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CONTRIBUTIONS FROM THE LABORATORY
OF THE
MARINE BIOLOGICAL ASSOCIATION OF SAN DIEGO

XIX

THE EARLY LIFE-HISTORY OF *DOLICH-*
OGLOSSUS PUSILLUS RITTER

BY

B. M. DAVIS



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Volume 4. No. 3

The Early Life-History of *Dolichoglossus Pusillus* Ritter, by B. M. Davis.

ERRATA: On page 214, last line, for *D. kowalevski*, read *B. kowalevskii*.

On page 218, explanation of Plate 4, last line, for $\times 1$, read $\times 4$.

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XIX

THE EARLY LIFE-HISTORY OF DOLICH-
OGLOSSUS PUSILLUS RITTER.

BY

B. M. DAVIS.

Benjamin Marshall Davis
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INTRODUCTION.

In November, 1902, I secured a few eggs and free larvae of *D. pusillus* Ritter, a short account of which is given in a recent paper (Ritter-Davis '04). In this account it is stated that owing to destruction of collecting grounds at San Pedro, California, it would be impossible to secure a complete series of stages necessary for detailed study, but that it was hoped to obtain sufficient material at San Diego, California. On January 18, 1905, I had the good fortune to find a small area of mud flats on the west side of Whaler's Bight, San Diego Bay, containing *D. pusillus* at the height of its breeding season. During the month following (January 18 to February 18) I succeeded in getting all stages from unsegmented egg to metamorphosis, and was able to study these stages in the living condition.

The only account we have of direct development of Enteropneusta is that by Bateson '84-'85. This has become one of the zoological classics. However, Bateson's series of early stages was not complete, and he did not give a detailed account of the animal's activities at different periods of its development.

Further investigation of the subject is an urgent desideratum. In this paper I give an account of the habits and activities of the early life of *D. pusillus*, and also the stages of development up to and including that of the formation of the body cavities.

At another time I hope to follow out the later stages of development, particularly of the connective tissue system and supporting framework. I take pleasure in acknowledging the helpful suggestions and directions which I have received in the preparation of this paper from Professor William E. Ritter.

MATERIAL AND METHODS.

The portion of the mud flats on which the animals live is uncovered at mean low tide so that collecting is possible for several hours during five or six days of each low tide period. This fact is important, for the work of collecting is slow and tedious, as a great many burrows must be examined before one is found containing eggs.

D. pusillus has its burrow near the surface, but there are no external indications of its presence except occasionally when the animal extends its proboscis from the burrow (Ritter '02). A certain amount of prospecting is therefore necessary before the animals are found in large numbers.

When such a place is found, a spadeful of mud is dug up and the burrow of each animal carefully examined for eggs. By breaking one side of the burrow and gently lifting the animal out or pushing it aside, the eggs, if present, may be seen clinging to the unbroken side. They are usually closely packed and sometimes extend over an area of several square millimeters. The position of the eggs in a burrow is shown in plate 4. Here the animal has been lifted up and to one side, leaving the eggs exposed. The eggs are somewhat flattened, whereas in water they are perfectly round. They also appear to be of a darker tint of yellow ochre than when seen in water. This is due to the dark background of the mud.

From the burrow the eggs are removed by means of a fine pipette to a shallow dish filled with clear water. By holding the dish against a white background, the eggs may be easily separated from the particles of sand, and then transferred to small vials of sea-water for transportation. As eggs or larvae of a single burrow are usually in nearly the same stage of development, when a large number in one burrow is found, they are kept in a separate vial. On reaching the laboratory the material is transferred from vials to small dishes filled with fresh sea-water. An occasional change of water is all that is necessary to keep the animals alive.

Zenker's fluid, corrosive-acetic mixture, Lo Bianco's chromic mixture, and osmic acid were used as killing and fixing agents. After fixation and washing the specimens were preserved in eighty per cent. alcohol. Eggs or larvae from single burrows were kept in separate dishes. Some were killed and preserved from time to time, the intervals depending upon stage of development. Fifteen such series were made.

Various stains were used, including Mallory's connective tissue stain, Meyer's acid haemalum, Benda's iron haematoxylin, Heidenhain's iron haematoxylin, Delafield's haematoxylin, and

borax carmine. Congo red, erythrosin, eosin, and orange-G were used as counter stains. Of these Meyer's haemalum counter-stained with congo red for the early stages, and Mallory's connective tissue stain for advanced stages that were fixed in Zenker's fluid proved to be the most satisfactory.

Living material was examined with a Zeiss-Greenough stereoscopic microscope. The depth of field and appearance of three dimensions afforded by this instrument added greatly to the accuracy and facility of observations, particularly in the detailed study of swimming.

PERIODS OF DEVELOPMENT.

The following statement made in a previous paper (Ritter-Davis '04, p. 173) in regard to periods of larval life of *Tornaria*, applies to *D. pusillus*: "In the larval life of the enteropneusta three periods should be recognized; namely, a period of *larval development*; a *climactic* period; and a *metamorphic* period; *i.e.*, a period of development again, but this time development toward the adult animal."

As I shall recognize these periods in my account of the early life history of *D. pusillus*, it will be necessary to modify somewhat the definition previously given, particularly since no account was taken of the earliest developmental stages of *tornaria*.

The first period, or period of larval development, includes the time passed wholly within the egg-membranes. It may be noted that my observations on the time of hatching do not reveal any such irregularity as described by Bateson for *B. kowalevskii*. After describing that stage of the larva where the collar area is definitely marked off by anterior and posterior grooves, he says: "The animal remains in this condition for some hours and is generally hatched without the occurrence of any further alteration. The time of hatching is, however, quite irregular. Larvae may frequently be found swimming freely whose organization is not much in advance of Stage C (the stage before either groove has appeared), and on the other hand, I have seen them in the condition of Stage G (after appearance of first gill opening) in the eggshell." (Bateson '84, p. 211.)

My observations agree substantially with the first part of this statement, but not with that part referring to irregularity of hatching. In his second paper (Bateson '85, p. 2) he says: "From further observation it seems probable that this period (Stage D) assigned as the time of hatching is too early; for embryos kept in aquaria do not break the membranous shell before Stage G is reached. Probably, therefore, the larvae found swimming in Stage D had escaped owing to an artificial rupture of the shell during the process by which they were found,¹ an account of which is given in an appendix."

I am inclined to think that the presence of late unhatched larvae is also abnormal and exceptional. As will be described in detail later, the stages of normal larvae immediately preceding hatching is one of great activity within the egg-shell. It might be supposed that in exceptional cases larvae would not be sufficiently active to effect an opening. In such cases there is no apparent reason why the larvae should not pass through the later stages of development within the egg-shell; no instance of this kind, however, has come within my observation.

The second or climactic period includes the period of active swimming.

The third or metamorphic period includes two phases: one of rapid crawling, mainly by means of the ciliary band, and one of slow crawling, by means of proboscis and body cilia.

These periods are not absolutely distinct in the sense of being delimited from one another. Period II gradually merges into period III by the active swimming cycles, as will subsequently be described, becoming less frequent. The same is true of the two phases of period III, for the movements by means of proboscis and small cilia are gradually substituted for those made by the ciliary band. The three periods above described will hereafter be referred to by their numbers: I, II, III.

¹ The essential steps of this process are as follows: (a) Shake up mud in vessel, avoiding rotary currents; (b) put in this, minced balanoglossus and allow to settle a few minutes; (c) siphon off lighter particles in suspension until balanoglossus fragments are reached; (d) draw this portion off and in it will be found the embryos.

EARLY HABITS AND ACTIVITIES OF *D. pusillus*.*Period I.*

As soon as the ciliary band is fully formed, the larva begins active swimming within the egg-capsule. Bateson '84 (p. 211) says: "The larva swims about very rapidly, rubbing the membranous shell with its anterior end until it gives way, and the animal escapes." The larva of *D. pusillus* does not escape as easily as one might suppose from the above statement. As soon as an opening is made, the anterior end is pushed through. The proboscis becomes flattened and the body within the capsule constricted. The large cilia cease to move, but the small ones are in active motion, producing a slow rotation. The crucial point in the process of hatching is in the passage of the ciliary band. After this portion of the body passes through the opening, the larva is soon free. The average time for the entire process after the opening is made, as observed in five specimens, was twenty minutes. Within a few minutes after escape from the egg the larva begins active swimming. Plate 7, figs. 17, *a*, *b*, and *c*, represents the passage of the larva through the egg case opening.

Period II.

As will be noted in connection with the description of period III, period II was not noticed at San Pedro. Bateson '84 (p. 211) says: "On leaving the egg it does not swim at the surface as pelagic larvae do, but creeps about in the mud, burrowing with its proboscis, in the walls of which muscle fibers soon appear, and also propelling itself by means of its ciliated band. If placed in a beaker of water it sinks to the bottom at once." This description, with the exception of one point, applies very well to the third period of *D. pusillus*, but not to the stage immediately following escape from the egg, or period II. The exception above noted is in reference to what Bateson says of "burrowing with its proboscis, in the walls of which muscle fibers soon appear." It is hard to understand how the animal could burrow with its proboscis before the muscle fibers appear and become active. As a matter of fact in *D. pusillus* when the burrowing stage is reached two sets of muscle fibers have appeared in the proboscis.

Period II in *D. pusillus* is a time of active swimming, and varies in length from twelve to twenty-four hours as shown in observation of twelve individuals. Intervals of rest occur, during the period, and these become longer as the larva grows older. When swimming actively the larva appears much like a miniature tornaria, both in outline and relative length of longer and shorter axes. However, the similarity goes further than this. Like tornaria it swims chiefly from below upward. It may occasionally take a horizontal course, but this is always undulatory and never in a straight line. Like tornaria, its swimming movements are produced by the cilia of the ciliary band. Like tornaria, also, it does not swim vertically upward but takes a spiral course, the direction of the spiral, as in tornaria, being clockwise.

Unlike tornaria, as far as has been observed, it has a regular cycle of movements which is repeated at rather regular intervals. Beginning at the bottom it swims vertically upward always describing a spiral. When near the surface of the water the direction becomes horizontal and the path undulatory instead of spiral; then the animal ceases to swim and falls rapidly to the bottom. The bottom having been reached, after a short period of rest, the cycle of movements is repeated. Sometimes a new cycle may begin before the bottom is reached.

The following is a tabulation of a typical series of swimming cycles:

Time of rising through distance of 25mm.	Time of horizontal swimming.	Time of falling.	Time of rest at bottom.
40 secs.	15 secs.	10 secs.	No secs.
35 secs.	15 secs.	8 secs.	60 secs.
30 secs.	Half way down and new cycle begun	90 secs.
30 secs.	105 secs.	20 secs.	1 sec.
25 secs.	10 secs.	14 secs.	No secs.
32 secs.	1 sec.	11 secs.	2 secs.
30 secs.	5 secs.	New cycle half way	
		New cycle one-third way down	
	8 secs.	12 secs.	6 secs.
	40 secs.	9 secs.	70 secs.
35 secs.	1 sec.	New cycle two-thirds way down	
25 secs.	2 secs.	way down	
	3 secs.	8 secs.	1 sec.
32 secs.	1 sec.	9 secs.	40 secs.

After several hours the intervals of rest at the bottom grow longer and longer until finally the animal ceases to rise. Light does not seem to have any influence in directing the animal's movements. It swims freely both toward and away from the light. A number of animals were put in a dish from which light was excluded except at a small opening in the cover of the dish. The dish was left undisturbed for five minutes. At the end of this time the animals were found scattered irregularly through the water. This experiment was modified in various ways, and for varying lengths of time, but always with negative results.

It is during period II that the tuft of apical cilia reaches its fullest development, although it appears in period I. There is reason to suppose that these cilia are of use to the animal at this time. It is impossible to ascertain their exact behavior when the animal is moving rapidly, but when it moves slowly along the bottom of a dish by means of the small cilia with which the body is covered, the tuft swings in a direction opposite that which the animal takes in turning, thus playing the part of a tiller or rudder for steering the creature. It is probable that these large cilia are used in a similar way during the animal's more active movements. But the direction is not determined entirely by the apical cilia, for only one part of the ciliary band may beat at a time; *e.g.*, slow rotation is caused when the cilia of one region on one side beat while the rest are inactive. Furthermore, the rate as well as the direction of swimming is determined by the number of cilia that beat in the ciliary band. The velocity of swimming may be said to vary with the number of cilia in action. When the velocity is increased, as it always is when the animal is rising, this is probably always accomplished by use of additional cilia. In one portion of the swimming cycle the descent is vertical. The cilia of the ciliary band are, at such times, entirely motionless.

Period III.

The few specimens examined at San Pedro in 1902 consisted of unhatched larvae (period I), and free larvae of period III. From the scanty material then at hand I concluded with Bateson that the first period after hatching was a crawling one, and that there was no free swimming period. My observations were de-

scribed in these words: "Animals do not swim freely, but glide about with proboscis pointed forward over the supporting surface. These movements are made mainly by means of the large cilia composing the posterior ciliated band, although their action ceases occasionally for short periods. At such times the animal continues to move by means of minute cilia with which the body is covered, but the motion is very slow." Ritter-Davis '04, pp. 201-202. This is correct for the first phase of period III. As the larva grows older there is less and less use of the ciliary band until finally it is not used at all; at least there is no more rapid creeping.

During the latter part of the first phase of period III, at times when the ciliary band is not in use, considerable muscular activity may be noticed in the proboscis, this member now contracting and extending alternately. These movements are characteristic of the second part of period III. By them, and also through the action of the small body-cilia, the animal is able to creep slowly about over the supporting surface. Bits of sand and other sediment readily adhere to the body in the region of the collar, collected there by the mucus or slime which is actively secreted at this time. The secretion in one instance was so much that two larvae that happened to meet were bound together, and so closely that neither could escape by its own efforts. In older stages the accumulation of sediment was so great as to necessitate its removal before the animals could be studied.

It is at this time that the larva begins to burrow. When placed in a dish of mud it soon disappears beneath the surface. The burrowing movements of *D. pusillus* are very similar to those of the larva described by Morgan '94, p. 17. He says: "It was very noticeable that so soon as the proboscis was thrust in the sand a thick mucus was thrown out from the surface of the proboscis and collar region to which the sand granules stick, forming an irregular tube around the animal."

Periods of development correlated with habits and distribution of adults.

The adult life of *D. pusillus* is passed in burrows in the mud. The animal does not, as many sand-dwelling animals do, come to

the surface and migrate to new areas. It is true that occasionally the proboscis is protruded above ground, but with the rising tide this is again withdrawn. Careful observations have failed to discover any instance where the animal's body is in any way exposed after the ground is covered with water. (Ritter '02, p. 255.)

It is also very probable that any individual animal is restricted to a comparatively small area; that it does not migrate from place to place in the mud. This is indicated by the fact that burrows are vertical for the most part, and that the burrowing movements are slow compared, for example, with those of certain nemertians and annelids. Furthermore, the adult animals are not uniformly distributed over the area in which they are found. In some places as many as fifty individuals may be found in an area of one square foot, while in others but one individual may be found in an area of a square yard.

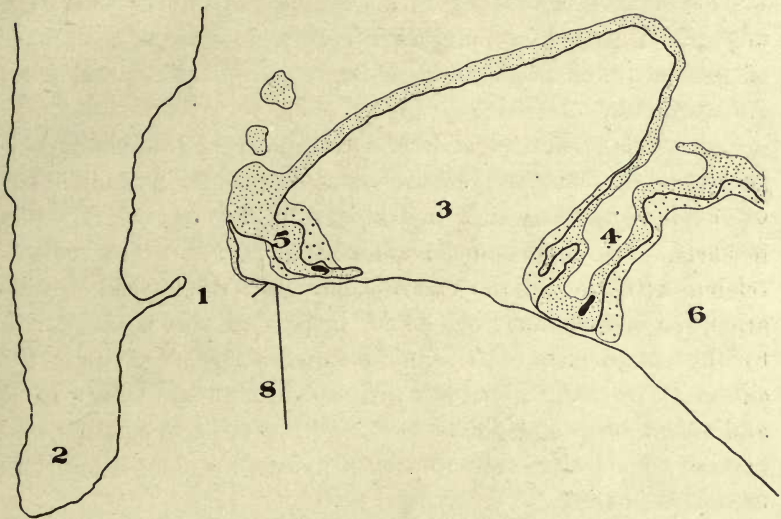
The distribution of *D. pusillus* during a period of six years at San Pedro, California, is described as follows: "The area of greatest numbers has gradually shifted toward the mouth of the harbor. None were found in 1902 in places where they were abundant in 1897. In 1900 there were two areas of distribution, one at low tide-mark, where large individuals predominated; the other considerably above low tide-mark, where small ones predominated." (Ritter-Davis '04, p. 200.)

Here are two points worthy of notice. The shifting must not be understood as migration in the sense of individuals moving toward the mouth of the harbor, but rather as plants migrate. For various reasons, among them being deposition of sediment, change of currents, and invasion of eel-grass, the region populated in 1897 became unfavorable for enteropneust life, and the animals died. The opposite end of the mud-flat strip which was the center of distribution in 1902 became so by the growth of new individuals. In 1900 the increased number of new individuals was great enough to be noticeable but in another direction of distribution. Here they appeared farther shoreward.

Before discussing means of dispersal for *D. pusillus* it will be worth while to consider more in detail the environment of the adult. The animal is always found in sheltered places, free from

swift currents and disturbing waves. It is in such places that fine, black mud, rich in organic matter, occurs. The animal is never found in even moderately clean sand.

The black-mud areas are associated with level surfaces which are uncovered at low tide, with freedom from wave action, and



MAP OF PORTION OF SAN DIEGO BAY.

1. Entrance to San Diego Bay.
2. Point Loma.
3. North Island.
4. Spanish Bight.
5. Whaler's Bight.
6. Coronado.
8. Government breakwater.

Heavy black shading in 4 and 5 indicates area of distribution of *D. pusillus*.

Region indicated by fine dots uncovered at low tide.

Region indicated by large dots covered at high tide.

with marginal plant growth, part of which is submerged at very high tides. Whaler's Bight and Spanish Bight of San Diego Bay are typical illustrations of just such conditions. The map (p. 197) is part of the San Diego Bay including the above bights. A reference to this map will indicate to what extent these conditions obtain in the area of distribution of *D. pusillus*.

I have already stated that such situations are free from swift currents and strong waves. The tidal currents in these extensive flats are unnoticeable on casual observation. With this environment in mind we are ready to consider how the early life-stages of the animal are correlated with these conditions. Period I is spent in the egg-capsule in the security of the burrow. Here all the developmental changes, fitting the animal for the active life of period II, take place, and the beginning is made of the organs of greatest functional importance for period III, *viz.*, museles and mucus glands.

Although no accurate determinations were made of the specific gravity of the organism, it was obvious from watching individuals of the three periods that this is least during period II, as in tornaria. The important characters of period II which are correlated with the animal's movements are: diminished specific gravity, brought about, no doubt, in part as will be indicated, by the enlargement of the middle and posterior cavities; the climax of efficiency of ciliary activity, in both the ciliary band and apical tuft; and absence of growth, except of museles and glands, whereby the animal's energy may be more completely used in swimming.

The activities of the organism at this period are directed, first, to escape from the burrow of the parent, and second, to its suspension in the water during short intervals of time. I say "in suspension," for a little calculation shows that it could not proceed by swimming more than two or three meters during this entire period, and when we consider that the direction of swimming is mostly vertical, the actual horizontal progress would be much less.

Obviously, this part of the animal's life is adapted to make use of the slow tidal currents for dispersal. Judging from the numerous laboratory experiments already described, the swimming cycles would prevent the animal from being carried very far into deep water, for the time of rest at the bottom is often greater than the time of active swimming. Besides we must also take into consideration the fact that the tide moves in a direction away from the deep water as well as toward it. The actual procedure from the burrow of the parent is somewhat as

follows: First, a cycle of swimming keeps the animal suspended for a short time, during which it is borne by the tidal current; second, a period of rest on the surface of the mud, until the beginning of the next cycle. This process being repeated for from twelve to twenty-four hours, the animal would, during this time, be carried a considerable distance from the parental burrow, at least far enough to account for such migrations as are described for San Pedro Bay. (Ritter-Davis '04.)

During the latter part of this period the intervals of rest are longer and longer, until by the time period III is reached the creature is safely located in the region of its future burrow.

What induces these swimming cycles is by no means clear. Two possibilities are suggested: one of stimulation, the other of rhythmical physiological states.

In the consideration of stimuli the factor of light must be excluded, since the organism has been shown to be indifferent to light. The only source of stimulation would seem to be contact with the surface of the mud, for temperature, salinity, oxygen content of the water and other conceivable factors are practically constant.

When Paramoecium comes in contact with a solid or other source of stimulation, a new cycle of movements is instituted (Jennings '04). At first thought it might seem that the free-swimming larva of *D. pusillus* behaves in the same way. If, as it touches the mud a new cycle were to begin immediately, the behavior of the two organisms would be essentially the same. But the larva crawls for awhile on the mud, and these crawling periods, even at the height of period II, vary in length. Again, a new cycle is often begun before the animal reaches the mud. This precludes the possibility of contact as a stimulus. It would seem, therefore, impossible to assign any particular stimulus as a cause for starting the swimming cycle.

As to these cycles being rhythmical and due to physiological states, the chief objection is that they are not regular either in point of time or extent of movement. In the behavior of Paramoecium and similar organisms where this explanation seems plausible, the animals are adults, and the physiological conditions are fairly constant. In the animal under present consideration,

the physiological conditions would not be expected to be constant, since they would be interfered with by growth, which, though reduced, is nevertheless going on. But might not the cycles which would otherwise be rhythmical due to one set of physiological conditions be modified by another set, *e.g.*, that of growth, so as to bring about just such irregularity of swimming cycles as is found? The fact that these cycles become more and more irregular toward the end of period II, when internal changes are becoming more active just previous to period III, lends some credence to this explanation, and makes it plausible but by no means certain.

As has already been stated, the first phase of period III is characterized by rapid crawling in which the now motile proboscis begins to take part. This is an adaptation which is important for preservation of the animal at low tide, for it is enabled to burrow in the soft ooze and thus readily escape destruction.

The second phase is really metamorphic. The animal's movements are mainly muscular. The mucous glands are sufficiently numerous and active to pour out abundant secretion for cementing the walls of its burrow and lubricating them with slime.

The yolk supplied by the egg is now nearly used up as indicated by the transparency of the animal (Bateson '84), and by the absence of yolk granules as shown by microscopical examination. But in the meantime a mouth, digestive tract, and gills have appeared, thus equipping the organism for self-support.

EARLY GROWTH STAGES.

External Features.—Period I.

Egg. The method of discharging the ova, and the general characters of the egg itself are adequately described by Ritter-Davis '04. The enteropneust egg has two membranes, a fact hitherto unnoticed. At first, these are so closely adherent to each other as to be indistinguishable, but later they are separated by a narrow space. The outer membrane may then be removed, leaving the inner one intact. This is often necessary in order to study the larva within the capsule when the outer membrane is

rendered partially opaque by accumulation of sediment. While no special observations were made on the nature of these membranes, it was noticed that, just before hatching, the outer one became less transparent and less resistant. The latter condition is correlated with the hatching process which has already been described.

The particular feature of the egg in this stage is its opacity. Bateson's statement that the egg of *D. kowalevskii* is "very opaque" is equally applicable to that of *D. pusillus*. The opacity is due to the yolk granules which are uniformly scattered throughout the cell. This distribution is so uniform that no difference such as described for the ascidian egg by Conklin and by Castle may be seen distinguishing one part of the cytoplasm from another.

Cleavage. Although I obtained many hundred eggs and larvae, I only succeeded in one instance in finding the earliest stages. One lot contained a few unsegmented eggs and several in early cleavage (four, eight, sixteen cells). The material was insufficient for a study of the cell lineage.

First cleavage. Bateson '84 (p. 209) says of the early cleavage: "The first furrow is formed in a median plane, dividing the ovum into two equal parts. It passes to a considerable depth. With regard to subsequent segmentation I have no certain observations; for though some of the ova divided into four and eight nearly equal parts, these were obtained by artificial fertilization, and the process of division was afterwards continued in an entirely abnormal manner as mentioned above. Judging, however, from the characters of the blastosphere, and from the fact that yolk granules are uniformly distributed through the whole tissue, there can be little doubt that the segmentation is regular and complete."

I am able to verify the above observations in regard to the first cleavage. (Fig. 3, pl. 5.)

Second cleavage. (Fig. 4, pl. 5.) The second cleavage is also equal, the blastomeres being arranged at first in perfect radial symmetry. In most cases this symmetry seems to persist, but in a few there is a slight irregularity. This irregularity was at first attributed to displacement due to manipulation. While this may

be the case it was more likely normal, especially in view of the position of the blastomeres of some eggs of the third cleavage. Wilson '94 has called attention to the same thing in *Amphioxus*: "Slight as they are," says this author, "they deserve attentive consideration, for they give, I believe, a key to the more considerable deviations of later stages."

Third cleavage. (Fig. 5.) The third cleavage is unequal and is of especial interest because of its similarity to that of *Amphioxus*. The blastomeres of the upper pole are smaller than those of the lower. The difference in size between those of the two poles is somewhat less than that shown by Wilson for *Amphioxus*; otherwise the two eggs are much alike even to the exception to the usual bi-lateral form of segmentation, for as in *Amphioxus* there occurs a deviation from the typical arrangement of blastomeres into the spiral form described by Wilson.

The slight irregularity noted in the second cleavage and the spiral form in the third cleavage were observed on preserved material. I am therefore unable to say whether or not the irregular second cleavage of *D. pusillus* gives rise, as Wilson found for *Amphioxus*, to the spiral third cleavage. The early cleavage stages of the two animals being thus far so much alike, and also in other respects to be described, suggest that they may be found to be alike in this particular also.

Fourth cleavage. (Fig. 6.) The typical fourth cleavage is shown in fig. 6. Here it will be seen that the cleavage is bi-lateral. All eggs observed in this stage, except one, were of this form. This one exception corresponds to Wilson's mixed form of cleavage.

Fifth cleavage. (Fig. 7.) In the fifth cleavage another point of similarity between *D. pusillus* and *Amphioxus* is to be observed: the presence of a cleavage pore. This and the bi-lateral form of cleavage is shown in fig. 7. A large per cent. of eggs of this stage and stages immediately following possessed cleavage pores.

Sixth and subsequent cleavages. The sixth and subsequent cleavages do not show clearly a bi-lateral symmetry. Two of these stages are shown in figs. 8, 9, pl. 5.

Comparison of D. pusillus with Amphioxus. Although my

observations as above recorded were made upon few individuals they are sufficient to warrant pointing out the great similarity between the early cleavage of this animal and *Amphioxus*. A reëxamination of plenty of material and a careful study of the cell-lineage of *D. pusillus* would no doubt show a greater likeness. The points of similarity are:

- (a) Bi-lateral form of cleavage, as a rule.
- (b) Occasional variation from this into spiral, and perhaps into radial and mixed cleavage.
- (c) Frequent occurrence of a cleavage pore.
- (d) Gradual obliteration, in later stages, of bi-lateral symmetry.

The points of difference are:

- (a) Absence of radial cleavage in *D. pusillus*. But since there was one instance of mixed cleavage, it is not unlikely that in a large number of individuals in early cleavage some of this form might be found.

- (b) Less difference in the size of the blastomeres of the two poles of the third cleavage in *D. pusillus* than in *Amphioxus*. This point, however, is unessential since the yolk is uniformly distributed in the eggs of both animals, and the stages subsequent to the third cleavage are alike as far as can be shown with the material at hand.

Comparison of D. pusillus and Amphioxus with Ascidia (Ciona intestinalis) in their early cleavage. Castle and Conklin were able to recognize that the unsegmented ovum of *Ascidia* is made up of two unlike hemispheres, one richer in yolk and the other richer in protoplasm. Castle '96 concludes: "The form and rate of cleavage are therefore manifestly predetermined by the internal constitution of the ovum." In the *Enteropneust* egg (*D. pusillus*) and in the egg of *Amphioxus* no such distribution of yolk and protoplasm occurs.

With reference to symmetry of cleavage Castle '96 (p. 233) says: "Wilson '94 observed that the cleavage of *Amphioxus* showed all gradations between a perfectly radial, a bi-lateral, and even a spiral form; and raised a query whether the same might not be true for *Ascidians*. In *Ciona* at least this does not seem to be the case. I have never observed an instance of deviation

from the regular mode of cleavage described in the foregoing paper." By "regular mode" he means bi-lateral. He adds further: "In having a perfectly definite and stereotyped manner of cleavage, the Ascidian egg resembles more closely the egg of Annelids, Mollusks, and the great majority of invertebrates than it does Amphioxus and the vertebrates, notwithstanding that the *end product* of cleavage shows unmistakably the now generally admitted closer affinity of the tunicates with the latter group of animals."

One must conclude from this and from what has been indicated in the comparison of *D. pusillus* with Amphioxus that, in so far as resemblance in cleavage may be relied upon for determining affinities, there is a closer relation between these two animals than between Ascidia (*Ciona*) and Amphioxus.

Blastula. The blastocoele appears as a segmentation cavity as early as the fourth cleavage, so that it is difficult to set apart the stages of cleavage, just described, from the blastula.

I shall regard the stage just succeeding that shown in fig. 9, as the early blastula stage, for it corresponds to the earliest stage that Bateson found beyond the first cleavage and which he calls the blastosphere. He describes it as spherical, with opaque walls, though "the outline of cells composing them could be faintly distinguished in a surface view."

I did not find among my living stages of the blastula the elliptical form described by him for the later stages; but in preservation some became elliptical. I did, however, observe the slightly flattened condition which he describes as just preceding gastrulation.

Gastrula. Estimated from the time eggs were taken from the burrow when segmentation no doubt was beginning, until the first evidence of gastrulation, about twenty hours elapsed (figs. 10 and 11). Bateson's account of external changes of this period agrees largely with my own observations.

The flattened condition of the gastrula remains but for a short time. Before the ring of cilia appears the gastrula is nearly spherical. In this respect it differs from *B. kowalevskii*.

The time between the beginning of gastrulation and the appearance of cilia is about twelve hours. The blastopore can no

longer be seen from the surface but sections show that it does not completely close until about six hours later.

Larva within the egg-capsule. The larval period may be said to begin with the closure of the blastopore and elongation of the body. This elongation is at right angles to the plane of the ciliary band. I was, unfortunately, not able to determine the relation of the axis of the embryo to the first cleavage plane.

The most important changes that take place are: growth in length, enlargement of the ciliary band, appearance of the posterior collar groove and later of the anterior groove; and development of the anterior, or apical cilia. (Figs. 13, 14, pl. 6.) As these changes are fully described by Bateson '84 and are so closely repeated in the present species I need not here discuss them.

Period II.

The larva remains through this period, in external appearance, just as when leaving the egg, except perhaps for a slight elongation of the body. (Fig. 15, pl. 6.) The significance of this quiescent stage has already been discussed in another connection.

Period III.

Like period I, this period is marked by many changes: further elongation of body, both of proboscis and body proper; appearance of gill openings; disappearance of apical cilia, and, later, reduction of the ciliary band; increase in diameter and width of collar. (Fig. 16, pl. 6.) I have nothing to add to Bateson's (pp. 211-213) full description of the external appearance of the stages of this period. My own observations, except for slight details, agree with his.

Internal Features.—Period I.

Blastula. The first internal change to be noticed is in the latter part of this period. The cells forming that portion of the blastosphere which is to be invaginated (endodermal portion) are somewhat irregular, having rounded ends projecting unevenly into the blastocoele. (Fig. 18, pl. 7.) They are relatively narrower than the cells of the opposite portion of the blastosphere

(ectodermal portion), probably indicating more rapid cell division in this region.

Numerous large round cells, many of them in mitosis, are to be seen near or at the surface of both the ectodermal and endodermal portions of the blastosphere. These cells are further distinguished from the remaining cells by having different staining properties of the cytoplasm. One of them is shown in fig. 18, *g. c.* None were noticed earlier.

Bateson found a greater difference between the ectodermal and endodermal portions of the blastosphere than I have just described. He did not, however, notice the presence of the round cells, above mentioned, which are very conspicuous in this and later stages of *D. pusillus*.

Gastrula. The gastrula is formed by the invagination of the endodermal portion of the blastosphere, and elongation in the direction of a line passing through the center of the blastopore and opposite pole. As elongation proceeds the endoderm approaches the ectoderm until the two layers meet. At the same time the blastopore is closing.

Fig. 19, pl. 7, shows the early stage of gastrulation before elongation. It will be noticed that the endoderm is somewhat thinner than the ectoderm, and that there is less difference in the histological character of the cells than in the stage just preceding invagination. The rounded ends of the endodermal cells and indications of their being amoeboid, as described by Bateson '84 (p. 213), I was unable to find.

Fig. 20 is a longitudinal section through the blastopore at the last stage of gastrulation. Here the blastopore is about closed, and the two layers are adjacent. The cells of the region of the blastopore are irregular and in rapid division as indicated by the numerous nuclei. The ectodermal part of the blastoporic rim is already in process of fusion. Later the endodermal part fuses, and thus the blastopore is completely closed. The ectoderm and endoderm of this region remain coalescent for a time (fig. 21), but finally separate (fig. 22, pl. 7).

In this manner the gastrula passes into what Bateson (p. 215) calls a "two-walled cylinder," the outside wall of which is the ectoderm, and the inside the endoderm. With the exception

already noted, my observations on this stage agree with Bateson's account. I have, therefore, omitted many details.

Mesoderm and body cavities. Bateson (p. 214) says of the two-layer stage just described: "The hypoblast of the middle region is seen to be more columnar in character than that of the anterior region, while the other appearances are the same." Fig. 22 shows this condition. It will be noticed that the cells of the extreme anterior are somewhat shortened. The cells at the transition from the anterior to the middle region tend to lose their columnar character and to become irregular. Here, as we shall see, is the beginning of a series of changes in the endodermal cylinder which is to give rise to the mesoderm.

Since Spengel '04 associates the formation of the mouth with the origin of the mesoderm in tornaria it may be worth while to state that according to Bateson's observations on *B. Kowalevskii* and my own on *D. pusillus* the mouth does not appear until after the mesoderm is formed.

By the time the two layers in the blastoporic region have become separated, that portion of the endoderm which has just been described as composed of irregular cells (fig. 22) has projected outward and backward somewhat, thus partially separating the archenteron into anterior and posterior parts. The former will be referred to as "anterior body cavity" (Bateson '84, p. 216). This stage of development is shown in fig. 23, pl. 8. It will be seen that the cells of the walls of the anterior cavity are becoming irregular, especially on the sides, and that some cells are being budded off into the cavity. Rapid growth and redispotion of cells continue until the condition shown in fig. 24 is reached. Here the anterior body cavity although irregular shows the beginning of a lateral and backward projection. The walls of the anterior body cavity and those of the archenteron are sharply differentiated in character of their cells. The archenteron will henceforth be referred to as *enteron*.

The lateral backward projections of the walls of the anterior body cavity continue to grow. The next two stages are shown in fig. 25 and fig. 26, pl. 8.

It must be here noted that the backward growth is not only lateral, but, for a short distance, dorsal as well. There is no

ventral extension, so that the mesoderm forms a broken ring around the anterior extremity of the enteron (fig. 27). From this point the mesodermal growth is confined to the sides. (See quotation from Bateson.)

The following description refers exclusively to the lateral backward growth of the mesoderm. In the section represented in fig. 24, pl. 8, it will be seen that the walls of the enteron are completely separated from the anterior body cavity except at one point. Complete separation is seen in stages represented by figs. 27 and 28. The walls of the anterior body cavity may now be called mesoderm.² The edges of the anterior portion of the enteric walls are approaching, leaving a small oblong communication (oblong dorso-ventrally) between the two cavities.

The growth of the mesoderm backward is taking place wedge-wise between the walls of the enteron and the ectoderm. The cells of the edge are irregular, tending somewhat to flatness at its narrow extremity. In this way the mesoderm continues to grow backward, the wedge of cells (as seen in longitudinal section) becoming somewhat longer and thinner, and reaching beyond the collar region. This stage of development is shown in fig. 25. At a little later period an important change takes place. The mass of cells extending backward between the enteric wall and the ectoderm loses its wedge-shape in longitudinal section. At a point corresponding to the anterior collar groove, the mass of cells is slightly constricted. In the collar region corresponding in longitudinal extent to the collar, the cells form two layers. Posterior to this region the mesoderm continues for a short distance as a single layer of somewhat flattened cells. (Fig. 26.) This is the beginning of the middle body cavity.

The mesoderm grows backward until it almost reaches the posterior extremity of the enteron. In the meantime a second constriction occurs at a point corresponding to the posterior groove of the collar (fig. 28, pl. 8). Posterior to this constriction the mesoderm forms two layers just as described above for the collar region.

In this manner the middle and posterior body cavities arise

² The term mesoderm has already been used in reference to the portion of this region which is reflected backward.

from the mesoderm. Their walls are one-cell deep, the cells becoming flattened. For a while the three body cavities, anterior, middle, and posterior, are continuous, though marked off by the constrictions already described. Fig. 29, pl. 8, shows the relation of these cavities to one another at this stage. Subsequently the constrictions deepen until finally they completely separate the cavities.

Prior to this period the middle and posterior cavities have extended ventrally somewhat, but more dorsally. This growth continues concomitantly with the later stages of longitudinal growth just described. They extend dorsally, meeting at the mid-dorsal line, but ventrally they do not quite meet.

As the above account of the origin of the middle and posterior body cavities in *D. pusillus* is at absolute variance with Bateson's description of the origin of these structures in *B. kowalevskii*, and with his diagram, familiar in text-books, I wish to review his account somewhat fully.

After describing the stage corresponding to that in *D. pusillus* shown in fig. 23, pl. 8, he says: "The mesoblast arises at this period of development. It is formed directly by differentiation of cells belonging to the archenteron. These differentiations occur in five regions. The first comprises a median and primitively-unpaired tract in the anterior end, which forms the lining of the body cavity of the praeoral lobe. Behind this anterior body cavity a pair of mesoblastic differentiations occur in the region of the collar, constituting lateral outgrowths of the archenteric walls, each containing a cavity which communicates directly with the cavity of the archenteron. Behind these, again, is another pair of regular archenteric diverticula, in the region of the trunk." He refers at this point to his well known diagram illustrating his description.

He then describes in detail (pp. 141-142) the development of the anterior body cavity. The latter part of his description (pp. 218-219) is as follows:

"Now, since the anterior body cavity is continued behind the end of the gut on all sides excepting the ventral, it is crescentic in shape, the concavity being directed downwards. This appearance exists only for a short distance. Behind it the continuity

across the dorsal surface ceases, and *the mesoblast exists as a pair of small, hollow cavities at the dorso-lateral sides of the gut, which is here much more fully developed, occupying most of the space enclosed by the epiblast. Still farther backward the cavities in these two mesoblastic tracts close up, and their walls are continued for a short distance as two solid cords of cells, and then disappear.*

“The mesoblast of the anterior body cavity is, therefore, formed directly from the walls of the hypoblast, which occupied the same situation. It is separated off from it by a process of constriction in the region of the external groove, dividing the proboscis from the collar. While this process of constriction is being carried out, *the pouch of mesoblast grows backwards, surrounding the gut except on the ventral surface, but especially forming the hollow horns.*” (Italics mine.)

The description which I have just quoted agrees substantially with my own observations of the origin and first stages of development of the mesoblast in its relation to the anterior body cavity. The portions of his description of the lateral backward growths of the mesoblast which I have italicized is almost an exact account of the stage which I have shown in fig. 26, and I believe must correspond to it. As I have shown, the mesoderm already growing backwards continues to do so at the sides, finally *forming the middle and posterior body cavities.* This conclusion is based on an examination of a large number of sections (about one hundred and fifty series of sections), a typical series of which I have shown in my figures. *Nowhere have I seen any indication of a communication between any one of the middle or posterior cavities with the enteric canal.* Neither have I seen any evidence of delamination of the enteric wall. Throughout all the stages, from the two-walled cylinder to that of the fully formed body cavities, the walls of the enteron in the region of the middle and posterior body cavities show no histological difference from other parts of the wall. Whereas the cells bounding these cavities are very different from the cells making up the wall of the enteron.

Concerning the connection between the middle body cavities and the archenteron Bateson '84 (p. 220) says: “This condition is only visible in a very few of the larvae, and may possibly be

due to the action of reagents. Since, however, the middle mesoblastic tracts in *Tornaria* are said to be archenteric diverticula (Spengel, etc.), it seems more likely that the rarity of their occurrence is due to the shortness of the time for which they are present."

Of the connection between the posterior body cavities and the archenteron he is more certain. He says (p. 221): "These mesoblastic pouches open by large foramina into the lumen of the gut." No such large foramina exist in any stage that I have studied in *D. pusillus*. Although Spengel '77, several years before Bateson's paper, held that the middle mesoblastic tracts in *Tornaria* are "archenteric diverticula," he later modified this view, for he says (Spengel '94, p. 431): "dass die beiden Cölome jeder Seite aus einer gemeinschaftlichen Anlage hervorgehen, die später in zwei Theile zerfällt."

I cannot but think that Bateson failed to notice the connecting links between the mesoderm of the anterior body cavities and that of the other cavities, for his observations of the early backward growth of the mesoderm, as well as of the cavities after being formed, agree with mine. Indeed, his fig. 36 shows a communication, or at least a close connection, between the middle and posterior body cavities just as I have shown in my fig. 28. The only essential difference between his figure and mine will be seen to be in the definite line which he has drawn separating the anterior from the middle body cavity. Such a separation does not occur in my sections of the same stage.

Furthermore the archenteric walls in the region of the middle and posterior cavities at no time show any evidences of outpocketing. If these cavities were outgrowths of the archenteric wall one would expect to find evidences of this growth in the disposition of the cells of these regions. In the early stages of the anterior cavity the cells of the portion of the archenteron giving rise to it are irregular both as to shape and arrangement. The same condition is observed at the blastopore at the time of its closure. But no such irregularity is ever noticed in the archenteric walls, where according to Bateson the middle and posterior outgrowths occur.

Morgan '94 does not accept Bateson's account of the origin

of the second and third body cavities as will be seen from the following (p. 70): "The collar cavities are said to arise by a pair of lateral evaginations from the archenteron. For the present I accept this account of the origin of the second pair only tentatively. The evidence furnished by Bateson does not seem to me conclusive for accepting his statement. I think the phenomena could be explained by a process of delamination or migration, and a subsequent opening (or perhaps the small openings are artifacts)."

If one may judge from his descriptions and figures, Bateson relied in his studies mainly upon transverse sections, whereas the relation of the cavities to one another may be more certainly followed on horizontal longitudinal sections. But these suggestions hardly suffice to harmonize our observations. The other alternative is that *B. kowalevskii* and *D. pusillus*, though so much alike in most respects, differ widely in the origin and development of their middle and posterior body cavities. But this is hardly probable.

Gland cells. One characteristic of the Enteropneusta is the presence of numerous unicellular glands in the epidermis. Allusion has already been made to the very active secretion of these glands which occurs in period III. I have also called attention to certain large round cells which make their appearance on the periphery of the blastula.

Those that happen to be on the endodermal portion of the blastula are invaginated during gastrulation. They persist a while in the endoderm, but I was unable to determine their subsequent fate.

The cells which are in the ectoderm increase in number throughout the subsequent stages. During the latter part of period I some of these are made deep blue by Mallory stain, indicating the mucous character of their contents.

In period II they become numerous in the collar region, and at the two extremities. Some are empty; others are full of secretion.

In the early part of period III the number in the collar region is so great as to form an almost continuous layer. Here, in Mallory-stained sections, the collar presents a deep blue border,

with here and there light spots indicating the position of cells from which secretion has been discharged.

OCCURRENCE OF HALF EMBRYOS AND DOUBLE EMBRYOS.

The blastomeres of the early cleavages are loosely held together. This fact may account for the appearance of various small embryos. No careful study was made of them; indeed a great many were destroyed or not preserved. From the few notes I made the following may be of interest, particularly in view of the recent studies in the development of egg fragments.

The occurrence of what may be called half embryos was noted in several stages. The earliest was where two blastomeres of the four-celled stage had been destroyed. The next stage observed consisted of eight blastomeres. This stage corresponds to the third cleavage except in the smaller size of the blastomeres. Another stage was an early blastula about half normal size. Within the egg capsule were the disintegrating remains of a blastomere, probably the companion of the one that had developed. The latest stage observed was one where the ciliary band had appeared. It was small but otherwise normal. In the egg capsule was a disintegrating blastomere.

The occurrence of double embryos was observed in a few stages, but none later than the blastula. The embryos seemed normal except in size.

These observations would indicate that isolated blastomeres in *D. pusillus* may develop into embryos, as they are known to be able to do in *Amphioxus* and some other animals.

COMPARISON OF *D. PUSILLUS* WITH *AMPHIOXUS* WITH REFERENCE TO ORIGIN OF BODY CAVITIES.

Since Bateson called attention to certain points of similarity between *Balanoglossus* and *Amphioxus* various attempts have been made to further homologize the two animals. In these discussions one point has been made much of, especially by MacBride '98, *viz.*, the similar origin of the body cavities. He says: "The mesoderm originates in *Amphioxus* as a series of true gut

pouches, viz., one anterior unpaired pouch and two pairs of lateral pouches. Of these, the first divides to form the two head cavities; the anterior pair give rise to the first pair of myotomes, and in addition to two long canals extending back ventrally; the posterior pair are gradually separated from the gut, and pari passu divided into a series of myotomes. The whole process of mesoderm formation is therefore referable to the type found in *Balanoglossus*, the main difference being that the pouch corresponding to the trunk coelom of *Balanoglossus* becomes segmented." Pp. 606-607.

He reproduces, though somewhat modified, the diagram which Bateson '84 (fig. 40) used to illustrate the body cavities of *Balanoglossus*. When seen side by side, Bateson's diagram of the body cavities of *Balanoglossus* and MacBride's diagram of the body cavities of *Amphioxus* are strikingly similar. If one were to judge by these the homology of the cavities of the two animals would seem to be well established.

Recently MacBride's work has been questioned. Cerfontaine, '05, says of MacBride's contention that in *Amphioxus* the mesoblast occurs in five diverticula: "Je dirai dès à présent, que, dan mes nombreuses préparations je n'ai jamais vu, quoi que ce soit, qui puisse avoir des rapports avec une semblable évolution du mésoblaste chez l'*Amphioxus*, et je dois encore une fois répéter que certaines figures, qui accompagnent le memoire de MacBride dénotent, évidemment, une mauvaise conservation du matériel." p. 364. "L'ébauche du mésoblaste, existe sur tout le pourtour l'ébauche notochorde. Cette ébauche du mésoblaste, au moment de l'achèvement des phénomènes de gastrulation, presente, avec l'orifice d'invagination des rapports tels, qu'on doit distinguer chez l'*Amphioxus*, mangré l'absence des cellules polaires de Hatschek, un mésoblaste gastral et un mésoblaste prostomial." p. 389. With such a disagreement as to the origin of the mesoderm in *Amphioxus* it would be difficult to homologize the body cavities of this animal and those of *Balanoglossus* even if Bateson's account of the latter be accepted. But the difficulty grows into an impossibility if I have made my case as to the origin of the cavities of *D. pusillus* and as to the improbability that they arise differently in *D. kowalevskii* from what they do in *D. pusillus*.

If we are looking for types having a development of body cavities similar to that found in *D. pusillus* we must take into consideration Annelids and Molluses. In both of these groups the general plan of mesodermal development as shown, for example, by Wilson, for Annelids and by Conklin for Molluses may be briefly stated as follows: The development of body cavities is from in front backwards. The mesodermal bands posterior to cavities already formed are undifferentiated and "new cell material continues to be formed here in the vicinity of the primitive mesoderm cells."

The development of the body cavities in *D. pusillus*, as I have shown, follows in a *very general* way the above plan. But I do not wish in making this comparison to suggest any homology. On the contrary, I wish rather to emphasize the great difficulty in the present state of our knowledge of establishing any homologies whatever for these structures.

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ABBREVIATIONS USED IN THE FIGURES.

- a. Archenteric cavity.
a.m. Anlage of mesoderm.
b.c. 1, 2, and 3: first, second, and third body cavities respectively.
ec. Ectoderm.
en. Endoderm.
ent. Enteron.
g.c. Gland cell.
m. Mesoderm.
m.f. Muscle fibers.
n. Nerve cord.



EXPLANATION OF PLATES.

PLATE 4.

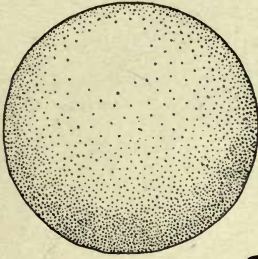
Fig. 1 shows adult animal partly in mud, with one end (anterior lifted and turned aside showing burrow and the eggs clinging to one side—the unbroken side of the burrow). $\times 1$, sketched from life.



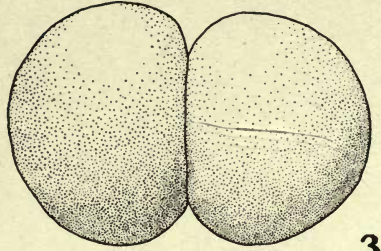


PLATE 5.

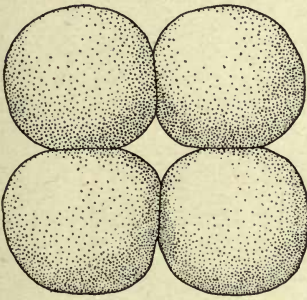
- Fig. 2. Unsegmented egg, actual size 264μ
- Fig. 3. First cleavage, greatest diameter 420μ
- Fig. 4. Second cleavage, greatest diameter 420μ
- Fig. 5. Third cleavage, greatest diameter 360μ
- Fig. 6. Fourth cleavage, greatest diameter 360μ
- Fig. 7. Fifth cleavage. Cleavage pore shown, 384μ
- Fig. 8. Sixth cleavage. Cleavage pore shown, 384μ
- Fig. 9. Seventh cleavage, 360μ



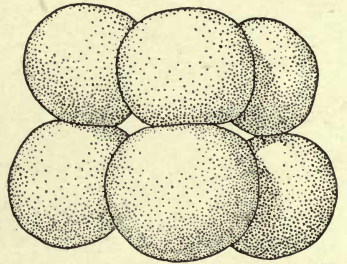
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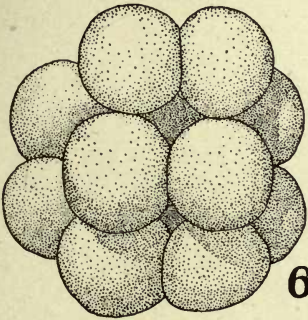
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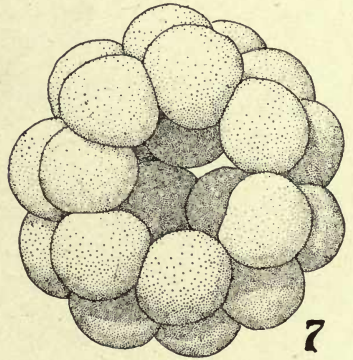
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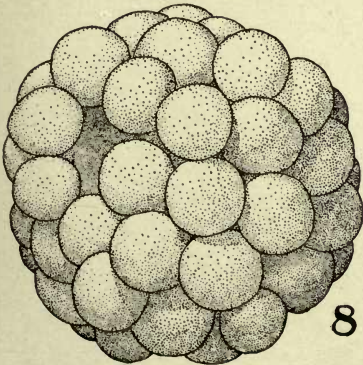
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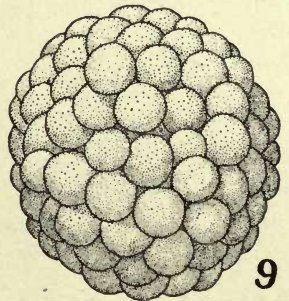
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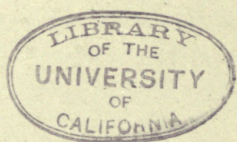
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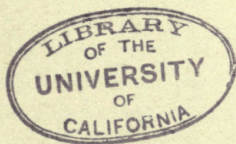
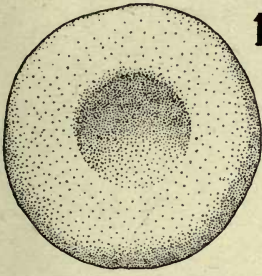
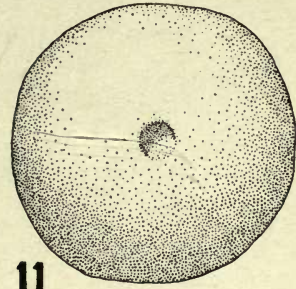
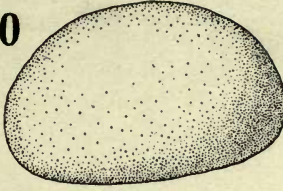


PLATE 6.

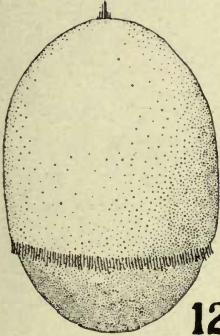
- Fig. 10. Gastrula, early stage. (a) Looking toward blastopore. (b) Side view, 300μ
- Fig. 11. Gastrula, late stage, looking toward the blastopore, 300μ
- Fig. 12. Period I soon after appearance of ciliary band, long axis, 300μ
- Fig. 13. Period I soon after appearance of groove, long axis 336μ
- Fig. 14. Period I at time of hatching, 384μ
- Fig. 15. Period II, latter part, long axis 384μ (partly contracted).
- Fig. 16. Périod III, second phase, long axis 540μ (average).



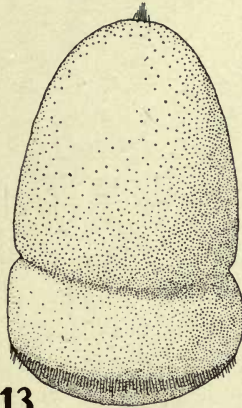
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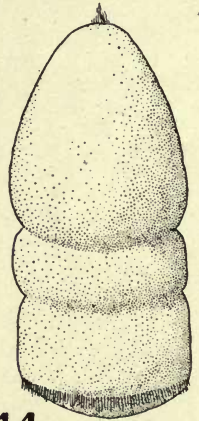
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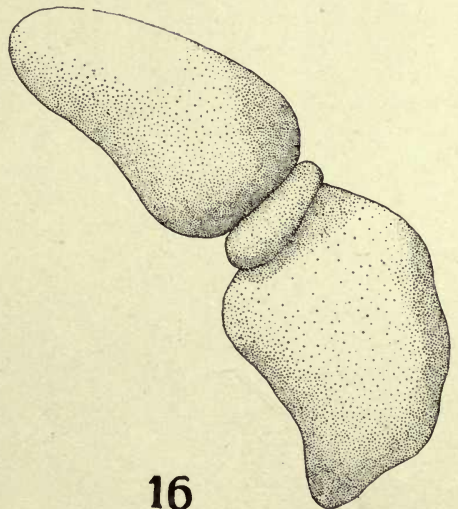
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16

PLATE 7.

Fig. 17. a. b. c. Diagrams showing escape of larva from egg capsule. × 190.

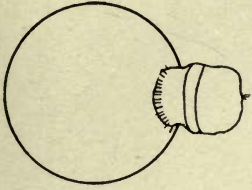
Fig. 18. Section of blastula. × 190.

Fig. 19. Section of gastrula, early stage. × 190.

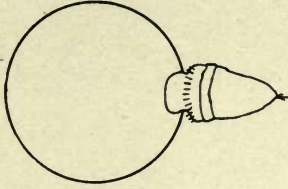
Fig. 20. Section of gastrula just before closure of blastopore. × 190.

Fig. 21. Section of larva immediately succeeding closure of blastopore. × 190.

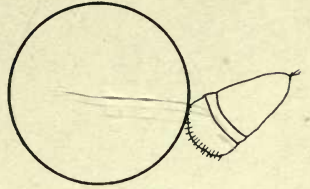
Fig. 22. Sagittal section of larva at the beginning of two-walled cylinder stage, showing rapid growth of cells in archenteric wall. × 190.



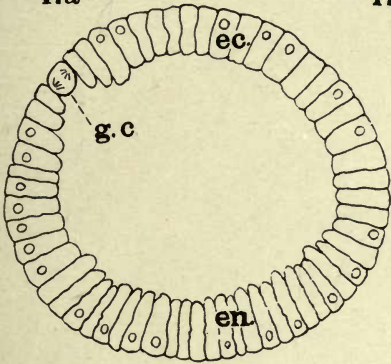
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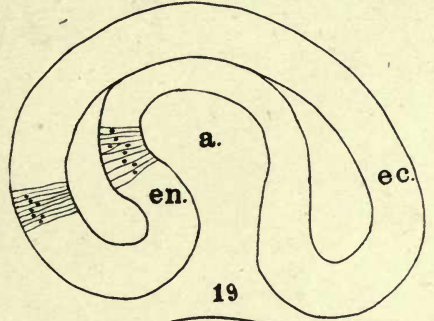
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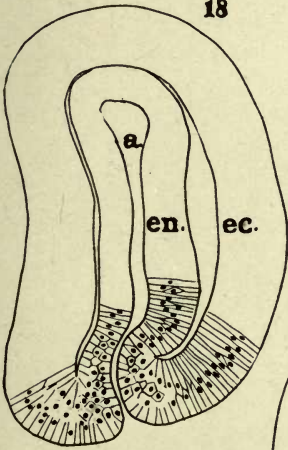
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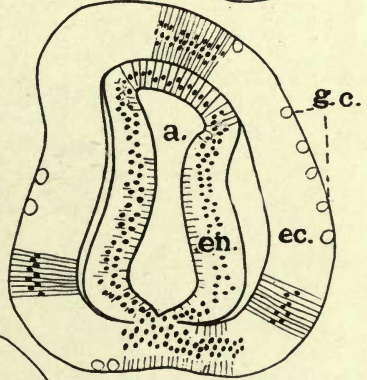
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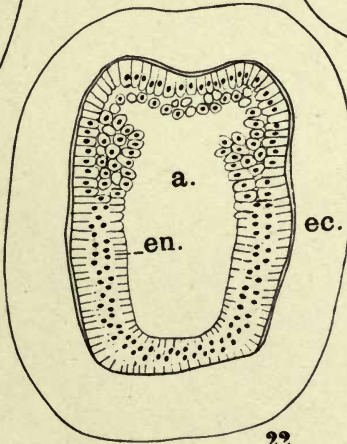
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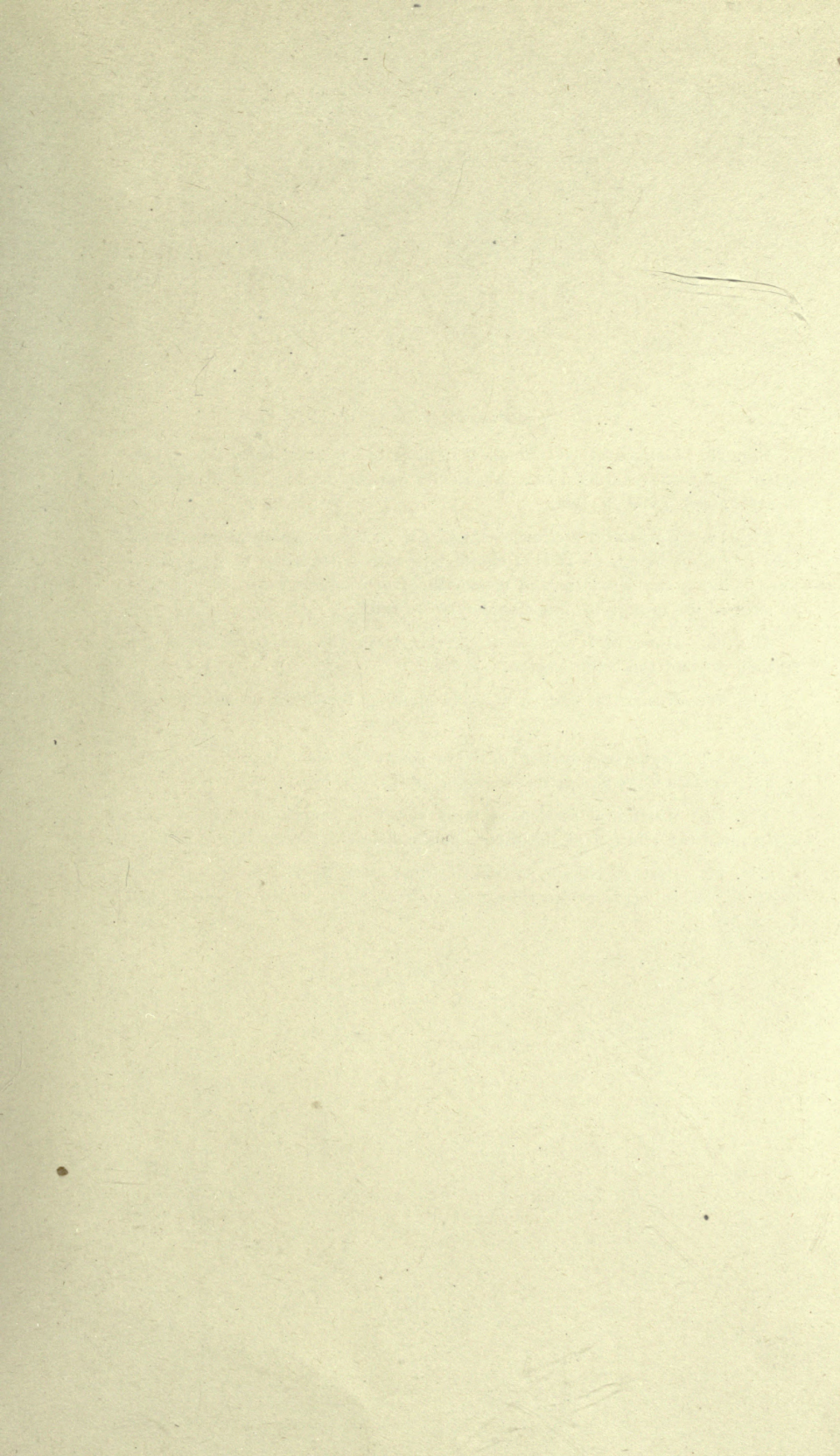


PLATE 8.

Fig. 23. Horizontal section of somewhat older larva than that represented in fig. 22. Here further irregularity appears among cells at anterior of archenteric wall. $\times 190$.

Fig. 24. Horizontal section of larva. Here the irregular disposition of cells indicated in fig. 23 is still shown but with a tendency to grow backward between the ectoderm and endoderm or archenteric wall. This wedge of cells is the anlage of the mesoderm. $\times 190$.

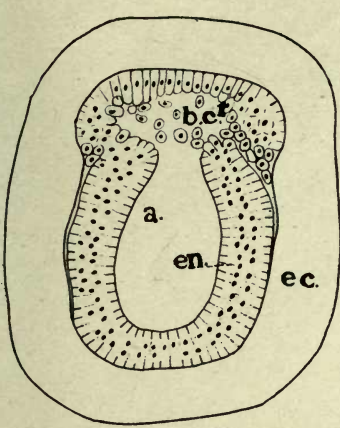
Fig. 25. Horizontal section of larva. Here the wedge of cells has reached beyond the collar region. $\times 190$.

Fig. 26. Horizontal section of larva showing formation of second body cavity. $\times 190$.

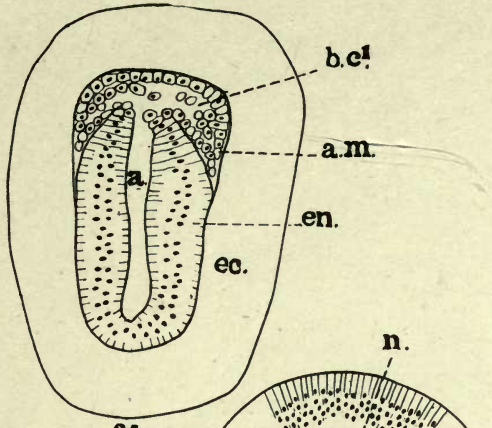
Fig. 27. Transverse section of larva somewhat older than that figured in 26. Section cuts across second body cavity. $\times 190$.

Fig. 28. Horizontal section of larva showing formation of third body cavity, and the relation of the three body cavities to one another. $\times 190$.

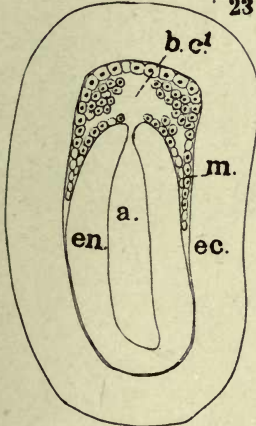
Fig. 29. Part of similar section of same larva figured in 28 but more enlarged. This figure shows communication between cavities 1 and 2, and 3. $\times 300$.



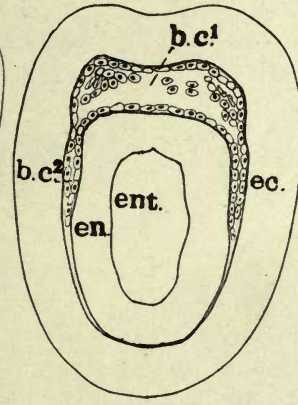
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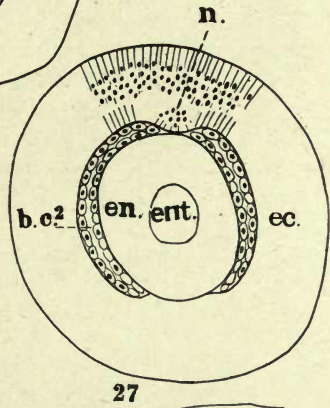
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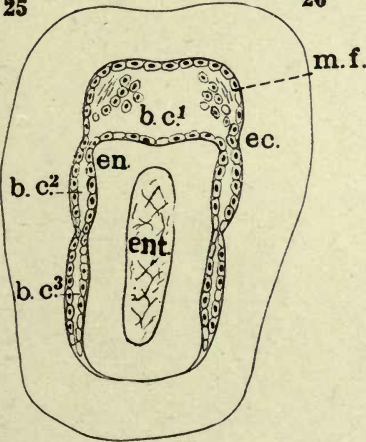
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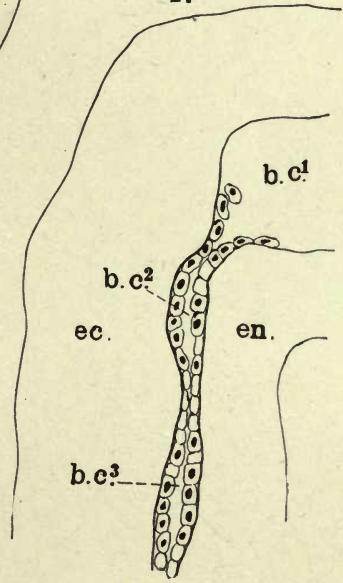
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