further out of shape; their effect is presumably augmented, however, by a centripetal tendency on the part of the larger nuclear granules. Both these forces seem to be occasioned by the cessation of larval nutrition, and their nature is not clear. It is fairly certain that the nucleoplasm itself does not disperse with the granules, from evidence of the staining reaction of the granules themselves as they pass into the cytoplasm. The dispersing forces must lie in the granules themselves, conceivably a like static charge, for instance.


17
22
27
32
18
23
28
33
19
24
29
34
20
25
30
35
21
26
31
36

SUBJECT AND AUTHOR INDEX