

FORMATION OF THE GERM LAYERS IN THE
AMPHIPOD MICRODEUTOPUS GRYLLO-
TALPA COSTA.

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THE interesting results which Dr. McMurrich obtained from a study of the cytogenesis of the isopods led him to suggest to me that I should undertake the study of amphipod development from the same standpoint.

This investigation was begun under Dr. McMurrich's direction in the summer of 1893, at the Marine Biological Laboratory, Woods Holl, Mass., was continued there during the two following summers, and was completed during my term of the Biological Fellowship at Bryn Mawr College, in the winter of 1895-96. I wish here to express my thanks to Dr. McMurrich, as well as to Dr. Whitman, Director of the Marine Biological Laboratory, and to Dr. Morgan, Professor of Zoölogy, Bryn Mawr College, for their kind interest and assistance.

Up to the present time observations which have been recorded upon the segmentation stages of the amphipod ovum have been made only upon the living egg. For a historical sketch of the literature I refer the reader to the *Monograph upon the Amphipods*, by Della Valle ('93). The only paper upon the subject which has appeared since then was published in December of the same year by Bergh ('93), who calls attention to the interesting rotation of the embryo upon the egg, and points out the necessity of studying the whole cleared egg before sectioning it. The paper does not go into detail, being intended rather as a suggestion for future work than as an exposition of amphipod development.

My observations were made upon the egg of a small marine amphipod, *Microdeutopus gryllotalpa* Costa, which lives in shallow water among decaying seaweed. This species is widely distributed, being found on the coast of New England as well

as on the European coast from Norway to the Mediterranean.¹ It is most plentiful and accessible, and, as I was able to collect a complete series of embryos, it proved a very favorable species upon which to work. For a description of the animal I refer the reader to Della Valle ('93), who places it in family IV, Corophiidae. To this same family belong also *Amphitoë* and *Sunamphitoë*, whose development has been described by Mlle. Rossiiskaya ('91). Judging from the resemblance of her figures to those I have of *Microdeutopus*, the modes of development of these species must be almost identical; our interpretations, however, differ widely.

The Corophiidae are placed in the sub-order Crevettina together with the Gammaridae and the Orchestiidae (Leunis), families whose development has received the most attention. *Microdeutopus* itself was studied by Della Valle. In his introduction to the embryology he says he studied not only the eggs of *Orchestia* and *Gammarus*, which he figures and describes, but also amphipods of other families, especially *Microdeutopus gryllotalpa*, as control observations. Della Valle is convinced from his studies that, on the whole, there is no essential difference in the development of these groups. There are many points in his description which I am not able to bring into harmony with what I found for *Microdeutopus*, but I shall defer the discussion of these points until I have given my own results.

Methods.

Microdeutopus lives in shallow water among decaying seaweed. By taking small portions of the seaweed at a time and squeezing them the animals came out of hiding and could be easily caught. They were then placed in glass dishes with fresh salt water, and kept in captivity for several days. It is very difficult to catch the animals with eggs in the early stages, though why this should be so I cannot tell. Quantities of females with the eggs twenty-four hours old could be found

¹ Prof. Sidney I. Smith, of Yale University, who very kindly identified the amphipod, made the statement about its distribution in a letter to Dr. McMurrich, to whom I am indebted for the information.

each day; but, although the seaweed was carefully searched, the number of eggs in the segmentation stages found in the material brought in on one day was out of all proportion to the number of eggs twenty-four hours old found in the material brought in the next day from the same place. The animals do not hide in the mud during the early period of development of their eggs, because the bottom of the pool in which they were collected was composed of a black refuse, which gave off so much marsh gas that the animals could not live in it. By carefully watching those kept in captivity, I observed that when there was a moulted amphipod shell floating at the surface of the water, an animal which had just deposited its eggs was almost always to be found in the dish, and in this way the early stages were obtained.

The females were caught and firmly held with a forceps while the eggs were removed from the brood pouch with a dissecting needle. The eggs were then killed in a modification of Kleinenberg's picro-sulphuric solution, in which sea water was substituted for the ordinary distilled water. This solution gave better results than the ordinary Kleinenberg killing fluid, which distends the egg. Corrosive sublimate, as suggested by Della Valle, also distends the egg and injures the protoplasmic structure. The living egg contains a fluid substance which exudes into the space between the surface of the egg and the chorion as soon as the egg is killed. This fluid substance coagulates and is stained by haematoxylin, the stain, however, being extracted by the acid alcohol before the protoplasm is decolorized. I could find no killing fluid which would prevent this exudation. Hot corrosive sublimate, Perenyi's, Flemming's, and Kleinenberg's fluids, alcoholic picro-sulphuric acid, and hot water all affected the egg, in this respect, in the same way. With the modified Kleinenberg solution the eggs shrink considerably, but the parts are not distorted, and good, clear nuclear figures were always obtained. The protoplasm showed no abnormal vacuolization, such as occurred when corrosive sublimate was used.

The chorion closely invests the fresh egg, but in the killed specimen there is a large space between it and the surface of

the egg. It was, therefore, found advisable to dissect off the chorion, since it collapses when the egg is placed in oil of cloves, and the resulting folds in it are easily mistaken for cleavage furrows.

The eggs were overstained in Kleinenberg's or Delafield's haematoxylin, washed out with acid alcohol, dehydrated, cleared in oil of cloves, and mounted under a cover slip supported by wax feet. By pushing the cover slip from side to side the eggs could be rolled into any desired position while they were being studied. Mlle. Rossiiskaya attempted to work upon the whole egg viewed as a transparent object, but says that she met with no success. I found that, unless a strong condensing lens is used, it is, as Mlle. Rossiiskaya ('88) says, almost impossible to distinguish the cellular structure, but with the condensing lens the cells can be distinctly and clearly seen. These same eggs which had been studied *in toto* were then imbedded in paraffin and sectioned. It was not possible to cut the segmentation and early blastoderm stages thinner than 10 μ , because the protoplasm at this time is so distributed that it does not seem to offer sufficient support to the yolk. Sections of the later stages were cut 5 μ thick, and preferably stained upon the slide. Eggs of the third day and after, when the yolk was partly digested, were stained with a $\frac{1}{2}\%$ aqueous solution of haematoxylin and washed out in iron alum, according to the usual iron-alum method. This stain could not be used for the earlier stages, because the undigested yolk becomes very black and totally obscures the structure of the egg.

I tried to orient the eggs for sectioning according to Patten's method. They were so small, however, that the least amount of celloidin which would hold them to the paper formed a coat over the eggs so that the paraffin did not penetrate. Knowing the relative position of the dorsal organ with respect to the rest of the embryo, from a study of the cleared egg, it was not difficult to orient sections.

Segmentation.

A complete account of the cell genesis of the amphipod egg has never been published. The segmentation has been followed only on the living egg. The most complete account was published by Van Beneden and Bessels in 1869, who carry their observations up to the time when the blastoderm begins to appear upon the surface of the egg. Other authors merely state that the segmentation is total; that the third cleavage plane divides the egg unequally; that after the 32-cell stage the segmentation becomes irregular; that just before the blastoderm appears on the surface of the egg the difference in size between the micromeres and macromeres is lost; and that the protoplasm rises to the surface and the cells migrate toward the micromere pole to form the blastoderm. In the present work the segmentation was followed on the living egg as far as the 80-cell stage, and eggs in all stages of development were also studied as transparent objects. The drawings were all made from stained and cleared eggs, which were afterwards imbedded in paraffin, cut as described above, and studied in section.

I have never seen the process of fertilization in *Microdeutopus*, but I have caught many pairs of a closely allied amphipod in the act of copulation. In these forms the male rests upon the dorsum of the female, clasping her with the large chelae. He probably assists the female to slough. Sometimes the sloughing takes place soon after the animals have come together, and sometimes I have seen them united for days before the female sloughed. Shortly before she sheds her shell the male leaves her, and as soon as it is shed he returns, and the animals unite in the same way that they did before the sloughing took place. They remain together a short time and then separate, and shortly after the male has left for the second time the eggs are extruded. I do not know how nearly the process of fertilization in *Microdeutopus* agrees with what I have observed for the other amphipod, but a female of *Microdeutopus* which had not sloughed, but whose eggs were just in a condition to be extruded, was isolated. After a time she sloughed, and the eggs were extruded in the

normal way; but the eggs, although apparently quite normal, evidently were not fertilized, because they did not segment. I concluded, therefore, that in *Microdeutopus*, as in the other amphipod, fertilization takes place between the time of sloughing and the time of extrusion of the eggs. It was also observed that when a moulted amphipod shell was found floating at the surface of the water a female which had lately extruded her eggs was almost always in the dish.

When the eggs are first extruded into the brood pouch they are of a bright opaque green. The chorion closely invests the egg, but no other membrane could be seen either in the fresh specimen or in the sections. The eggs seem to be covered with some sticky substance, which causes those coming from a single ovary to cling to one another; but the groups of eggs from the two ovaries are separate. This substance is subsequently either absorbed or loses its sticky properties, because after the first cleavage the eggs separate readily as soon as they are removed from the brood pouch. The protoplasm is found at the center of the egg. It is irregular in outline, sending out long pseudopodia-like prolongations, which ramify throughout the egg, very much as Dr. McMurrich ('93) has shown to be the case in the egg of *Jaera*. No protoplasmic layer could be seen around the periphery of the egg, however; if it is there, it must be very thin. Fig. 19, which represents an egg passing from the 2-cell into the 4-cell stage, shows the manner in which the protoplasm ramifies throughout the yolk mass. The nucleus is found in the center of the protoplasmic area.

The segmentation in the early stages is total, but not equal; later it is superficial. The protoplasm loses its control over the inner ends of the blastomeres as it moves nearer the surface in the succeeding divisions, and the inner ends of the blastomeres fuse, so that the blastocoel, which is at first present, is obliterated.

2-cell stage. — About three hours after the eggs have been extruded into the brood pouch, the protoplasm in the center of the egg divides, the nuclear spindle lying in the long axis. After the two halves of the central protoplasm have separated a furrow appears at the surface of the egg and gradually deepens,

dividing the egg into two equal parts. The two blastomeres then flatten against each other, and the living egg presents the same form as it did before cleavage. I have never seen the blastomeres unequal in size at this stage, as Van Beneden and Bessels and Della Valle describe for the gammarids.

4-cell stage. — One hour elapses before the completion of the second division. Fig. 19 represents an optical section of an egg which was killed half an hour after the first division. The protoplasm has almost divided and the second cleavage furrow has begun to appear. This cleavage plane makes an acute angle with the first plane, giving rise to two small and two large blastomeres, the smaller blastomeres being even less than one-half the size of the larger ones, as shown in Fig. 1. When the egg comes to rest the two large cells flatten against each other, pushing the smaller ones apart in such a way that one lies above and the other below the plane of the equator, this plane being supposed to pass through the long axis of the egg and at right angles to the first and second cleavage plane; *i.e.*, in Fig. 1 it lies in the plane of the paper. No rotation of the blastomeres, as described by Wagner for *Melita* ('91), has ever been observed; the blastomeres always flatten against one another without changing their position. I always find, at this stage, two cells smaller than the other two, and not three of the same size and one somewhat smaller, as in the gammarids described by Van Beneden and Bessels ('69) and Della Valle ('93). Sometimes one of the two smaller blastomeres is larger than the other small one, but never as large as the large ones.

For convenience in describing the later stages, I shall name the larger cells *AB* and *CD*, the small cell above the equator *EF*, and the remaining one *GH*.

8-cell stage. — At the end of the fifth hour an equatorial furrow divides the egg into four micromeres and four macromeres (Fig. 2). The four micromeres bear to each other the same relation in size as the four macromeres, and the larger micromeres are smaller than the smaller macromeres. When the egg comes to rest the two large macromeres flatten against each other, and likewise the two large micromeres, while the two small macromeres and the two small micromeres are forced

apart. The larger micromeres stand just above the larger macromeres, and the same holds true for the smaller micromeres and macromeres. In the figures the macromeres and their descendants will be designated by the large letters, and the micromeres and their descendants by the small letters. The macromeres *AB*, *CD*, and *EF* and their descendants are drawn in red ink, while *GH* and the micromeres are in black.

From this time the *macromeres always divide before the micromeres*, and the larger macromeres before the smaller ones. The subsequent cleavage planes which divide the two larger macromeres are alternately meridional and equatorial, while the planes dividing *EF* and the micromeres are always meridional. This may be due to mechanical causes; since these blastomeres, lying upon the larger ones, are somewhat flattened, as shown in Fig. 25, the spindle would find more room in the horizontal than in the vertical plane.

16-cell stage.—After the sixth hour two vertical cleavages at right angles to each other give rise to *A, B, C, D, E, F, G, H, a, b, c, d, e, f, g, h* (Figs. 3, 4). When the egg comes to rest *A* and *C* flatten against each other, but *B* and *D* are forced apart by *G* and *H* (Fig. 3). It is to be noticed that the zone of small cells lies obliquely over the oval egg. Mlle. Wagner ('91) finds the same oblique zone of small cells in *Melita*, but the obliquity was, in that form, brought about by a rotation of the cells in the 4-cell stage. Her account agrees in part with the results of Van Beneden and Bessels ('69), whose Figs. 10, 11, and 12 show exactly the same oblique arrangement. In the text Van Beneden and Bessels make no mention of this oblique zone of cells, but in their Fig. 9, which represents the 8-cell stage, the cells are arranged symmetrically with reference to the long axis of the egg, while in their Fig. 10, where the macromeres are just beginning to pass into the 16-cell stage, the oblique position of the micromeres is manifest; therefore, it would seem that a rotation occurred just at this time. In *Microdeutopus* the obliquity is occasioned by the angle which the second cleavage plane makes with the first (Fig. 19).

22-24-cell stage. — Figs. 5-8 show four views of an egg of the 22-cell stage, Fig. 5 representing the macromere pole of the egg. In this stage *A*, *B*, *C*, *D*, *G*, and *H* have given rise to *A^a*, *A^b*, *B^a*, *B^b*, etc., by an equatorial cleavage (Fig. 8), while *E* and *F* are still in the act of dividing and the micromeres show no trace of division.

30-cell stage. — Figs. 22-24 show three views of an egg of thirty cells passing into the 42-cell stage. The cells *E* and *F* have given rise to *E^a*, *E^b*, *F^a*, and *F^b* (Fig. 22). The cells *a* and *c*, *b* and *d* have all divided, forming *a^b-a^a*, *c^a-c^b*, and *b^b-b^a*, *d^a-d^b*, while *g^a* and *h^a*, *x* and *y* are the descendants of *g* and *h*. Instead of the division being by an equatorial plane in these cells, as it is in the macromeres, and as Van Beneden and Bessels ('69), Rossiiskaya ('90), Della Valle ('93), and Ulianin ('81) found it for the micromeres of other amphipods, it is vertical and at right angles to the last plane of division of these cells, which also was vertical (Figs. 3, 4). That the division of the micromeres at this time is vertical and not equatorial in *Microdeutopus* is shown by the spindles in *g* and *h* (Fig. 11). I have also seen spindles in *b* and *d* lying in the equatorial plane and at right angles to those shown in *g* and *h*; i.e., in the direction of the arrows in the cells *b* and *d* (Fig. 11). Figs. 11, 12 show the protoplasm in *A^a*, *A^b*, *B^a*, *B^b*, *C^a*, *C^b*, *D^a*, *D^b* divided and the yolk deeply constricted; the nuclei of *G^a*, *H^a* in the aster, and of *g*, *h* in the diaster stage. In Figs. 24, 25 *G^b*, *H^b* are beginning to divide; in *G^a*, *H^a* the process is further advanced, and *g* and *h* have divided completely. The daughter cells of *g* and *h* I shall name *g^a*, *h^a*, *x*, and *y*, the *x*, *y* cells being those lying next to *b^b-b^a*, *d^a-d^b*. These are always present before *G^a*, *H^a* have divided, although *e* and *f*, the cells corresponding to *g* and *h*, upon the other side, show no trace of division until a much later period (about the 72-cell stage). It is interesting to note that the cells of the *EF* and *ef* groups divide later than those of the *GH* and *gh* groups. It may be that the *EF* cell corresponds to the smaller cell which Della Valle ('93) found to result from the second division in *Orchestia*. He describes this cell as lagging behind the others in development.

At this stage x and y appear smaller and at a lower level in surface view, as though they were being pushed inward (Fig. 13). Sections just after this time (Figs. 9, 10) show forty-three cells arranged around a blastocoel, within which, at one end, lie two cells x' , y' (Fig. 9). The nucleus in one of these cells may still be seen (Fig. 9); in the other it is no longer present. I have also sections of one egg (Fig. 20) showing forty-one cells arranged around a blastocoel, and two of the cells have spindles whose equatorial planes are parallel to the surface of the egg. (Only one of these is shown in the figure.) The question now arises, Do the *two cells which are, after this stage, ALWAYS found in the center of the egg*, go in by a division parallel to the surface, or are they x and y which have been pushed in during the later division of the other cells? Unfortunately, I was not able to tell whether the two cells dividing inwards (Fig. 20) were the cells g , h or not. Although I have cut a number of eggs passing from the 32-cell into the 42-cell stage, I have never seen spindles directed radially when x and y were present. I hoped by tracing the cells into the next division to determine whether x and y were pushed in; if these two cells in that place were wanting, they might be accounted for in this way. I did not succeed, however, because the descendants of G^a , H^a , and G^b , H^b take such different positions that it is impossible to be sure of the following generation. For instance, in one egg the arrangement was

$$\begin{array}{c} G^a H^a \\ G^{a'} H^{a'} \\ G^b G^b H^b H^b \end{array}$$

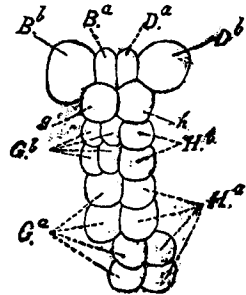
as shown in Fig. 23. In another

$$\begin{array}{c} G^a \\ G^{a'} H^a H^{a'} \\ G^b G^b H^b H^b \end{array}$$

while in a third it was

$$\begin{array}{c} G^a H^a \\ G^{a'} H^{a'} \\ G^b H^b \\ G^b H^b \end{array}$$

so that in the next stage I was not able to decide from their position whether g^a , h^a had divided again or whether certain of the other cells had divided. I did find, however, one egg with about sixty-four cells, in which the two cells next to b^b - b^a , d^a - d^b were as large as g and h are before dividing. The arrangement of the other cells is diagrammatically sketched in the cut. It was necessary to make a diagram, because all the cells could not be seen in the same field of view. In this one egg I did not find the two cells in the center. I cut sections to see if I could find spindles going in, but the critical section was completely broken. I know there were no cells in the interior for the reason that when they are present they can always be distinguished in optical section in the whole cleared egg.



After this time the two cells in the interior are found in various stages of disintegration. As late as the 112-cell stage, after the blastoderm has appeared on the surface of the egg, two deeply stained patches can still be seen in the interior. Shortly after this stage they disappear altogether, and no other cells are seen in the yolk until the egg is about forty-eight hours old (Fig. 39). Weismann and Ischikawa ('87) have described three secondary polar bodies in *Bythotrephes longimanus*, which are carried into the interior of the egg during the early segmentation stages. These polar bodies subsequently disintegrate, though as late as the 32-cell stage remnants of them could still be detected in the axial space between the blastomeres. Dr. Mead ('95) also has found that the polar bodies of *Amphitrite* are taken into the axial cells, where they are absorbed. These results of Weismann and Ischikawa and of Mead led me to suppose that the two cells found in the interior of the *Microdeutopus* egg were polar bodies. I therefore made a careful study of the eggs before the 32-cell stage. Since I found no cells which had not been derived from one of the two blastomeres of the 2-cell stage, I conclude that the cells in the interior cannot be polar bodies. In the literature which

I have seen I have found nothing to which I might compare these two cells. Dr. McMurrich, however, found two cells in the interior of an advanced isopod egg; but as they were seen only once, and no traces of disintegrating nuclei were found in the later stages, Dr. McMurrich supposed it to be an abnormality. It may be, however, as Dr. McMurrich suggested to me, that the two cells found in the interior of the isopod egg are comparable to those which I have described for *Microdeutopus*. Dr. Conklin has kindly permitted me to state that he, too, finds that two cells (the tip cells in one arm of the cross) in *Crepidula* are lost in the later stages. However, they are *not*, as I understand, absorbed, but thrown out. It would be interesting to know if these cells in *Crepidula* could be compared to those in *Microdeutopus*. Comparisons have been made between the amphipod and molluscs before (Ulianin '81), with how much right is still to be decided. But certainly the loss at an early stage of two blastomeres is very remarkable, and has as yet not been described for other forms.

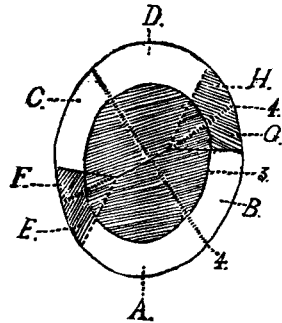
44-cell stage.—The blastomeres E^a , F^a divide, giving rise to E^{a1} , F^{a1} and E^{a2} , F^{a2} . The cleavage is parallel to the planes which divided E and F before. Fig. 21 shows an egg passing from the 44-cell stage into the 46-cell stage. The blastomeres E^{a2} and F^{a2} are dividing for the last time before they rise to the surface of the egg.

73-cell stage.—Figs. 14–16 represent three views of an egg of seventy-three cells. In this egg E^{a1} , F^{a1} , E^{a2} , F^{a2} , E^b , and F^b have all been divided by vertical planes at right angles to the last, and the micromere f has also divided in the same way. The protoplasm in these cells has come up to the surface, and the nuclei have become very large and clear. This is the first appearance of the blastoderm. The sixteen large macromeres have divided into thirty-two by an equatorial cleavage. a^b – a^a , c^a – c^b , and b^b – b^a , d^a – d^b have not changed, and there are seventeen cells derived from the gh and GH groups. The descendants of A and C lie next to each other and border upon the EF group, while the B and D groups are forced apart by the GH group. Sections (Fig. 26) show the protoplasm quite near the surface at this time, the cell boundaries breaking down at their

inner ends, and the blastocoel becoming obliterated. The section shows one of the two cells which have wandered in. Its nucleus appears as a dark, homogeneous mass, showing signs of disintegration.

106-cell stage. — The thirty-two large cells all divide once more, by vertical cleavages, into sixty-four. I should state here that, although I have always spoken of the descendants of the four large macromeres of the 16-cell stage as large, I have done so merely to distinguish them from the descendants of the small macromeres. Somewhat before the 73-cell stage, owing to their more rapid cell division, the difference in size has disappeared, and in the living egg, as stated by Mlles. Pereyaslawzewa ('88), Rossiiskaya ('88), and Wagner ('91), the macromeres and micromeres cannot be distinguished. In the cleared egg they are recognized by the different appearance of their nuclei and the smaller amount of protoplasm contained in the micromeres.

It will be recalled that in the 16-cell stage *A* and *C* were flattened against each other, while *B* and *D* were forced apart by *G* and *H*. Because of the obliquity of the second cleavage furrow and the flattening of the larger macromeres against each other, the group of small cells lies obliquely on the oval egg, as shown in the diagram. Since, as stated before, no change in the position of the cells takes place, their descendants have exactly the same position as the cells themselves originally had. So the *AB* group lies over the pole nearest the *EF* group, while the *CD* group lies over the opposite pole, and the *A* and *C* groups lie nearest each other and border the *EF* group. At this time the protoplasm of all the blastomeres rises to the surface (Fig. 17). The protoplasm of the macromeres becomes more concentrated, while that of the micromeres spreads over the yolk, thus making such a thin layer that only the dark nuclei remain visible (Fig. 18). Since the concentration of the



protoplasm of the macromeres is toward the *EF* group, the cells of the *AB* group, as can be seen by the diagram, would come to lie over the lower pole, whereas the cells of the *CD* group would be drawn away from the upper pole and lie on the side of the egg, and the whole ventral plate so formed would lie eccentrically over the oval egg. Figs. 17 and 18 show an egg at this stage. The eleven cells at *EF* are the descendants of the *EF* group, the twelfth cell of this group lying beneath the surface, as was shown by sections. The end cells of the *CD* group can be seen in Fig. 18 on the side of the egg, while the end cells of the *AB* group cover the lower pole. The cells of the *EF* group will form the head region and the dorsal organ. Bessels ('70) is inclined to believe that the dorsal organ arises exactly at the same point where the blastoderm first appears on the surface of the egg, but, according to my observations, it does not appear exactly at this point, but a little lower down, as the cells of the *EF* group, which are the first blastodermic cells to appear, spread during their growth. In sections of an egg at this stage cells are seen underneath the surface in the *EF* region, and I think these are the descendants of *ef*, because the sections of the egg represented in Fig. 17 show five cells beneath the surface in this region. One of these is, I think, the twelfth cell of the *EF* group, as only eleven were seen on the surface. It is only in this way that the *ef* group can be accounted for. It will be remembered that only four cells were derived from this group. Another reason which led me to this conclusion is that all the other micromere cells are overgrown as the embryo develops and come to lie in the lower layer.

Summary.

The first cleavage plane appears three hours after the deposition of the egg. The three succeeding divisions, vertical, equatorial, and vertical, occur at intervals of an hour each, and after this the large macromeres divide synchronously and regularly, a vertical cleavage alternating with an equatorial cleavage; but the micromeres no longer divide synchronously with the macromeres.

The *EF* group and the micromeres divide only vertically.

The ventral plate is formed by the descendants of the large macromeres and of the *EF* group, and has an oblique position upon the egg, owing to the obliquity of the second cleavage plane.

After the 42-cell stage two cells are found in the interior of the egg in different stages of disintegration.

Formation of the Embryo.

In the last section I have described the ventral plate as being formed by the macromeres on the lower pole of the egg. I am well aware that in this I stand alone, all previous writers describing the descendants of the micromeres as the first which rise to the surface. Further, they describe the embryo as forming over the micromere pole, and the macromeres as gradually added to the outer layer during the growth of the ventral plate over the egg. Della Valle, who studied *Microdeutopus* as a control observation, agrees in this point with what has heretofore been published. He describes the blastoderm as arising on the micromere pole in *Orchestia*, and makes no exception in the case of *Microdeutopus*. The results which Mlle. Wagner obtained for *Melita* agree most nearly with what I found for *Microdeutopus*. According to her account, when the cells emerge from the yolk they lie on the sides as well as on the oral pole of the egg, and later grow over the dorsal face. This corresponds almost exactly to what I have found. The figures 4, 5, and 6 of *Sunamphitoë*, by Mlle. Rossiiskaya, are almost identical to those I have for sections of eggs in the stage figured on Pl. XXVI, Fig. 17. Her figures show that the blastoderm probably arises in the same way as in *Microdeutopus*.

The *EF* group lies above the plane of the equator on the side of the micromeres, and the cells of this group are the first which appear on the surface to form the blastoderm. Previous investigators may have been led by this to suppose that the ventral plate forms on the micromere pole, but, having traced the development cell by cell on the stained and cleared egg, I

am convinced that *the ventral plate is formed from the descendants of the macromeres and over the macromere pole.*

The pole upon which the ventral plate is formed will become, as in all other Crustacea, the ventral side of the embryo. The cells after reaching the surface rapidly increase in number, and as the embryo grows backward over the egg it shifts its position, so that its long axis finally corresponds with the long axis of the egg. In Fig. 29 we can still see that one side of the ventral plate is a little nearer the posterior pole than the other side, the shifting at this time not having been completed. Fig. 35 shows an egg in which, for some reason, the shifting has not taken place, so that at this late stage the embryo is still oblique upon the egg. In Fig. 33, a stage which is a little later than that shown in Fig. 29, the ventral plate has grown almost over the posterior pole (Fig. 32), and the axis of the embryo lies parallel to the long axis of the egg.

The rotation of the embryo upon the egg has been described by Bergh ('93) for *Gammarus pulex*, in which the embryo lies at first parallel to the shorter axis of the egg, and rotates during development through an angle of 90° . Bergh said he could not explain why it arose in that position. Della Valle also figures the embryo as lying at first obliquely over the egg, then parallel to the short axis, and, lastly, parallel to the long axis. I could not make out, however, whether he supposed a rotation to take place, or whether he considered the short axis to be drawn out at the expense of the long axis. In *Microdeutopus* we have seen that the cause of the oblique position of the embryo upon the egg is due to the obliquity of the second cleavage plane.

In the stages represented in Figs. 33 and 34 the outlines of the cells are quite sharply marked off from the yolk. Their arrangement in definite lines is partly due to the fact that the blastodermic cells appear on the surface of the egg arranged in definite rows, on account of the regular cleavage of the two large macromeres. Yet this cause cannot hold good for the sides of the embryo, for in Fig. 28, the side view of an egg shown in Fig. 29, the spindles lie at all sorts of angles to each other, and the cells have at this time no definite arrangement,

although later they fall into line. In the isopods and in Mysis teloblasts give rise to regular rows of cells in the postnaupliar region. In *Microdeutopus*, however, I could find neither ectodermal nor mesodermal teloblasts. Bergh supposed that the regular arrangement of ectoderm cells, which is found in the amphipods, arose from some indistinguishable teloblasts, but I think this assumption is not necessary for *Microdeutopus*, since the regular arrangement is found also in the naupliar region (Figs. 27 and 31), where no teloblastic growth occurs.

The dark patches (*d*) in Figs. 28, 29, 32, and 33 represent cells below the surface, which, I think, have been overgrown by the ventral plate as it extends over the egg. In the isopods, as described by Dr. McMurrich, and in Mysis, as described by Bergh, the cells scattered over the dorsal pole are added to the ectodermal layer of the ventral plate as it grows over the egg, and with these facts in mind I searched carefully to see if this was the case with *Microdeutopus*. Further and careful examination only strengthened the view that in *Microdeutopus* the dorsal cells are overgrown and so form part of the lower layer. At a little later stage (Fig. 34) the head region has increased in extent, a few ectodermal cells are beginning to differentiate at the sides, and the edge of the ventral plate is even more sharply marked off from the yolk than in the preceding stages. In the next stage (Fig. 30) the patch of dark cells has become very characteristic in appearance. The dorsal organ (*d.o.*) has begun to differentiate, and the outline of the ventral plate is no longer distinct. It appears as though a layer of protoplasm had spread over the yolk, and through this layer the nuclei are found irregularly scattered. This appearance is due, I think, to the lack of definite cell walls, for I have never been able to distinguish them in *Microdeutopus*. In the earlier stages the cells seem to be "räumlich centriert," as Flemming expresses it; therefore, in eggs of stages represented in Figs. 29, 33, and 34, the cell boundaries can be distinguished, but in the later stages, where the cells become closely packed, cell outlines are lost. In eggs after the stage represented in Fig. 34, it seems as though the cells at the edge of the ventral plate have no longer the power to assume the spherical form, so making

the outline of the cell distinct, but their protoplasm spreads out over the surface of the yolk, as seen in section (Fig. 47, *ppl.*). As the cells in this region multiply, the protoplasm of one cell fuses with that of the surrounding cells (Fig. 47, *r.*), so that it appears as though a layer of protoplasm, through which the nuclei are scattered, was spreading over the surface of the egg, as shown in Fig. 30. The large cell (Fig. 30, *d'*) near the region of the differentiated cells lies below the two small nuclei which are imbedded in the protoplasmic layer. The *d'* cell is almost always seen in this position, even before the ventral plate has reached that point (Fig. 34). In this case, therefore, it is unmistakably one of the dorsal cells which has been overgrown.

As the embryo develops, the cells which are spreading dorsally arrange themselves in definite rows corresponding to the rows of cells of the ventral plate. During the second day the yolk is completely overgrown by the ventral plate, the edges of which meet at the dorsal organ. At this stage the dorsal organ has moved to the center of the dorsal pole of the egg, and is composed of large triangular cells, whose apices are at the surface of the egg; the antennules are clearly defined, and the appendages could be seen just beginning to form. Fig. 31 represents an egg of the second day. The appendages have appeared as a series of ridges on the ventral surface, gradually shading off dorsally, and it will be observed that the series extends over the posterior pole and dorsal surface of the egg, reaching almost to the dorsal organ (Fig. 36). The appendages are formed by the pinching off of from eight to ten parallel rows of cells, and while being pinched off each ridge includes some of the cells of the lower layer from which the mesoderm of the appendage develops. This mode of origin of the limbs and their musculature recalls what has been figured by Dr. Bumpus ('91) for *Homarus*.

The embryo now elongates, the area of growth being chiefly in the dorsal region, posterior to the dorsal organ, as shown in Figs. 36-43. By comparing these figures it may be seen that the distance between the dorsal organ and the last appendage increases in extent, in consequence of which a fold appears on

the ventral surface (Fig. 38, *abdf.*) in the region of the first abdominal appendage. Figs. 40-42 are three views of the same egg. At this stage the appendages no longer extend over the dorsal surface (*cf.* Figs. 36 and 40), although they still cover the lower pole of the egg (Fig. 41). The anlagen of the seven abdominal appendages are pushed inwards, and finally all lie in the abdominal fold (Fig. 43), whereas the space between the dorsal organ and the last appendage extends over the whole lower hemisphere of the egg.

The mode of formation of the abdominal fold in *Microdeutopus* differs widely from that in *Orchestia*, judging from the comparison of Figs. 36-43 for *Microdeutopus* with those of *Orchestia*, as figured by Della Valle ('93).

Entoderm.

The entoderm in *Microdeutopus* arises as a true invagination at the hind end of the embryo. During the second day, when the ventral plate has completely overgrown the egg, the cells immediately behind the dorsal organ invaginate. These cells migrate into the interior of the egg, and there arrange themselves to form the liver tubes and the greater part of the digestive tract. The entodermal invagination is shown in optical section in Fig. 37 (*en. inv.*), and Fig. 53 represents a transverse section passing through the center of the entodermal sac, while in Fig. 54, which represents a small portion of a sagittal section, the relative position of the entodermal invagination and the dorsal organ are shown. In this egg the cells of the dorsal organ have the characteristic bottle shape which is peculiar to them, but the ends of the cells still contain granular protoplasm; later they are filled with some clear, unstainable substance.

A section of an egg a little older (Fig. 55) shows the cells of the entodermal sac migrating into the interior as an irregular mass. These cells distribute themselves throughout the yolk area, the greater number, however, remaining in the region at which they entered. These are the first cells which appear in the center of the egg, excepting the two blastomeres

described above, which were absorbed. In eggs at about the stage represented in Fig. 33 I have seen cells in the anterior part of the head region which were apparently migrating off into the yolk area, as the one shown in Fig. 49, but these lay very near the surface, and in the stages shortly before the entodermal invagination had appeared cells were never seen in the yolk area. I conclude, therefore, that these were probably dorsal cells which were overgrown, and had not taken their final position at the time when the egg was killed. Cells which appear to be similar to these were described by Dr. Pereyaslawzewa ('88), who considers them to be entoderm cells derived from the ventral plate and migrating into the yolk area. I do not believe, however, that in *Microdeutopus* they take any part in the formation of the entoderm.

Ulianin ('81) finds a number of cells scattered through the yolk area which he supposes are derived from the dorsal organ, as at first they are found in that region. He leaves the origin of these cells an open question, however, as he was not able to secure a complete series of embryos, and, therefore, could not trace them to their source. The whole entoderm, according to Ulianin, is derived from these cells, and the lower layer cells of the ventral plate form mesoderm only. Dr. Pereyaslawzewa ('88) believes that the entoderm is derived from two sources, from the ventral plate and from the dorsal invagination, which she considers to be the dorsal organ, as the following passage states: "À mesure que l'organe dorsal se développe, l'ectoderme avoisinant s'épaissit visiblement et garde pour longtemps cette configuration, vu qu'il ne détache aucun organe nouveau. Ce rôle passif qui lui est propre, me permet de le comparer à la plaque dorsal chez les Insectes. La dissemblance consiste en ce que chez ces derniers la formation de la plaque précède celle du tube, tandis que chez les Crustacés nous remarquons le contraire. D'après les recherches de M. Korotneff sur le développement de *Gryllotalpa* les cellules qui dérivent en grande nombre de la plaque dorsal s'introduisent dans les masses nutritives et après les avoir élaboré de manière à les préparer pour l'assimilation, qui aura lieu, dans les cellules de l'intestine, elles se détruisent complètement.

Il est indubitable que chez les Gammarus et de plus chez les Orchesties l'organ dorsal, ainsi que l'ectoderme avoisinant, détachent de cellules; leur nombre n'est pas grand, elles sont tout à fait libérées et s'enfoncent dans le vitellus nutritif. Or, tandis que les cellules en question se logent dans les masses vitellines, les cellules entodermiques, d'une parfaite ressemblance avec les premières, sont aussi en voie d'y chevaucher; leur résidence simultanée dans le vitellus ne nous permet d'affirmer aucunement que les cellules issues de la plaque dorsale s'atrophient; aucune de mes préparations ne le prouve pas."

I think the plaque of cells from which, Dr. Pereyaslawzewa writes, no organ is derived is the dorsal organ, and what she calls the dorsal organ is the entodermal invagination. At a later stage a cavity is found in the dorsal organ in *Microdeutopus* which agrees, in this respect, with what Korotneff found in *Gryllotalpa*. The characteristic appearance of the dorsal organ cells led me to recognize the plaque of cells as the dorsal organ. Dr. Pereyaslawzewa, it would seem, occupies the middle ground between Ulianin ('81), who supposes the entoderm is derived from the dorsal organ alone, and Bergh, who derives all the entoderm from the ventral plate. Bergh does not hold that the entoderm is formed at many points on the ventral plate, as Dr. Pereyaslawzewa holds, to judge from her figures, but he supposes that "vielmehr entsteht dasselbe durch Einwucherung von Blastodermzellen an einer bestimmten Stelle die also dem Blastoporus entsprechen dürfte." In his figures Bergh marks the cells in the second layer of the ventral plate, in the naupliar region, *en*. My own results agree with those of Ulianin, who believes that *all* the cells in the second layer of the ventral plate give rise to mesoderm, and that *only* the cells carried in by the dorsal invagination form the entoderm. It would seem, then, that as far as the entoderm is concerned the amphipod agrees most nearly with what Bobretzsky has found to be the case in *Palaemon*, according to the statement of Heider.

Mesoderm.

At the time when the entodermal invagination takes place the mesoderm is completely laid down and the appendages have begun to pinch off, each ridge containing mesoderm cells, as described above and figured in Figs. 36, 37, and 55.

To judge from what takes place in the great majority of other Crustacea heretofore described, we should naturally expect to find the mesoderm arising from the region where the anterior lip of the entodermal invagination will be formed. In *Microdeutopus*, then, the extreme posterior end of the ventral plate would be the region where we should look for a proliferation of mesoderm cells, since that is the region of the entodermal invagination. However, the posterior end of the ventral plate is exactly the region where the smallest number of lower layer cells are found; in fact, except for the few dorsal cells which were overgrown, that region is composed of only one cellular layer, whereas the head region at the time is composed of two or even three layers, and patches of cells are found irregularly scattered under the ventral plate, decreasing in size and number as they approach the posterior end.

When the protoplasm rises to the surface of the egg to form the blastoderm the four cells of the *ef* group were found under the cells of the *EF* group. The dorsal cells, which are the descendants of the remaining micromeres, are overgrown by the ventral plate, and, subsequently, form part of the lower layer of cells. In Fig. 18 only the dark nuclei of the dorsal cells can be seen, their protoplasm being spread over a large surface, making such a thin layer that it cannot be distinguished. As the ventral plate grows over the egg the protoplasm of the dorsal cells concentrates around their nuclei, and the cells present the stellate appearance shown in Figs. 27 and 34. Fig. 46 shows a section of the head region of an embryo at the stage shown in Fig. 30. A large cell (*d*) is seen just below the edge of the ventral plate. The beginning of the yolk area is shown at *Y*, and this area was greater in extent in the next section, showing that the cell above the large stellate one is at the edge of the ventral plate. Fig. 48 also shows

a large, clear nucleus (*d*) below the outer edge of the ventral plate, while in Fig. 47 the protoplasm (*ppl.*) of a cell also at the edge of the ventral plate has spread out over the surface of the yolk for some distance, and a large stellate cell (*d*) is seen beneath it. I do not think that these cells arise by division from the ventral plate, as Della Valle ('93) supposes. I draw this conclusion because there is no cell above the one shown in Fig. 36 from which it could divide, and especially because these cells at this stage are very different from those of the ventral plate, and their nuclei are similar to those of the dorsal cells. It may be that they are analogous to the vitellophags of the isopods, which arise from the *D* cell, described by Dr. McMurrich. The dorsal cells of *Microdeutopus* and the vitellophags of *Jaera* resemble each other in three points: in that they appear during the segmentation stage, in that their nuclei have a characteristic appearance, and in that they are overgrown by the ventral plate. The vitellophags in *Jaera* also give rise to mesoderm. The dorsal cells in *Microdeutopus*, however, take no part in the digestion of the yolk. After the ventral plate has completely inclosed the egg the dorsal cells have lost their characteristic appearance, and they cannot be distinguished from the other cells of the second layer.

I have still to add that, to judge from the appearance of embryos like the ones shown in Figs. 29, 32, and 33, the cells of the *GH* group also are overgrown by the descendants of the *AB* and *CD* groups.

In the head region of the embryo, in the stages shown in Figs. 29 and 33, numerous cells are found in the lower layer,¹ whereas only a few large stellate cells (overgrown dorsal cells) are found under the ventral plate in the postnaupliar region. At a little later stage (Fig. 30) there are more cells in the lower layer of the postnaupliar region than could be accounted for by the division of the stellate cells. They are found in patches irregularly scattered throughout the region of the ventral plate. As I had a very complete series of embryos, I do not believe that

¹ The mesoderm cells are not represented in Figs. 27 and 31, because they were so numerous at these stages that they could not be well represented, and because the regular arrangement of the ectoderm would have been obscured.

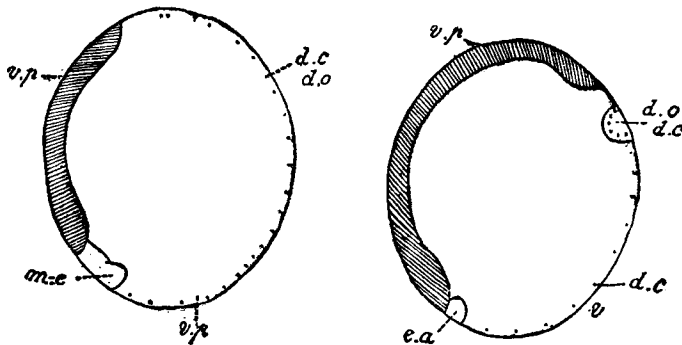
a plug of mesoderm cells arises at any one point, and that this stage had escaped my notice, but rather that in *Microdeutopus* the mesoderm is formed at many points in the ventral plate. I have a section of a cell dividing obliquely inwards in the anterior end of the head region, and Fig. 51 shows another case in which there are two cells in the aster stage. The equatorial plate of each spindle makes an acute angle with the tangent to the surface of the egg at that point. Had *A* divided, the greater part of one daughter cell would have lain in the second layer. In Fig. 52 we see a cell (*m*) which may have been derived from a cell like *h*, or it may be one of the cells of the outer layer drawn under the surface; from the appearance of the nucleus I am rather inclined to the latter view. I never have seen spindles whose axes were parallel to the radius of the egg in cells at the surface, although I have seen radially directed spindles in the second layer (Fig. 50). Cells which appear as though they were drawing in or had arisen by oblique division were found at any point on the ventral plate. I therefore conclude that part of the mesoderm in *Microdeutopus* is derived from the ventral plate.

When the dorsal pole of the egg has been completely overgrown by the ventral plate the ventral portion of the blastoderm, posterior to the head region, is composed of two layers of cells. With the digestion of the yolk by the entoderm cells, which begins during the third day, all the cells of the embryo rapidly increase in number. Cell boundaries are entirely lost at this stage, the protoplasm of the cells fusing and making it impossible to distinguish where one layer ends and the other begins. Since the nuclei of both ectoderm and mesoderm appear exactly alike, I could not tell whether the ectoderm was only one layer deep and the mesoderm many layered, or whether the ectoderm consisted of more than one layer. Towards the end of the third day numerous spindles are found in all the layers making any angle with the tangent to the surface. In sections of the fourth day (Figs. 59-64) the muscle cells show their characteristic striations.

Bergh ('93), in his paper on *Gammarus*, suggests that the cells of the muscle plates are derived from teloblasts, as he

found to be the case in Mysis. In *Microdeutopus* I find no evidence of a teloblastic growth. The mesoderm cells seen in optical section in the stained and cleared egg are irregularly scattered under the ventral plate.

Morphologically, the isopods and amphipods are very closely allied, and it is, therefore, all the more interesting to note how similar are their modes of development. If we compare a diagram of Jaera, after the germ layers have been laid down, with

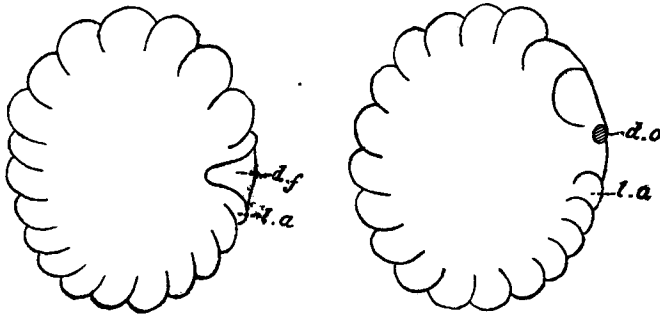


one of *Microdeutopus*, somewhat before the entodermal invagination has taken place, the similarity will at once become apparent (see diagrams).

Heider ('91), in writing upon the formation of the isopod embryo, states that the dorsal cells, as the embryo develops, become pushed together, and these cells later undergo degeneration. On page 352 he adds: "Es ist die Möglichkeit, die wir oben andeuteten nicht ausgeschlossen, dass in dem Dorsalorgan bloss die Involutionsform des Nahrungsdotter bedeckenden Blastodermtheils vorliegt. Die Involution wurde sich dann bei den Amphipoden-Typus durch Einstülpung, bei den Oniscus-Typus durch Amputation einleiten." If such is the case, then in both forms we have a ventral plate covering the ventral pole, at the posterior end of which the entoderm arises. Behind the entoderm lie the vitellophags or the dorsal cells in the amphipods, the similarity between these has been pointed out, and beyond the vitellophags the dorsal cells of the isopods or the dorsal organ of the amphipods, according to Heider.

The great difference lies in the origin of the mesoderm, and more work will have to be done upon other amphipods before this can be decided. It, however, is a great question to my mind if the dorsal cells in the isopods and the dorsal organ of the amphipods can be homologized. Heider himself merely suggests the possibility, as his conclusion was drawn solely from the work of Bobretzsky ('74).

If we compare embryos of the later stages we find that in both cases the embryo is folded dorsally (see diagram and Figs.



36, 37). In the amphipod, however, when the cells which will form the dorsum of the animal develop, the least resistance to the pressure exerted through their growth seems to be on the ventral pole, and, therefore, the embryo folds over ventrally (Figs. 38 and 42), whereas in the isopods the least resistance is in the dorsal region. Might not the different mode of folding in the two cases be due to the fact that by the time the cells which will form the dorsum of the animal develop the ventral pole in the isopods is further differentiated than is the case in the amphipod, and, therefore, offers greater resistance to the pressure exerted upon it by the growing region?

Formation of the Liver Tubes and the Intestine.

As has been described above, the entoderm arises as a true invagination. After the closure of the blastopore the cells migrate into the yolk area as an irregular mass, and by amoeboid motion migrate to both sides of the body in much the

same way that Bobretzsky ('74) describes for *Oniscus*. These become the liver tubes. Fig. 51 represents a section of an egg which is in about the stage shown in Fig. 38. The section passes somewhat obliquely through the region marked *a-a*. In the region of the dorsal organ the cells are arranging themselves to form a tube on the left side of the body. The sections (Figs. 57, 58) of a stage between Figs. 38 and 42 pass through the lines *a-a* and *b-b*, represented in Fig. 42. In the section lying nearest the dorsal organ (Fig. 58), where the greater mass of invaginated cells was found, the tubes are almost complete, and a mass of cells is still seen in the center of the egg. Only the dorsal walls are formed in the section nearer the anterior end, although in this case there are as many cells found in the part of a tube as there are in the whole tube of section (Fig. 58). This, however, is not always the case. The tubes are always complete in the region of the dorsal organ before they are complete in any other region of the body. Although no cell walls could be seen, the entoderm cells can be distinguished readily from those of the ectoderm and mesoderm by their nuclei, and in one egg there was a little yolk space between the cells of the body wall and the liver tubes. In other eggs the tubes have been seen in various stages of formation; for instance, in one section, where there was a break in the wall of the tube, like the one shown on the right-hand side of Fig. 58, three cells were lying in the gap, but they had not sent out protoplasmic prolongations at the time to complete the tube. In a section of an egg somewhat older (Pl. XXVII, Fig. 45), the liver tubes are complete in the region of the dorsal organ, and a few cells are found in the center of the yolk mass. Only in one instance have I seen an entoderm cell dividing. It was in the ring of the liver tube, and the plane of cleavage was parallel to the radius of the tube. I feel confident, however, that the cells must increase in number by division, because, as I shall show, most of the digestive tract is formed from entoderm cells.

By this time the elongation and consequent folding of the embryo, described above, has been completed. The area between the dorsal organ and the last appendage now extends

over the whole dorsal region of the abdomen. The blastopore, evidently, is carried over the posterior pole of the egg, and finally lies at or near the extreme tip of the abdomen (*cf.* Figs. 36-43). In consequence, the proctodaeum, which invaginates just posterior to the last appendage, forms either in the blastoporic area or just anterior to it, as is the case in the decapods. In the decapods, however, the stomodaeal and proctodaeal invaginations form the greater part of the intestine, while in the amphipods both stomodaeum and proctodaeum are very short. Figs. 59-64 represent sections of an embryo of the fourth day cut parallel to the line $x-x$ (Pl. XXVII, Fig. 44). Owing to the folding of the embryo, the sections pass through stomodaeum and proctodaeum, cutting them transversely, while the dorsal sections cut the digestive tube horizontally. In Fig. 59 we see both stomodaeum and proctodaeum. The cells are closely packed and columnar. In the next section (Fig. 60) the stomodaeum is still seen as a round tube, but the proctodaeum has broken through. Another section showed that the stomodaeum has also broken through. On examining Fig. 61 it will be seen that the part of the digestive tract immediately adjoining the stomodaeum and proctodaeum is not formed at this time. Six large nuclei are seen bordering the yolk area in the thoracic region, whereas only three long, spindle-shaped cells could be seen bordering the yolk of the abdominal region. Two sections beyond this (Fig. 62) show the anterior ends of the liver tubes. The cells have large vacuoles, and inclose the whole yolk area. In the next section (Fig. 63) the liver tubes are pushed somewhat apart, and the irregular mass of cells between them represents the digestive tract just beginning to form in this region. In Fig. 64, where the section passes through the dorsal organ, the digestive tract is completely formed of large, vacuolated cells, with large, clear nuclei looking exactly like those of the liver tubes. Another section shows the digestive tract shading off into the large yolk areas of the thoracic and abdominal regions. All the yolk in these sections is inclosed in the liver tubes and the digestive tract. I have never seen any outside of them being digested by special vitellophags as Dr. Pereyaslawzewa ('88)

figures in Gammarus, or as Dr. McMurrich ('95) describes for the isopods.

We have seen how the liver tubes formed first in the region of the dorsal organ, where the greater mass of the invaginated cells lay; now we find the digestive tract completely formed in that region, whereas, anteriorly and posteriorly, it has not begun to form. The cells also present exactly the same appearance as those of the liver tubes; therefore, I conclude that the whole digestive tract, except the short anterior and posterior portions, is formed from the invaginated cells. If cells from the ventral plate or from the stomodaeum or proctodaeum were carried in to form the digestive tract, then I should expect to see the ends formed before the central portion.

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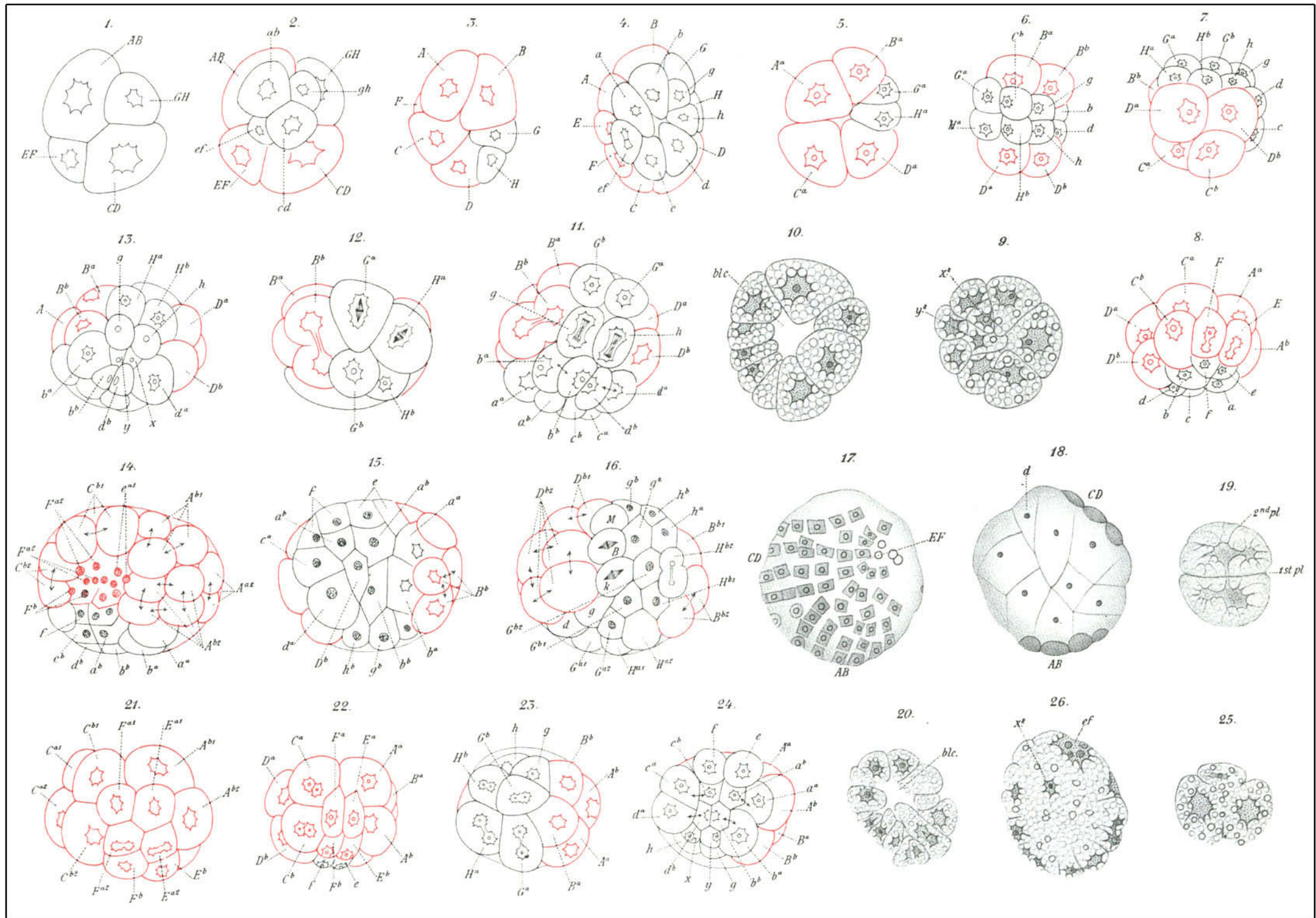
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LETTERING USED THROUGHOUT THE PLATES.

<i>abd.</i>	abdomen.	<i>en.inv.</i>	entodermal invagination.
<i>abdf.</i>	abdominal fold.	<i>L.</i>	liver tube.
<i>an.</i>	antennae.	<i>l.a.</i>	last appendage.
<i>ap.</i>	appendage.	<i>m.</i>	mesoderm.
<i>blc.</i>	blastocoel.	<i>ppl.</i>	protoplasm of cells at the
<i>c.th.</i>	cephalothorax.		edge of the ventral plate.
<i>d.</i>	dorsal cell.	<i>pr.</i>	proctodaeum.
<i>D.O.</i>	dorsal organ.	<i>st.</i>	stomodaeum.
<i>ec.</i>	ectoderm.	<i>x and y.</i>	degenerating cells.
<i>en.</i>	entoderm.	<i>Y.</i>	yolk area.

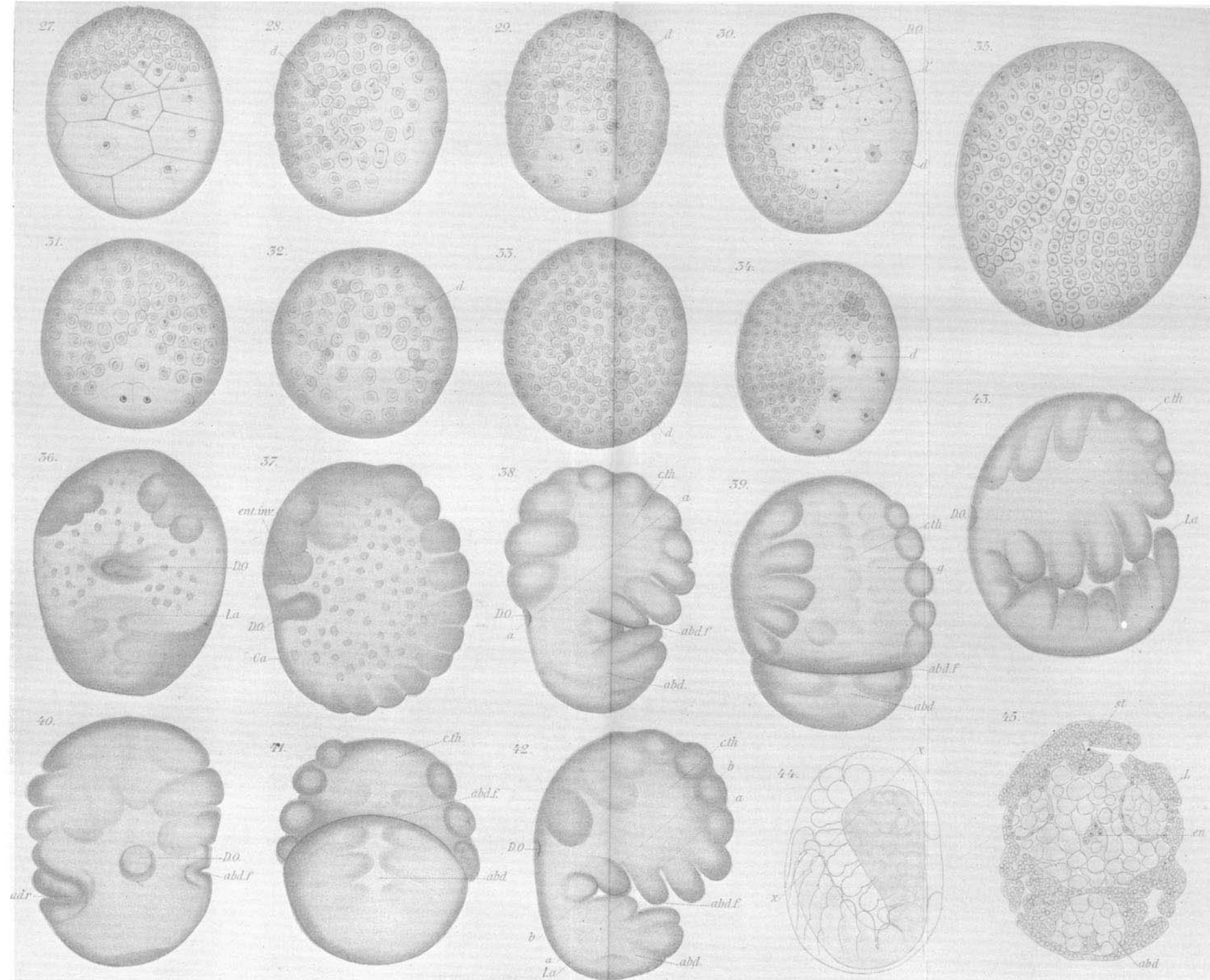
EXPLANATION OF PLATE XXVI.

- FIG. 1. 4-cell stage.
FIG. 2. 8-cell stage from micromere pole.
FIG. 3. 16-cell stage from macromere pole.
FIG. 4. 16-cell stage from micromere pole.
FIG. 5. 22-24-cell stage from macromere pole.
FIG. 6. 22-24-cell stage looking down upon *GH* cells.
FIG. 7. 22-24-cell stage seen from *CD* side.
FIG. 8. 22-24-cell stage looking down upon *EF* cells.
FIG. 9. Section of an egg of forty-five cells passing through the two blastomeres found in the blastocoel.
FIG. 10. Section of the same egg shown in Fig. 7 passing through the blastocoel.
FIG. 11. 28-40-cell stage looking down upon the *gh* cells.
FIG. 12. 28-40-cell stage looking down upon the *GH* cells.
FIG. 13. 40-cell stage showing *x* and *y* cells.
FIG. 14. 73-cell stage looking down upon the *EF* group.
FIG. 15. 73-cell stage from micromere pole.
FIG. 16. 73-cell stage looking down upon the *GH* group.
FIG. 17. 102-cell stage from the macromere or ventral pole after the blastoderm has risen to the surface of the egg.
FIG. 18. Micromere or dorsal pole of the egg shown in Fig. 17.
FIG. 19. Optical section of an egg passing from the 2 into the 4-cell stage.
FIG. 20. Section of an egg of forty-three cells, showing one cell with the spindle at right angles to the surface of the egg.
FIG. 21. 44-46-cell stage looking down on *EF* group.
FIG. 22. 30-42-cell stage looking down on *EF* group.
FIG. 23. 30-42-cell stage looking down on *GH* group.
FIG. 24. 30-42-cell stage seen from micromere pole.
FIG. 25. Section of an egg of the 8-cell stage.
FIG. 26. Section of an egg of 72-cell stage.



EXPLANATION OF PLATE XXVII.

- FIG. 27. Dorsal view of an egg of about twenty-four hours.
FIG. 28. Side view of the egg shown in Fig. 27.
FIG. 29. Ventral view of the egg shown in Fig. 27.
FIG. 30. Side view of an egg of about thirty-six hours.
FIG. 31. Anterior pole of an egg of about thirty hours.
FIG. 32. Posterior pole of the egg shown in Fig. 31.
FIG. 33. Ventral view of the egg shown in Fig. 31.
FIG. 34. Side view of an egg of a stage between those shown in Figs. 30 and 31.
FIG. 35. Ventral view of an abnormal egg of about the same stage as that shown in Fig. 33.
FIG. 36. Dorsal view of an egg of the second day.
FIG. 37. Side view of the egg shown in Fig. 36.
FIG. 38. Side view of an egg about the end of the second day.
FIG. 39. Ventral view of the egg shown in Fig. 38.
FIG. 40. Dorsal view of an egg of about the beginning of the third day.
FIG. 41. Ventral view of the egg shown in Fig. 40.
FIG. 42. Side view of the egg shown in Fig. 40.
FIG. 43. Side view of an egg at the end of the third day.
FIG. 44. Side view of an egg of the fourth day.
FIG. 45. Section of an egg of a stage shown in Fig. 43.



EXPLANATION OF PLATE XXVIII.

- FIG. 46. Transverse section of the head region of an embryo shown in Fig. 30.
- FIG. 47. Transverse section of the same egg about the middle of the ventral plate.
- FIG. 48. Transverse section of the same egg nearer the posterior pole.
- FIG. 49. Transverse section of the head region of an egg of the first day.
- FIG. 50. Transverse section of an egg of the first day.
- FIG. 51. Part of a section of an egg of the first day.
- FIG. 52. Part of a section of an egg of the first day.
- FIG. 53. Transverse section passing through the center of the entodermal invagination.
- FIG. 54. Small portion of a sagittal section, showing the relative position of the dorsal organ and the entodermal invagination.
- FIG. 55. Sagittal section of an egg of the second day.
- FIG. 56. Oblique section of an egg of the stage represented in Fig. 38 passing through the region *a-a*.
- FIG. 57. Section of an egg of a stage between 38-42 passing through *a-a*, Fig. 42.
- FIG. 58. Section of the same egg passing through the line *b-b*.
- FIG. 59. Section of an egg of the stage shown in Fig. 44, passing through the stomodaeum and proctodaeum and cut parallel to the line *x-x*, Fig. 44.
- FIG. 60. Another section of the egg shown in Fig. 59.
- FIG. 61. A section dorsal to the one shown in Fig. 60.
- FIG. 62. Thoracic portion of the second section beyond the one shown in Fig. 61.
- FIG. 63. Thoracic portion of the next section to the one shown in Fig. 62.
- FIG. 64. Thoracic section beyond the section shown in Fig. 63.

