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# BIOLOGICAL BULLETIN

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## ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

## PART II.

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CONTENTS.



The egg is covered by a chitinous chorion and the eggs are packed (and much compressed) in a tough chitinous tube (*e. c.*, Text Fig. 1), which is attached to the mother. In order to allow

[1] Thesis accepted by the Faculty of the Department of Philosophy of the University of Pennsylvania toward the degree of doctor of philosophy.

removal from the tube, the eggs must be fixed in fluids which cause them to draw away from the chorion (partly by increasing the osmotic pressure inside the chorion) and which do not make them friable. The only fluids which served contained alcohol and nitric acid. Nitric acid of 5 per cent.–10 per cent. in alcohol of about 30 per cent. served well enough for cell lineage but allowed the chromosomes to swell (partly re-dissolve in water?). The addition of chromic acid (Perenyi's formula) prevented the swelling of the chromosomes and made a good fixitive if manipulated properly. The chromic acid in this mixture is changed to blue chromic oxide, and P. Mayer in the first German Edition of Lee's "Vade Mecum,"[1] says it contains 30 per cent. alcohol, 5 per cent. nitric acid and a little nitric ether and chromic oxide, the last two having no effect in fixation. However, it has been my experience that the chromic oxide was necessary to prevent swelling of the chromosomes. Fischer ('99) says that nucleinic acid is not precipitated by dilute nitric acid, and that its precipitate formed by alcohol is soluble in water. It is possible that the swelling of the chromosomes is due to the solution of nucleinic acid or its compounds in the aqueous staining bath. In addition to its other qualities, nitric acid bleaches the eggs of *Pandarus*, and others that contain pigment, and although it is difficult to wash out (in 70 per cent. alcohol) it was found to be an indispensable ingredient.

I tried various standard fixatives for eggs to be sectioned, and found them little better than Perenyi's fluid; but for whole adults to be sectioned for the ovaries, etc., the latter fluid seemed to be much inferior to some others. It was probably too much diluted by the body fluids.

For staining whole eggs Delifield's hæmatoxylin diluted and acidulated (Conklin's formula) was the most convenient, but for sections various stains were used. In Hermann's safranin-gentian violet, besides the usual differentiations of chromatin, the centrosomes (centrioles) stained red and the archoplasm blue (unless the sequence of stains was reversed). Iron hæmatoxylin gave the sharpest stains for chromosomes.

It is probable that there can be no fixation without some

[1] "Grundzüge der Mikroskopischer Tecknik," by Lee and Mayer, Berlin, 1898.

artefacts, and that the differentiation shown by many stains is due to differences in the size and density of particles of protoplasm coagulated by fixation as claimed by some writers. I have paid special attention to the structure of coagulated proteids, and repeated experiments of Fischer and others by producing granular and "curdled" precipitates, and aster-like formations in albumin, and by staining the same. These led me to believe that probably none of the finest structures seen in the fixed protoplasm could be relied upon as representing structures in the living cell, but that such large bodies as chromosomes, spheres, etc., could not be considered artefacts, though their finer structure may be changed.

In the cell lineage I have used the quartet system of nomenclature (of Kofoid, '94) as applied by Bigelow to *Lepas* (the only crustacean whose cell lineage has been described beyond the 16 cell stage) to facilitate comparison among crustacea, but do not think this type of cleavage closely related to that of annelids and molluscs, in fact the cleavage of the parasitic copepods does *not* follow a quartet system and I hope no one will be misled by the inappropriate nomenclature.

The cells of the 4 cell stage are designated *a*, *b*, *c*, *d* in a dextral order, *a* being the left anterior cell. An exponent denotes the order of the generation starting with the ovum as the first. A second exponent is used to distinguish a cell from other cells of the same generation and derivation. The odd numbers refer in cases of equatorial division to cells nearer the vegetal pole; of transverse, to cells nearer the anterior end; of longitudinal, to cells nearer the sagittal plane, or in case the cleavage coincides with that plane, the right side. Thus *equatorial* refers to the equator of the chief axis of the egg while *sagittal* and *transverse* to the axes of the embryo that will develop therefrom, but which may be distinguished in the egg as early as the 2 cell stage.

To determine the second exponent of the two daughter cells of any cell division, multiply the second exponent of the mother cell by two and the product is the second exponent of that daughter cell which has an even number for this exponent, and is one greater than the second exponent of the daughter cell which has an odd number for a second exponent.

For cell lineage, whole eggs had to be used, and it was exceedingly difficult to get them out of the egg tube. The best way is to separate the eggs by cutting the tube between them with a sharp "spear head" dissecting needle under the microscope, which increases in difficulty in proportion to the flattening of the eggs. When the eggs are separated the polar bodies are lost and other means of orientation are necessary. In stages before the origin of the primary germ cell it was necessary to lay the eggs on the slide with a determined pole uppermost. The eggs, except when abnormally placed, have the vegetal pole turned toward the mother, and by placing the mother and attached egg strings in cedar oil on a slide under a Zeiss binocular dissecting microscope, it was possible to lay the eggs with vegetal pole up as they were separated, place a cover glass over them to prevent turning, and run balsam under from one side.

Schimkewitz ('96, '99) concluded that pressure was an important factor in determining the form of the cleavage of parasitic copepods.

Pedaschenko ('93, '97, '98) worked for a number of years on the embryology of *Lernæa branchialis* and traced the cell lineage to the 16 cell stage, but was mistaken in the orientation, thinking the first protoplasmic cell to be formed at the animal pole and not distinguishing between the two flat surfaces (dorsal and ventral) of the egg in early stages. He found the germ cells to arise from four cells at the edge of the blastopore and considered two of these to be male and two female. The identity of two of these cells was lost (incorporated in the other two) and the remaining two gave rise to the sex glands. If such were the case, it seems to me that we should expect frequent occurrence of bilateral androgyny (hermaphroditism). The close relation of Lernæa to the forms I studied has made Pedaschenko's work of great service as a hand-book.

Grobben ('79) had long before found the germ cells to arise from four cells of the anterior lip of blastopore of the phyllopod, *Moina*.

C. B. Wilson ('05) includes in his excellent monograph of the Caligidæ, a description of the general embryology of these parasitic copepods.

The only crustacean whose cell lineage has hitherto been carried beyond the 16 cell stage is *Lepas*, as described by Biglow ('02). Pedaschenko pointed out the resemblance between the segmentations of *Lepas* and parasitic copepods and I believe this resemblance is fundamental. The endoblast arises one generation earlier in *Lepas* than in parasitic copepods but this may be due to larger amount of yolk in the latter, which causes a retardation in the segregation of organ-forming substances, and of gastrulation.

Canu ('92) published a paper which I have not seen, which included embryology of copepods. Further consideration of the literature may be found in the text.

## I. Cleavage of the Egg.

### 1. *Læmargus muricatus* Kröyer.

*A. First Cleavage.* — At the earliest stage I have (Fig. 19) the male and female pronuclei lie side by side at the center of the egg and at the equator of the spindle. At this stage the egg throws out a number of yolk spherules into the space between the egg and the chorion, but the exact nature of this process seems obscure. Each pronucleus contains a nucleolus, and the chromatin is being aggregated into chromosomes. The pronuclei are of the same size and apparently similar in every respect. There is a deeply staining centriole at each pole of the spindle, surrounded by a layer of hyaloplasm that is drawn out into astral rays connected with the surface of the egg, and mantle fibers connected with the pronuclei. The astral rays of one pole are thicker (stronger) than those of the other. Where the mantle fibers come in contact with the nuclear membrane, the latter is pushed in (and partially dissolved?) and finally becomes dissolved, and the mantle fibers become attached to the chromosomes. The spindle thus formed is elongated. The astral rays of one pole shorten more rapidly than those of the other, drawing this pole nearer one edge of the egg than the other. The hyaloplasm is drawn from between the yolk globules and the astral rays increase in thickness. Not only is the hyaloplasm drawn into the astral rays, but small lumps of hyaloplasm adhere to their surfaces and move toward the centrosomes. Thus the

spheres increase in size by thickening of the hyaloplasm layer around the centrosome and become surrounded by protoplasm drawn in along the rays. One sphere grows larger than the other and moves to the surface of the egg. Some yolk granules are caught between the astral rays and form a clear space between the sphere and the egg membrane and push the astral rays outward. We thus have a central space almost free from rays, surrounded by an annular area in which the rays are especially aggregated. The center is bulged out and the annular area sunken in by the stress (Fig. 20). Some astral rays connect with those of the other pole, forming "spindle fibers" outside the mantle fibers. The sphere at the surface of the egg soon pulls all the hyaloplasm from between the yolk granules in that half of the egg and the astral rays that pass through the yolk break and are drawn into the sphere. The mantle fibers connected with the outer pole shorten more than those of the opposite pole, and the equatorial plate moves to the plane of the ensuing cell division. The cell division is very unequal, separating a cell containing very little yolk ($ab^2$) from one containing practically all the yolk ($cd^2$, Fig. 21).

Going back a little, by the dissolution of the nuclear membranes a good deal of nuclear sap is liberated. This fluid is hard to follow, but I have some slides that seem to show that most of it goes toward the sphere that reaches the surface, and is therefore included in the cell $ab^2$. The first cleavage plane is parallel to the chief axis, but is very eccentric because of the great inequality of the division. (See the section on orientation of the egg.)

*B. Second Cleavage.* — After fusion of the chromosomal vesicles in the two cell stages (Fig. 21), the nuclei thus formed remain connected for a short time by interzonal fibers. I have no stages in the division of the centrosome, but soon after this division the two centrosomes are at the ends of a spindle shaped sac or centrodesmus (Fig. 21, small figure to right below). Mantle fibers become attached to the nuclear membrane and the chromosomes gather in that side of the nucleus nearest these points of attachment. From this stage on, the histories of the protoplasmic cell and the yolk cell are different and will be treated separately.

The nuclear membrane of the protoplasmic cell dissolves in the region of attachment of the mantle fibers, which then become attached to the chromosomes. The remainder of the nuclear wall, and the nucleolus dissolve and the chromosomes are arranged in the equatorial plate (Fig. 23), which is at first far removed from the central spindle, but later the central spindle assumes its normal position in the center of the peripheral spindle (Fig. 24). The division divides the cell by a meridional (sagittal) cleavage into equal daughter cells ($a^3$ and $b^3$).

In the yolk cell, as the attraction spheres separate they grow in size (Figs. 21–24). The central spindle presses against the nucleus, forming a groove (Fig. 22) which makes it appear as though the nuclues was divided (maternal and paternal elements distinct), but sections show that this division does not pass completely through the nuclues. The nucleus is drawn out to about four times its original length (Fig. 23), one sphere moving faster than the other and reaching the surface of the egg, on the right side of the protoplasmic cell.

This elongated nucleus is bent considerably and suggests that it is being elongated by a force applied internally, and is bent by external resistance, but I think the bending may be due to the unequal pressure of the yolk, and the elongation of the nucleus may be due wholly or in part, to the contraction and separation * of the mantle fibers. The nuclear membrane dissolves, and the equatorial plate is formed (Fig. 24). It is to be noted that whereas the elongated nucleus is bent the fully formed spindle is straight. In Fig. 23 it is seen that the end of the nucleus attached to the peripheral sphere is enlarged and nearer to its sphere than the other end is, probably due to increased tension of the mantle fibers at this end, accompanied by pressure of the yolk on the sides of the nucleus.

On dissolution of the membrane all the nuclear sap goes into the peripheral sphere. A yolk spherule is often caught between the astral rays and the cell wall (Fig. 24, $c^3$). Protoplasm migrates along the astral rays to the spheres. The division cuts off a small protoplasmic cell from a large cell containing practically all the yolk ($d^3$). I have not worked out the cell lineage any further in this species, though it appears to be essentially the same as the dichelestid.

* By elongation of the central spindle?

## 2. *The Dichelestid.*

*A. First Cleavage.* — The earliest stage I have of this is an anaphase of the first cleavage (Fig. 25). It is similar to the same stage in the preceding species save that the centrosomes if they exist at all are larger and less dense, and the sphere reaching the surface collects a considerable mass of cytoplasm around it. The cleavage plane is "meridional" or more correctly, it is perpendicular to the equator of the egg, but owing to the great difference in size of the protoplasmic and yolk cells thus formed, it does not pass through the animal or vegetal pole (Fig. 26).

*B. Second Cleavage.* — The yolk cell ($cd^2$) is sometimes retarded in division — in Fig. 26 its nucleus is yet a mass of chromosomal vesicles while that of the protoplasmic cell ($cd^2$) has reached a late prophase. Already a thickened layer of protoplasm marks the place where $c^3$ will be cut off.

The protoplasmic cell ($ab^2$) divides by a meridional (sagittal) furrow into two cells, $a^3$ and $b^3$, almost equal in size (Fig. 27). The yolk cell produces an elongated spindle similar to that in the preceding species, one pole of which reaches the surface of the egg to the right (left, when viewed from the vegetal pole) of $b^3$ (Fig. 27). The protoplasmic cell that is cut off ($C^3$) often contains a considerable quantity of yolk (Fig. 28). Already a thickened layer of protoplasm (Fig. 33) marks the place where $d^{4.2}$ will be cut off.

In this and the two succeeding cleavages, the poles of the yolk cell spindle are differentiated by the appearance of larger granules on the astral rays of the posterior side of the sphere that is to remain in the yolk (Fig. 27). This probably occurs also in the first cleavage but I have not the right stage to show it. These granules are probably homologous to lumps of cytoplasm on the astral rays of *Læmargus muricatus*.

*C. Third Cleavage.* — The division of the protoplasmic cells $a^3$, $b^3$, and $c^3$ is equatorial (parallel with the face of the disc) (Fig. 28). The yolk cell gives off a protoplasmic cell, $d^{4.2}$ to the left of $a^3$. Large granules appear on the astral rays of the posterior side of the sphere left in the yolk. A thickened layer of protoplasm marks the place where $d^{5.2}$ will be cut off.

*D. Fourth Cleavage* (Figs. 29–30). — In this cleavage the divi-

sion of the yolk cell ($d^{4.1}$) cuts off a protoplasmic cell $d^{5.2}$ (Fig. 31) to the right of the cap of protoplasmic cells; $d^{4.2}$ divides equatorially, $C^{4.1}$ and $b^{4.1}$ transverselsy; $a^{4.1}$ divides obliquely but the daughter cells ($a^{5.1}$ and $a^{5.2}$, Fig. 31) come to lie one behind the other.

On the dorsal side $a^{4.2}$, $b^{4.2}$, and $C^{4.2}$ divide transversely.

Fig. 30 is an enlarged view of the spindle in the yolk cell, constructed from the two consecutive sections. The astral rays are only partially shown, and, connecting them, the alveolar (or reticular ?) hyaloplasm between the yolk spheres is represented by dotted lines. This is a little later stage than Fig. 29 and the spindle has shortened more. The distinctness of the centrosomes is exaggerated in the figure, in fact it is doubtful whether we deal here with centrosomes, but that the sphere is denser in the center can be shown in some cases with Hermann's safranin-gentian violet stain.

*E. Fifth Cleavage* (Figs. 31–35). — Of this cleavage I have not enough stages to be sure of the lineage of every cell. There are many disarrangements due to the cells extending over the yolk and slipping on one another, which makes their lineage extremely difficult to follow. In the figures I have divided the derivatives of *a*, *b*, *c* and *d* by heavy lines, and by comparing Figs. 31 and 32, one can see the great change that has come about.

In this cleavage the yolk cell divides, giving off (near the center of the ventral side) the last protoplasmic cell $d^{6.2}$ (Fig. 32), which is the primary germ cell.

*F. Sixth Cleavage.* — In the fifth cleavage the divisions were not synchronous, in the sixth cleavage the division of two cells, $d^{6.2}$ (the primary germ cell) and $d^{6.1}$ (the primary entoderm cell) is delayed until some of the other cells are dividing for the eighth time. After cleavage of the majority of the cells (Fig. 33) the blastoderm stretches over the yolk until it has half covered the latter (Fig. 34). During this process some derivatives of *d* at each side of the egg and at the end of the blastoderm, come to lie under the others and give rise to mesoderm. The yolk cell (entoderm) divides totally by a sagittal furrow (Figs. 34, 35) and the primary germ cell sinks beneath the blastoderm and divides by a sagittal furrow into two cells of unequal size.

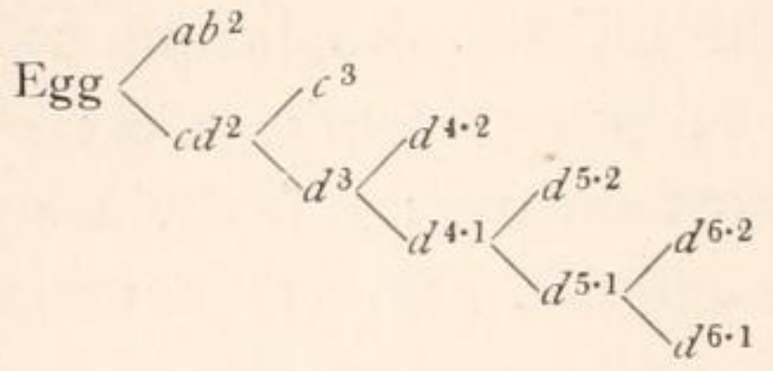


TABLE OF CELL LINEAGE TO 32 CELL STAGE.

## II. ON THE NATURE OF THE CLEAVAGE PROCESS.

Since Van Beneden in 1883 put forward the hypothesis that separation of the chromosomes and division of the cell was caused by contraction of the fibers of the karyokinetic figure, the question of the mechanics of mitosis has aroused a great deal of interest. The large size and peculiar form of the spindles in the early cleavage of this egg, make them favorable objects for observations on this point. I have attempted to harmonize observations on the structure of coagulated colloids, and the modern theory of the ultramicroscopic structure of colloids, with the observations on artificially produced asters in colloids, and asters and spindles appearing during mitosis in living cells. A theory of Rhumbler and others as to the mechanics of the formation of asters seems in general to be the only one applicable to the observations I have made, yet I do not believe that this theory is inseparable from the alveolar theory of the structure of protoplasm. Asters can be produced in colloids which we have no reason to believe have the alveolar structure in the strict sense. According to Mann ('06), colloids consist of minute or ultramicroscopic particles suspended in a thin fluid. On congealing (Hardy, Jour. of Physiol., V., 24), these particles by mutual attraction form rows which make up a meshwork (or interalveolar structure?) giving consistency to the mass. When colloids are coagulated with substances (electrolytes) that act strongly and quickly, the particles are large enough to be seen with the microscope and are at first distributed homogeneously through the fluid, but soon arrange themselves in rows which make up a meshwork ("gerinnselbilder" of Fischer). This passing of a colloid from the "sol" to the "gel" or congealed state may be hastened by addition of a fragment of coagulated colloid to the former, in which case the rows of drops or particles arrange themselves radially around the fragment, and an aster is

formed. Fischer ('99) varied this experiment in a number of ways, and one of his experiments modified slightly, might be tried by every one interested in the subject without much trouble: Spread a layer of egg albumin (which consists chiefly of albumin and a little globulin) on a slide and through a capillary tube introduce a small drop of a fixing solution into the albumin, observing the changes that take place under the microscope. The albumin immediately around the drop is coagulated into a membrane through which the fixing solution diffuses and from which radiations begin to form, giving the whole structure the appearance of an aster with the drop of fixing solution and the membrane around it as the centrosome. If the rays form, as they seem to, by mutual attraction of the drops or particles in the fluid, such rays or rows of drops would exert a pulling force, and if their ends were released should shorten by synæresis into a spherical mass. This may be the nature of the fibers of the karyokinetic figures in the cleavage of these copepods but does not explain the direction of movement of the asters.

## III. Mesoblast.

### 1. *Nauplius Mesoblast* (*Pandarus sinuatus*, Pl. IV. and V.).

When the cap of the protoplasmic cells has covered about one third of the yolk some of the marginal cells (lip of the blastopore) become differentiated as mesoblast. Of these one or more on the right and left edge will give rise to mesoderm of the first and second antennæ, and one near the middle of the ventral side and distinguished by its large nucleus (Fig. 37) will give rise to the sex or germ cells.

*A. The Germ Cells.* — This primary germ cell is turned under the rim of the blastopore (Fig. 38) and divides by a sagittal furrow into two (Figs. 39–40), which lie about the center of the ventral side just under the ectoderm. About the time of the closure of the blastopore these two divide by transverse furrows into four (Fig. 41). This group of four cells rotates until one cell is anterior, two lateral and one posterior. (In Fig. 42 the rotation is not quite completed.) But there is considerable variation in the amount of rotation (Figs. 41–48). The four germ

cells lie just beneath the ectoderm until the *Metanauplius* stage, when by concentration of the ventral nerve chain, the latter is pushed under them and they rest on top of it (Fig. 56). I have said the germ cells have large nuclei — the nucleus is further characterized by the fact that the chromosomes remain distinct as oval masses just inside the nuclear membrane (Fig. 52). The chromosomes can be counted and are sixteen, just twice the number in the female pronucleus. The cytoplasm is much vacuolated. The germ cells are flattened against each other, and are flattened against the ectoderm in the early stages (Figs. 49, 50). In later stages they detach from the ectoderm, and round up (Figs. 52, 56). And still later (during the *Metanauplius* stage from 24 to 72 hours after hatching of the larva) they separate and pass laterally and upwards into the yolk and two of them come together dorsal to the intestine, and I have not traced them further than the fourth day after the larva hatched, when they were still two in number. Pedaschenko says that two of the four genital cells pass to the right and two (one lateral and one median) to the left. Each pair fuse and, probably by degeneration of one nucleus, becomes a single cell, which finds its way upwards and posteriorly and by division forms the ovary of that side. The fusion of each pair he considers of great significance and the basis of a theory on the origin of the sex of the adult. He believes that one cell of each pair is male and one female and the one whose nucleus persists after fusion of the cytoplasm determines the sex of the animal. His belief that two cells of the four are male and two female is based on comparison with O. Hertwig's account of *Sagitta* in which this condition exists, with difference however in the later history. In *Sagitta* the two female cells give rise to the ovaries (in the anterior part of the animal) and the two male cells give rise to the testes (in the posterior part of the animal).

Sex is said to be determined in some animals by amount of food (of the individual, the parents, or the grandparents) in others by fertilization *vs.* parthenogenesis, in others by dimorphism of egg or spermatozoön, in others by temperature, etc. Pedaschenko proposes an additional factor.

Haecker ('97) found in *Cyclops* the primary germ cell differen-

tiated from the somatic cells at the close of the fourth cleavage or one generation earlier than in the parasitic copepods.

Boveri ('92) in *Ascaris*, and Haecker ('97) in *Cyclops* traced the "Keimbahn" from the first cleavage. In *Ascaris* the visible difference between germ cell and somatic cell was in the chromosomes, in *Cyclops* in the cytoplasm. Early differentiation of the germ cells has been noticed in a large number of animals, but the causal factors in their differentiation are yet unknown. From Boveri's account of *Ascaris*, it seems that the cells of the "Keimbahn" preserve all the characters of the fertilized egg, while the somatic cells lose some characters. Yet the mature ova and spermatozoa of most animals are possibly as highly differentiated as any somatic cell.

In the dichelestid the germ cells have the same origin as in *Pandarus sinuatus* but they differ in appearance. Fig. 57 shows the primary germ cell beginning to be turned under the blastoporal rim. Fig. 58 shows a stage after the division of the germ cell into two cells (of unequal size). If we followed Pedaschenko's theory we might consider the large cell as female and the small cell as male as it is always true that one is larger than the other. The nuclei of the two germ cells lie in their ends that are nearest the free border of the blastoderm (blastopore). These two cells divide into four and the nuclei of two are larger than of the other two, but the cell boundaries between them are extremely difficult to make out.

*B. The Mesoblastic Rudiments of the Nauplius Appendages* (*Pandarus sinuatus*) arise from cells turned under the rim of the blastopore during epibole. When the cap of protoplasmic cells has covered about one third the yolk (Fig. 38) a few cells are turned under the rim at the extreme right and left, that is to say at the edge of the disc shaped egg. These cells are the mesoblastic elements of the first and second antennæ and divide on each side into two masses (Fig. 40, $an^1$, $an^2$). The time of this division varies slightly, the elements being sometimes widely separated before closure of the blastopore (Fig. 40) and sometimes close together just after the closure of the blastopore (Fig. 41, $an^1$, $an^2$). Just before closure of the blastopore, a few cells are turned under its lip on each side (Fig. 40, *md*) and

are the mesoblastic rudiments of the mandibles. This completes the rudiments of the nauplius appendages. After the close of the blastopore the post nauplius segments are laid down by teloblastic growth at the posterior end, and the nauplius is pushed (compressed) forward, carrying the rudiments of the second antennæ and mandibles forward (Figs. 47–48), and causing the three pairs of appendages to lie closer together. In stage *D* (Figs. 45–6) the appendages begin to grow out and at the same time the muscle cells elongate into fibers. I think it more profitable to follow these latter backward in development, as it seems doubtful whether they have a single or a double origin. Observe the muscle cells in Fig. 45 elongating radially and attached peripherally to the rudiments of the appendages. In Fig. 44 (Stage *C*) the muscle cells (one shown at *m*) are just beginning to differentiate from the mesoblastic rudiments of the appendages, and two of them have begun to elongate (compare Fig. 50, *m*). The question arises whether all or only some of these muscle cells arose from the mesoblastic rudiments of the appendages.

Just after the closure of the blastopore a few cells similar to these muscle cells are seen considerably removed from the mesoblastic rudiments of the appendages (Fig. 41, *m*). And just before closure of the blastopore minute cells with scarcely any cytoplasm are seen budding off from the ectoderm in this region, (Figs. 40, 49, *x*). There is a slight probability that some of the cells *m* arise by growth of the cells *x* which would be a case of muscle cells arising from ectoderm as in cœlenterata, etc. But small cells with hardly any cytoplasm are found in the yolk at many stages of the embryo (Figs. 44 and 47, *x*) and although I have not closely traced them from cells like *x* in Fig. 40, I think their resemblance in structure indicates a likeness in origin. I think the evidence indicates that all the mesoblast arises from cells turned in from the lip of the blastopore, as is the case in other copepoda, phyllopoda, decapoda and cirripedia.

The muscle cells when first elongated push the ectoderm toward the center and mass it in a sort of structure which somewhat resembles the "dorsal organ" which disintegrates, and the elements of which wander into the yolk. The muscle cells are thus arranged radially just beneath the extremely thin dorsal

ectoderm (Fig. 45) but the forward movement of the appendages carries their peripheral ends forward (Figs. 45–48) until they assume a longitudinal direction. Contractile fibrillæ begin to form in the muscle cells in stage *E* (Fig. 47) and the nucleus and undifferentiated cytoplasm is pushed to one side. In the liberated nauplius the muscle fibers run almost the whole length of the animal and show cross striations (Fig. 51). Each appendage then has at least one muscle fiber attached to the anterior and one to the posterior border of its base. Muscle cells that go into the hollow appendages as they grow out, form muscles attached to the bifurcated ends of the appendages (Fig. 51, left side).

The same description in general holds good for the dichelestid and *Læmargus*. In these the mesoderm of the appendages is clearly derived only from marginal cells. In *Læmargus* the ectoderm massed in the middle of the dorsal side by growth of the dorsal muscle fibers forms a more conspicuous "dorsal organ" than in the other species and the elements arising from its disintegration are more numerous.

In relation to the formation of the appendages might be mentioned the segmentation of the nauplius of *Læmargus*. Soon after the closure of the blastopore the embryo is divided by bands of thinner ectoderm into three segments corresponding to the three pairs of nauplius appendages. This segmentation slowly disappears with the development of the nauplius. Other species show it but to a less degree than *Læmargus*. This segmentation might be used as evidence that the nauplius of ancestors of crustacea was segmented or it might be considered as cœnogenetic and associated with the development of the appendages and neuromeres of the nauplius.

### 2. *Post nauplius mesoblast* (*Pandarus sinuatus*).

At the closure of the blastopore some of the marginal cells are turned in (Fig. 49, *Mp*) and become the mesoblast of the post naupliar segments. These cells are much larger than the surrounding cells (Fig. 41) and form a mass at the posterior end of the animal that is destined to develop mesoblastic somites by teloblastic growth. By rapid division the cells become small and

by this time the ectoderm has completely closed over them (Fig. 42). This mass of cells divides in the sagittal plane into two masses (Fig. 44), which begin to grow forward as a pair of broad bands under the neural thickenings of the ectoderm (Figs. 46, 47, *Mp*). From the anterior ends of these bands oval masses are cut off that are the mesoblastic somites (Fig. 48).

## IV. Entoblast.

The entoblast is segregated one generation later than in Lepas. In the 32 cell stage the entoblast consists of one cell that contains practically all the yolk and which does not divide until the majority of the cells have completed the seventh cleavage and some are in the eighth. It then forms a very long transverse spindle with the poles inclined anteriorly. The daughter nuclei are widely separated, but in *Pandarus*, *Læmargus* and other Caligidæ the yolk does not segment. The next (second) division occurs about the time the "blastoderm" has covered half the yolk. The spindles extend longitudinally and the poles are inclined outward. Each of the two spindles is shorter than that of the previous division (Figs. 35, 38). The next (third) division occurs about the time of the closure of the blastopore (Fig. 40). There is much variation in the direction and curvature of each of the four spindles, but the daughter nuclei are about equally distributed through the yolk as they are after each division. The fourth division occurs in stage *B* (Fig. 42) and the fifth in stage *C* (Fig. 43).

In *Eudactylina* the yolk segments in the first three cleavages of the entoblast, (forming eight cells) after which the entoblast forms a syncytium. In the dichelestid the yolk divides into four cells and is then transformed into a syncytium. In the remaining species studied a syncytium is formed from the first. This omitting of the cleavage of the yolk is probably not entirely due to the amount of yolk present, which is as great in the dichelestid as in *Læmargus*, but largely due to the extent of compressions of the egg, for it has gone farther in those eggs which are compressed the most. The entoblast nuclei migrate to the surface of the yolk and form the enteron or mid gut, as described by Pedaschenko.

## V. Polyspermy.

In *Læmargus muricatus* I have found many eggs into which a number of spermatozoa had entered. In one case the whole egg string was of such eggs; in the other cases only a few such eggs were found in a string. The "development" of these eggs falls under three classes:

1. The ♀ pronucleus and the ♂ pronuclei fuse to form one nucleus in the center of the egg which does not develop further.

2. In the center of the egg a multipolar spindle is formed usually of three principal poles and one minor pole. The resulting division in all observed cases cleaves the egg into three subequal cells, in each of which a bipolar spindle with a very large number of chromosomes is formed. Further development is very irregular.

3. A bipolar spindle with an immense number of chromosomes is formed in the center of the egg. Apart from the number of chromosomes the cleavage approaches the normal type, especially up to the 4 cell stage after which it diverges more and more from the normal type. I am led to believe by certain eggs that show an intermediate stage between a multipolar and a bipolar spindle, that the bipolar spindles in the first cleavage of these eggs are formed out of multipolar spindles.

As all of these eggs were already mounted (by Professor Rynearson) I was not able to observe whether the axes of these polyspermous eggs that approached the normal type in development were the same as in normal eggs. I have not observed whether maturation takes place in polyspermous eggs — the cleavage spindles are very different from normal cleavage spindles, and are very similar to normal maturation spindles. This may be due to a tendency to throw out the excess of chromatin, and in some cases I have found a mass of very small cells extruded from the egg, not always, however, in the position in which polar bodies normally form. There are often many asters in the egg unconnected with chromosomes, and this may account for rounded masses of yolk that are sometimes cut off from the egg.

## VII. Relation of Pressure, Etc., to the Type of Cleavage.

When the eggs are released from the oviduct in sea water, they begin slowly to round up and separate one from another. The eggs adhere together so strongly that their tendency to assume a spherical form is greatly impeded, and it always takes several hours for them to round up. The majority of the eggs liberated begin to disintegrate before they proceed very far toward becoming isodiametrical. This is due to their very low surface tension, their cohesion being less than their adhesion for the surface film of sea water or for glass. This is shown by the fact that the eggs tend to stick to the bottom of the glass dish containing the sea water, and when the dish is tilted so that some eggs come in contact with the surface of the water, they quickly spread out over that surface. All these experiments support the direct observation that the oöcyte is surrounded by no other membrane than its surface film.

If eggs are left standing in sea water more than two to four hours their surfaces begin to disintegrate. This is probably caused by partial solution in sea water. The nuclei remain intact after a great deal of the egg has disintegrated. If the eggs are placed in hypotonic solutions they swell, if in hypertonic solutions, they shrink, without any other change that can be observed. I tried solutions of magnesium chloride, ether, and sodium hydroxide, of varying strengths in sea water containing eggs alone or eggs and sperm but could neither induce parthenogenesis nor fertilization. The spermatozoa are very similar to those of cirripedia, being thread-like and each containing a homogeneous thread of chromatin running the entire length. The sperm of many crustacea are non-motile when examined in sea water or serum, but some of them have been observed to perform movements in the female genital ducts. Cano ('93) saw decapod spermatozoa move lively in the Rec. seminis. It is therefore probable that I did not find the proper stimulus to cause fertilization in sea water. Immediately after fertilization and passage into the egg strings the egg secretes a chitinous chorion that resists all attempts at freeing the eggs so that they will round up, without mutilating them, so I had to resort to looking for eggs that by accident were not flattened in the usual manner. In the Di-

chelestid the egg at each end of the string is hemispherical in shape, due to the fact that it is pressed on only one side (Fig. 36). In the proximal egg the ventral side is rounded and in the distal egg the dorsal side is rounded. The first protoplasmic cell (*ab*) is cut off at one edge of the hemisphere. The second cleavage results in the formation of three protoplasmic cells (*a*, *b*, *c*) whose centers form the apices of a triangle on the spherical surface at its edge (Fig. 36, *A* and *B*). We should assume that this arrangement is nearer the ancestral type, which was probably a sphere, and that the first three protoplasmic cells being in the equatorial plane (Fig. 27) is due to the pressure. Fig. 36, *A* and *B*, shows a similar arrangement of cells to the same stage in the cleavage of *Lepas* as figured by Biglow, save that in the dichelestid the yolk is much greater in amount and one side of the egg is flat. In both cases *d* (the yolk cell) extends under *a*, *b*, and *c* but in the dichelestid the yolk cell is so large as to push *c* over *b* (in the distal egg).

This altered arrangement of the protoplasmic cells does not seem to affect the normal development of the embryo. The ectoderm grows over the yolk in the usual manner, except that it is stretched more on the rounded side of the egg (Fig. 36, *C*). The four entoderm cells are thicker, and in the distal egg of more volume, than normally and after the entoderm forms a syncytium the nuclei have not exactly their normal arrangement, but when the ensuing nauplius escapes from the egg membrane everything is apparently restored to its normal relation, save that a nauplius developing from a distal egg is larger.

This is contrary to the idea of Schimkewitz, who attributes many abnormalities in parasitic copepod embryos to slight differences in pressure in the egg string; but the eggs he studied had less yolk than those considered in this paper. Differences in pressure in the dichelestid egg result principally in differences in form of the yolk mass. This yolk mass does not, save to very small extent, enter as such into the composition of cells, but is dissolved and used as food by the cells. The protoplasmic cells always being on the surface of the yolk, their relation to the food supply remains unchanged.

Experiments on the effect of unequal compression on cleavage

have been made on *Ascaris* eggs by Auerbach ('74); on amphibian eggs by Pflüger ('84), Roux ('85, '93), Born ('93, '94), O. Hertwig ('93); on echinoderm eggs by Driesch ('92, '93, '95), Morgan ('93), Ziegler ('94); on ctenophore eggs by Ziegler ('94); and on *Nereis* eggs by Wilson ('95). These experiments show that if the egg is pressed more on certain sides than on others, as when it is pressed between two plates of glass and forced to assume a flattened shape, that the direction of the cleavage spindles (and consequently cleavage furrows) will be affected. Hertwig formulated the law that the spindle lies in the longest axis of the protoplasmic mass of the cell. This rule probably applies in the majority of cases, but there may be some exceptions, and there are evidently other and unknown factors which enter into the polar differentiation of the cell. Bigelow found in *Lepas* ('02) that the polarity of the egg was not affected by the oval form of the rigid chorion. The principal axis of the egg coincided with the long axis when the chorion was secreted and during the prophase of the first cleavage spindle the egg rotated through a right angle so that the first cleavage spindle was made to coincide with the long axis of the egg determined by the form of the chorion, and the principal or primary axis was perpendicular to it.

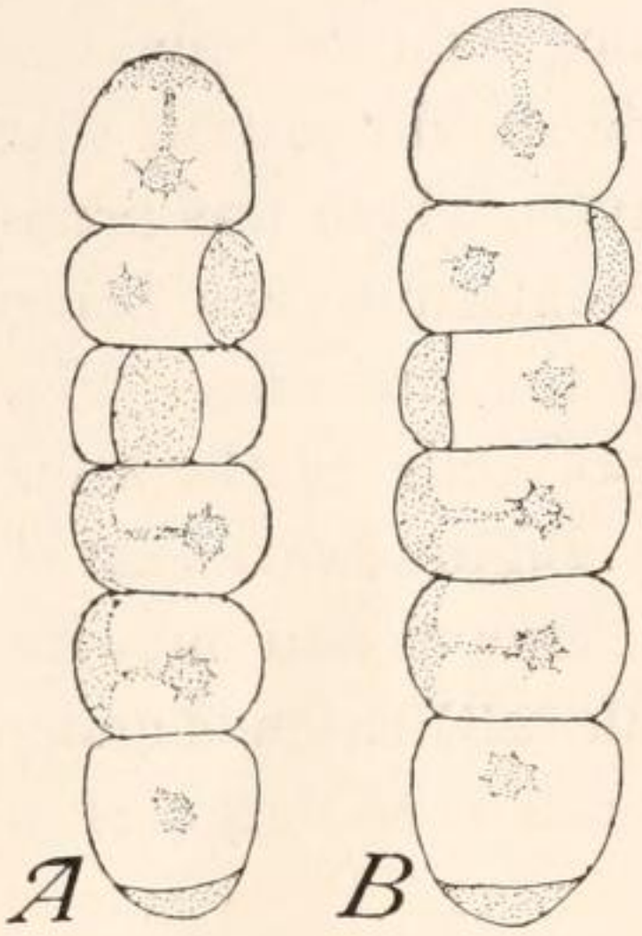


FIG. 4. Egg strings of *Endactylina nigra* Wilson. The protoplasm is stippled and the yolk white, the distinctness between the two being accentuated. *A*, Living egg string during first cleavage. *B*. The same compressed between the slide and cover glass.

I found a similar condition in *Eudactylina nigra* Wilson. If the egg string of this copepod be placed in sea water under the microscope during the first cleavage stage, the majority of the eggs will have their spindle axes, or in case the division is complete, common cell axis, nearly in the same plane. Often, however, some of these axes are considerably inclined to this plane as is shown in Fig. 4, *A*. If the egg string be pressed between slide and cover glass the above axes will rotate sufficiently to bring them all in a plane midway

between slide and cover glass and therefore in the same plane (Fig. 4, *B*). Thus the first cleavage obeys Hertwig's rule whether the compression be applied during or after the metaphase and possibly during the ensuing resting stage.

Hertwig says that if frog eggs are thus compressed normal embryos will develop, although a totally different distribution of nuclei results.

Born found that if a frog's egg were inverted before formation of first cleavage spindle the relative density of protoplasm and yolk would cause streaming movements in the egg, the protoplasm and nucleus rising through the yolk to the upper pole. But it is probable that these streaming movements would be hindered by the astral rays after formation of the spindle.

In the parasitic copepods the direction of many of the spindles is influenced by the pressure, and Hertwig's law applies in most cases. But the peculiar form of cleavage seems well adapted to variations in pressure. The blastoderm lying on the yolk may be compared to a rubber bag divided by lines into polygonal areas. The bag may be pressed into various shapes without altering the mutual relation of adjacent polygons. The only cell whose form is changed very much by pressure is the yolk cell.

I have said that the distal egg (the one at the free end of the egg string) is larger than the others. This is due to the fact that in the oviduct it presented more surface for absorption of nutriment through the wall of the oviduct. While the other eggs present only a thin edge toward the source of food, this last egg of the series presents this edge and one whole flat side in addition. Usually it does not remain flat, but becomes more or less hemispherical on the free side while still in the oviduct.

The cytoplasm of many cells is formed in large part from the substances that escape from the nucleus at the first maturation and early cleavage divisions. Dr. Conklin traced a similar process in gasteropods and in the living eggs of ascidians, and it may be a general phenomenon. In other words, a quantity of chromatin is dissolved and escapes into the cytoplasm in many and perhaps all cell divisions.

In Paramœcium the macronucleus and a large part of the substance of the micronucleus escapes into the cytoplasm at each copulation and may constitute necessary ingredients of the cyto-

plasm, as copulation is necessary to long continued existence of Paramœcia. It is probably chiefly thus that the heritable qualities residing in the chromosomes are conveyed to the cytoplasm. I do not mean to say that this is the only way the nucleus affects the cytoplasm, for with a few exceptions (*i. e.*, red blood corpuscles of higher animals) cytoplasm not containing a nucleus soon dies, but if the heritable qualities are stored up in the chromatin, part of this chromatin bearing these qualities could be transferred to the cytoplasm more easily during the absence of a nuclear membrane.

After the close of the fifth cleavage (32 cells) the embryo is composed of three types of cells that differ visibly.

1. The primary germ cell.
2. The primary entoblast cell.
3. Thirty cells of the blastoderm all similar in appearance.

The primary germ cell when first separated from the entoderm looks like the other cells of the blastoderm, but during the rest grows larger than its neighbors and is delayed in mitosis. In this character of delayed mitosis it resembles its sister cell (primary entoderm cell). There is nothing characteristic of its position that could cause it to become different from its neighbors, so we must ascribe this difference to the difference in the substances entering into it which in turn may be caused by unequal cleavage.

## VII. Summary.

1. My observations on the cell lineage agree in general with those of Pedaschenko (who worked it out to the 16 cell stage) save in regard to the orientation. Pedaschenko used no means to distinguish between the two flat sides of the egg and was mistaken in regard to the location of the animal pole, as I have shown (p. 50). At the fifth cleavage the yolk cell buds off the last protoplasmic cell, which is the primary germ cell. After extrusion of the germ cell (32 cell stage) the yolk cell is purely entoblastic. The segregation of the entoblast takes place one generation later than in *Lepas* (Biglow '02), and the segregation of the germ cells one generation later than in *Cyclops* (Haecker). The delay in the segregation of these two elements is probably due to the large amount of yolk present and the compressed condition of the egg, which cause delay in gastrula-

tion (epibole). It is probable that all the mesoderm arises from cells turned under the lip of the blastopore.

2. The entrance of supernumerary spermatozoa into the egg so greatly disturbs the process of development that the latter is either prevented or so distorted that it never progresses very far, and then in an abnormal manner.

3. The compressed condition of the egg affects the cleavage: (1) by altering the arrangement of the protoplasmic cells, (2) by necessitating increased length of the spindles in the yolk cells, (3) by preventing cleavage of the yolk, and (4) by increasing the surface of the egg and retarding gastrulation (epibole). But it is very improbable that slight alterations in the amount and direction of compression have as great an influence on development as supposed by Schimkewitz. I found nauplii which were apparently normal (save perhaps in size) hatching from hemispherical eggs. As the nauplius hatches it immediately rounds up, and assumes the same form whether it arise from a hemispherical or from a very flat egg.

## VIII. Explanation of Plates.

### Abbreviations.

$an^1$ = First antenna.
$an^2$ = Second antenna.
$b$ = Blastopore.
$e$ = Entoblast cell.
$en$ = Entoblast nucleus.
$ec$ = Ectoderm cell.
$f$ = Deeply staining protoplasm.
$g$ = Germ cell.
$m$ = Muscle cell.
$m^2$ = Mesoblast of first antenna.
$m^3$ = Mesoblast of second antenna.
$m^4$ = Mesoblast of mandible.
$m^{5-10}$ = Mesoblast of post nauplius appendages.
$md$ = Mandible.
$mp$ = Postnauplius mesoblast.
$n^1$ = Procerebrum.
$n^2$ = Neuromere of first antenna.
$n^3$ = Neuromere of second antenna.
$n^4$ = Neuromere of mandible.
$n^{5-10}$ = Neuromeres of post nauplius segments.
$o$ = Rudiment of mouth.
$o^1$ = Rudiment of lateral eye.
$om$ = Rudiment of median eye.
$x$ = Darkly staining cell in yolk.

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## PLATE II.

(*Figs. 19–24, Læmargus muricatus Kröyer.*)

FIG. 19. First cleavage spindle, prophase.

FIG. 20. First cleavage spindle, metaphase.

FIG. 21. Early prophase of second cleavage. To the right, below, is a highly magnified section of the centrosomes in which the centrodesmus and nucleus of the yolk cell are shown.

FIG. 22. A little later prophase of the same. In the protoplasmic cell the nuclear membrane has begun to dissolve.

FIG. 23. Later prophase showing the elongation of the nucleus of the yolk cell. Viewed from the animal pole.

FIG. 24. Late prophase (the protoplasmic cell is in the metaphase) viewed from the vegetal pole.

(*Figs. 25–30, The Dichelestid. All eggs viewed from vegetal pole.*)

FIG. 25. Anaphase of the first cleavage (fixation poor?).

FIG. 26. Two cell stage. The protoplasmic cell is in the anaphase of the second cleavage.

FIG. 27. Anaphase of the second cleavage viewed from the vegetal pole (the protoplasmic cell is in the telophase).

FIG. 28. Prophase of third cleavage.

FIG. 29. Late prophase of fourth cleavage $a^{4.2}$, $b^{4.2}$ and $c^{4.2}$ are almost completely hidden by cells lying over them.

FIG. 30. Spindle in the yolk cell, metaphase of fourth cleavage, from two consecutive sections and magnified more highly than Fig. 29. Stained with safranin-gentian-violet. The distinctness of the "centrosomes" is exaggerated. The dark granules on the astral rays of the sphere to the right are lumps of hyaloplasm. The delicate network of hyaloplasm between the yolk spherules is represented by dotted lines but the yolk itself is not shown.

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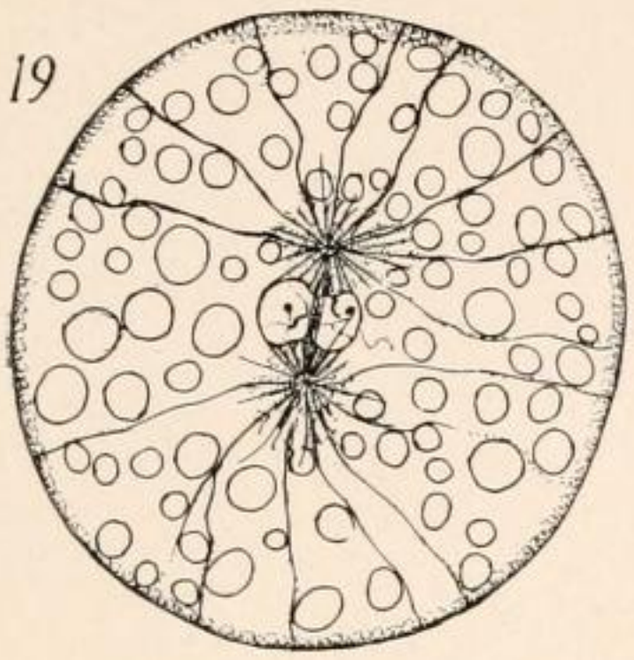

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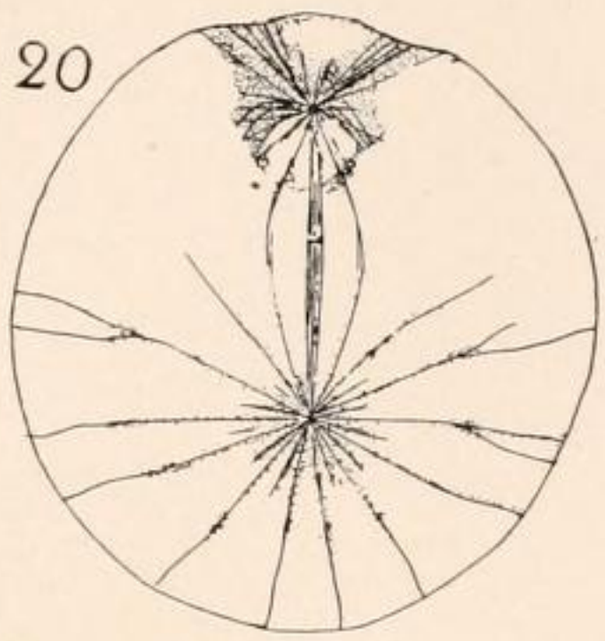

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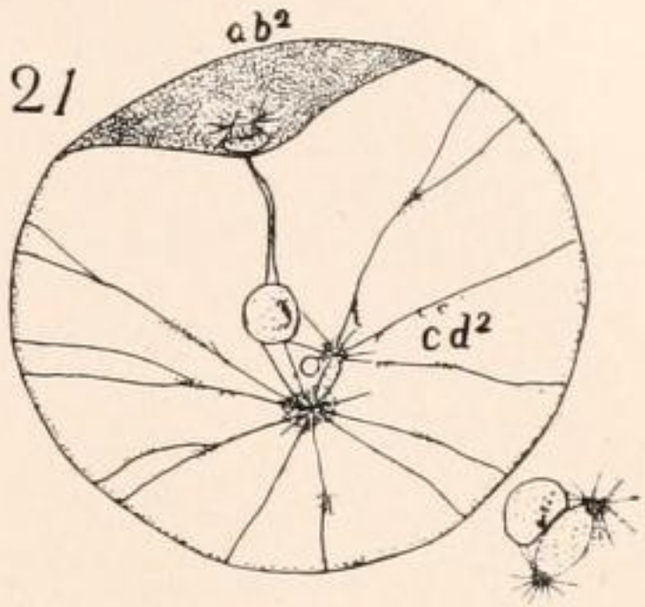


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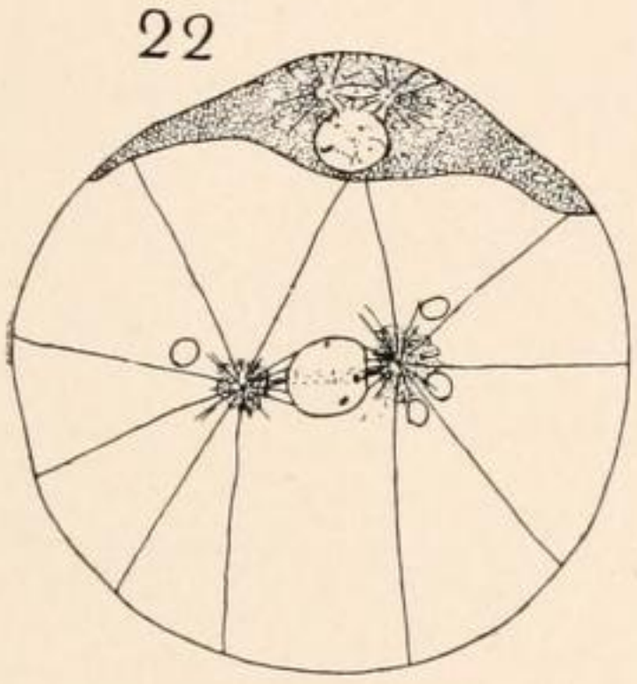

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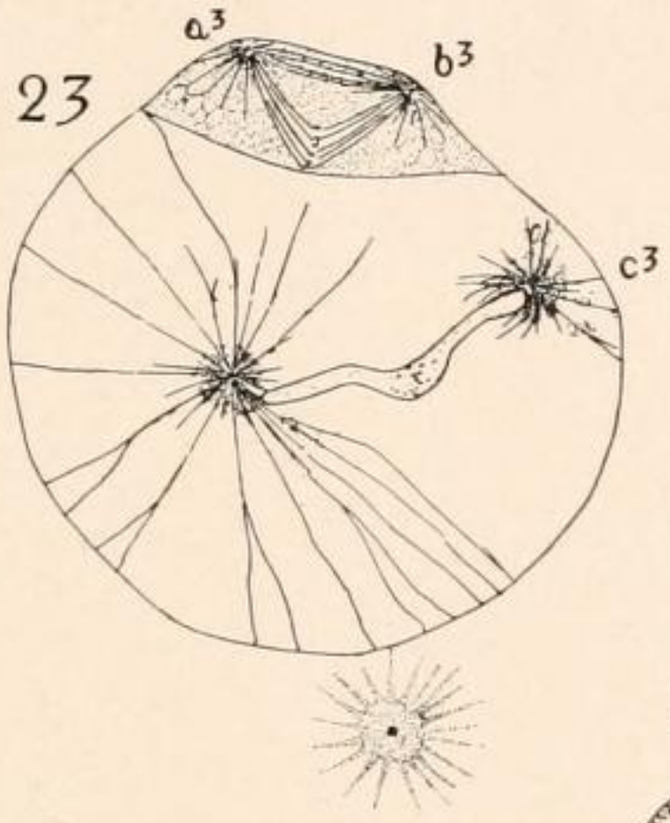


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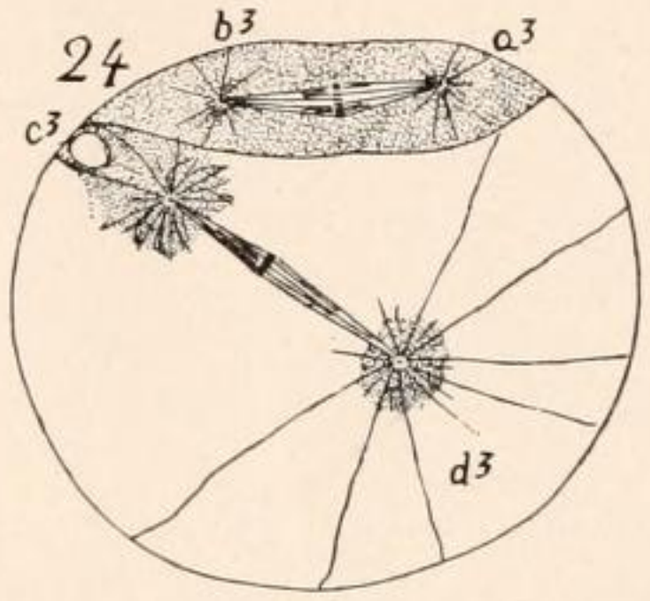


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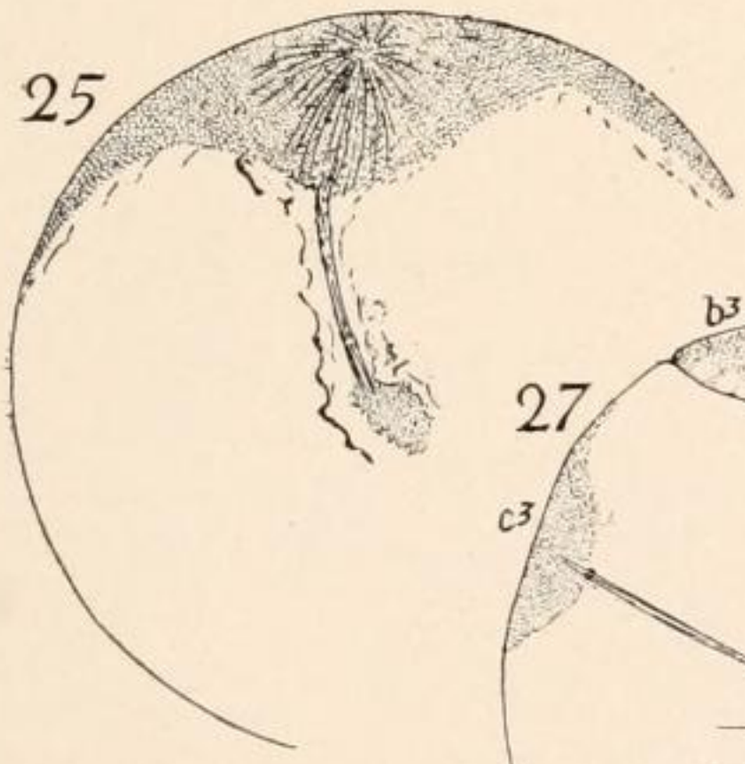

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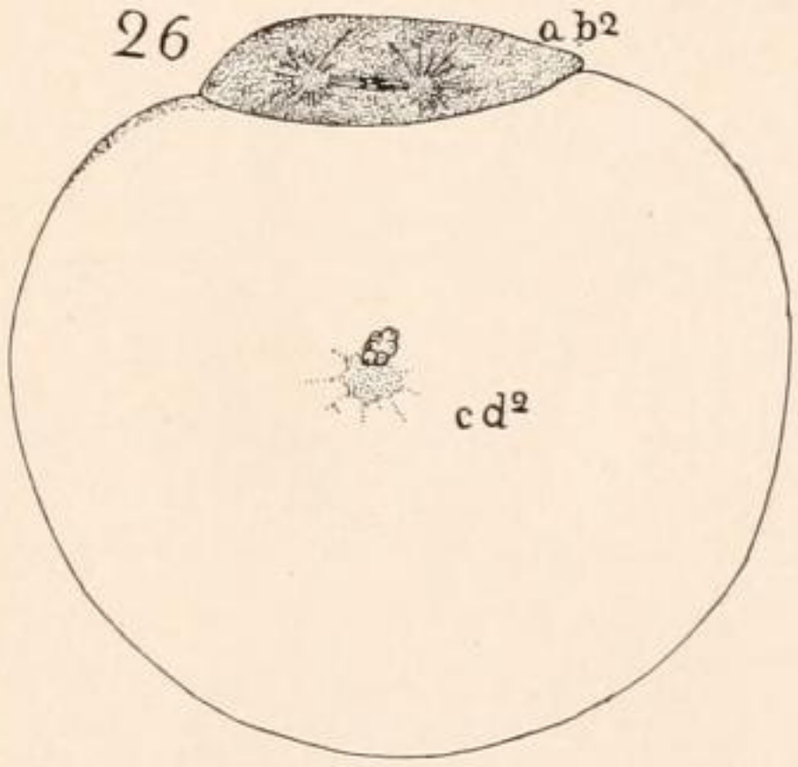


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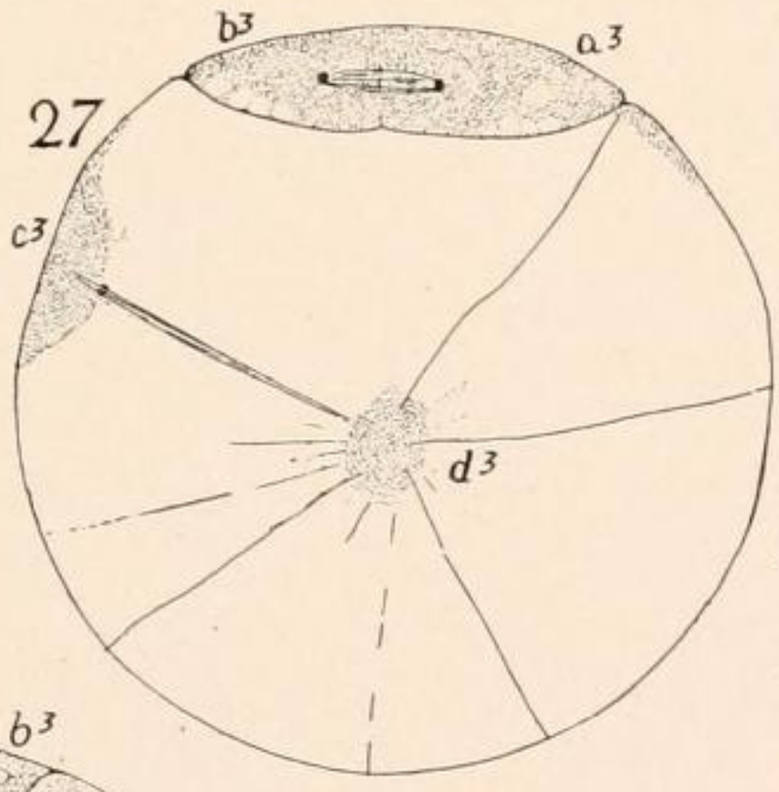


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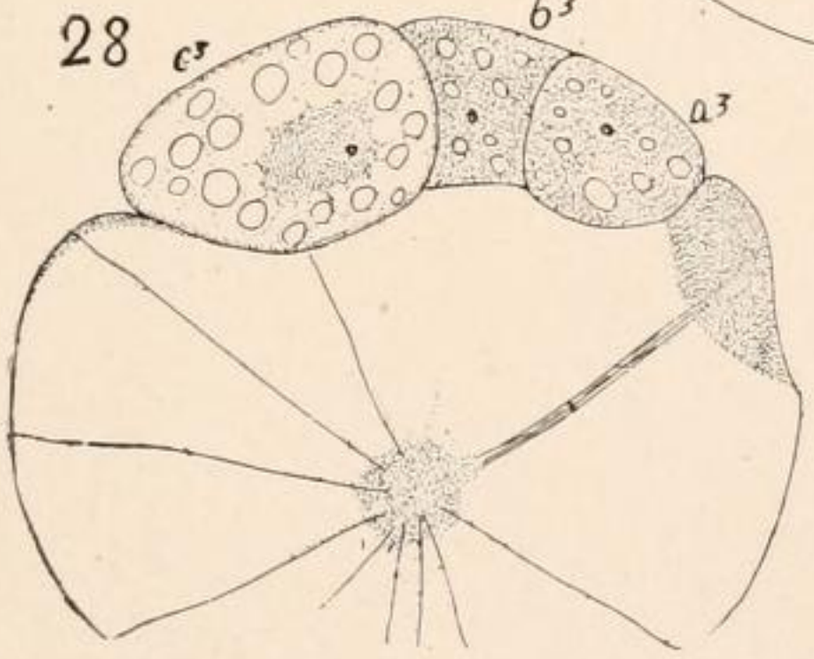


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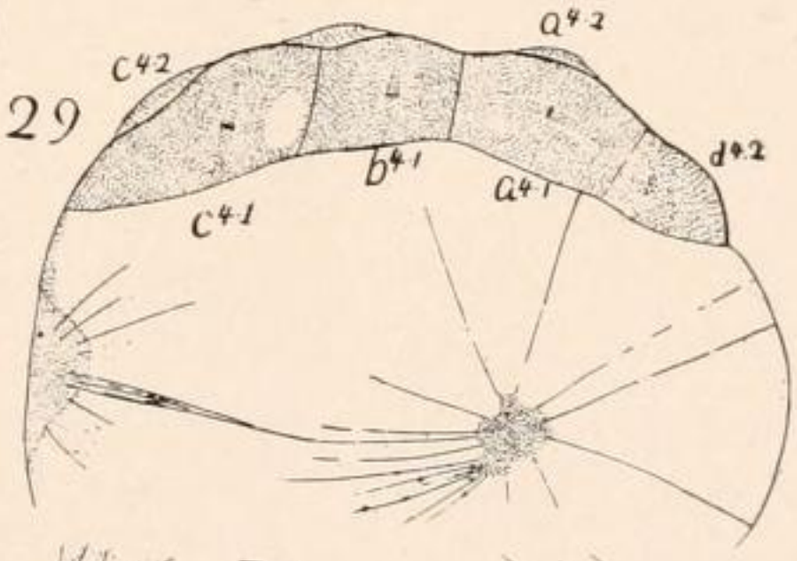


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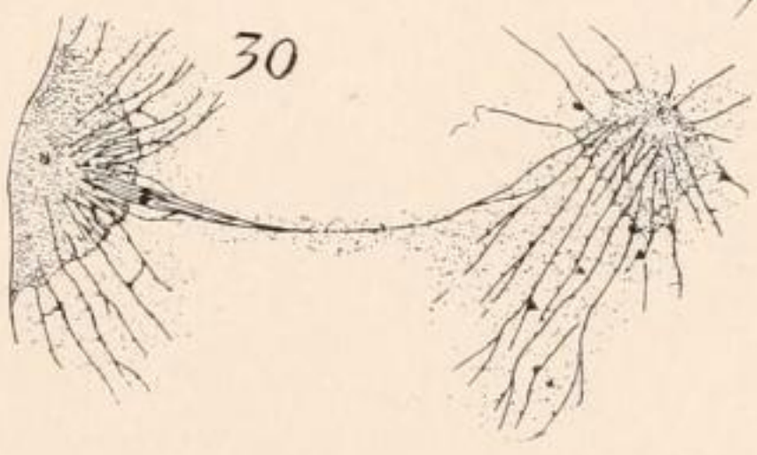

## PLATE III.

(In Figs. 31, 32 and 33 the derivatives of *a*, *b*, *c* and *d* are separated by heavy lines.)

FIG. 31. Sixteen cell stage. The cells of the animal-pole-side are shown by dotted lines. $a^{5.1}$, $b^{5.1}$ and $b^{5.2}$ are in the metaphase of the fifth cleavage.

FIG. 32. Thirty two cell stage. The cells of the animal-pole-side are shown by spaced lines.

FIG. 33. Sixty two cell stage. The primary entoblast, $d^{6.1}$, and the primary germ cell, $d^{6.2}$, have not begun the sixth cleavage while $d^{7.10}$, $d^{7.11}$ and $d^{7.12}$ are in the metaphase or anaphase of the seventh.

[*Figs. 34–36, The Dichelestid. Both embryos seen from ventral (vegetal) side.*]

FIG. 34. A later stage than the one shown in Fig. 36, *B*, Plate VI. The primary entoblast cell is dividing. The primary germ cell (*g*) has grown to large size and the blastoderm is beginning to grow over it. At the sides of the figure some mesoblast cells have been turned under the rim of the blastopore ($m^{2+3}$).

FIG. 35. A later stage than Fig. 34. The entoblast is in the telophase of the second division. The yolk is cut through completely by both divisions of the entoblast. The primary germ cell has divided (*g*) and the blastoderm has grown over it.

FIG. 36. Hemispherical eggs from the ends of egg strings.

*A*. Dorsal view.

*B*. Anterior view.

*C*. Lateral view of stage in which the blastoderm (stippled) has covered half the yolk ; the entoblast nuclei are stippled heavily.

(*Figs. 49–56, Pandarus sinuatus.*)

FIG. 49. Sagittal section of gastrula just before the closure of the blastopore (*b*). *p* = first polar body. *mp* = a cell of the post nauplius mesoblast. *x* is taken from another section of the same series and shows a small cell budded off into the yolk from an ectoderm cell. *g* is from one of the same series of sections near the median line, and represents a germ cell in its relation to the ectoderm.

FIG. 50. Part of a median cross-section of an embryo of stage *C* (Fig. 44). The section passes through two germ cells (*g*) a muscle cell (*m*) and two entoblast nuclei. The thickened portion of the ectoderm on the ventral side is the ganglionic rudiment of the second antenna.

FIG. 51. The nauplius just hatched. The ventral aspect is shown in the left, the dorsal in the right half of the figure. The median eye is seen at *om*, and the stomodæum at *o*. The rudiments of the post nauplius ganglia ($n^{5-10}$) and appendages ($m^{5-10}$) are clearly differentiated. The entoblasts (*en*) are still scattered through the yolk.

FIG. 52. Section of one of the four germ cells of the nauplius showing the sixteen chromosomes.

FIG. 53. Enlarged view of section of divided cell from rim of blastopore in Fig. 40. All the chromosomes are not included in the section.

FIG. 54. Prophase from same region.

FIG. 55. Telophase.

FIG. 56. Cross-section through germ cells, ventral ganglia, and ectoderm of nauplius twenty-four hours after hatching.

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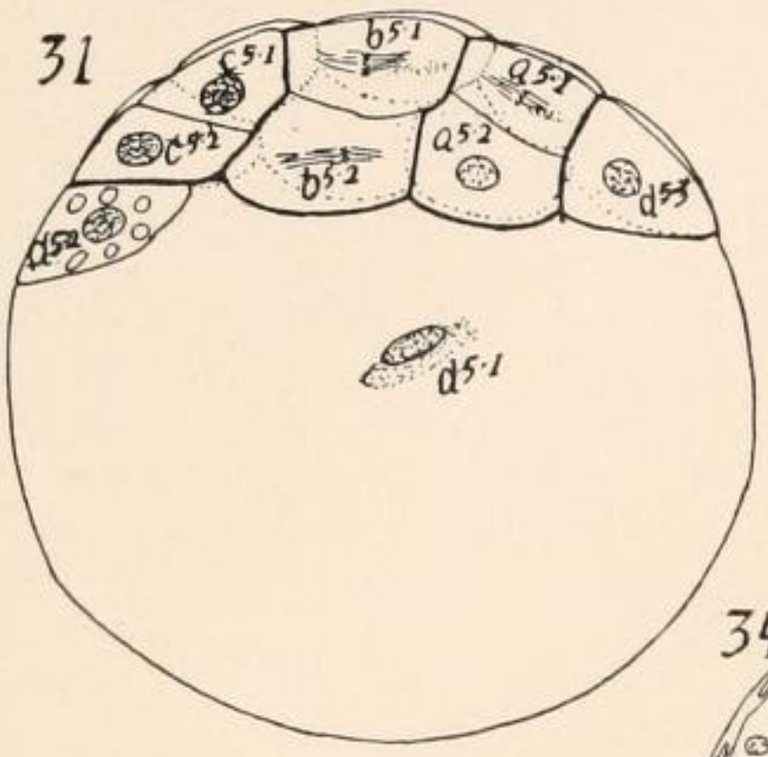


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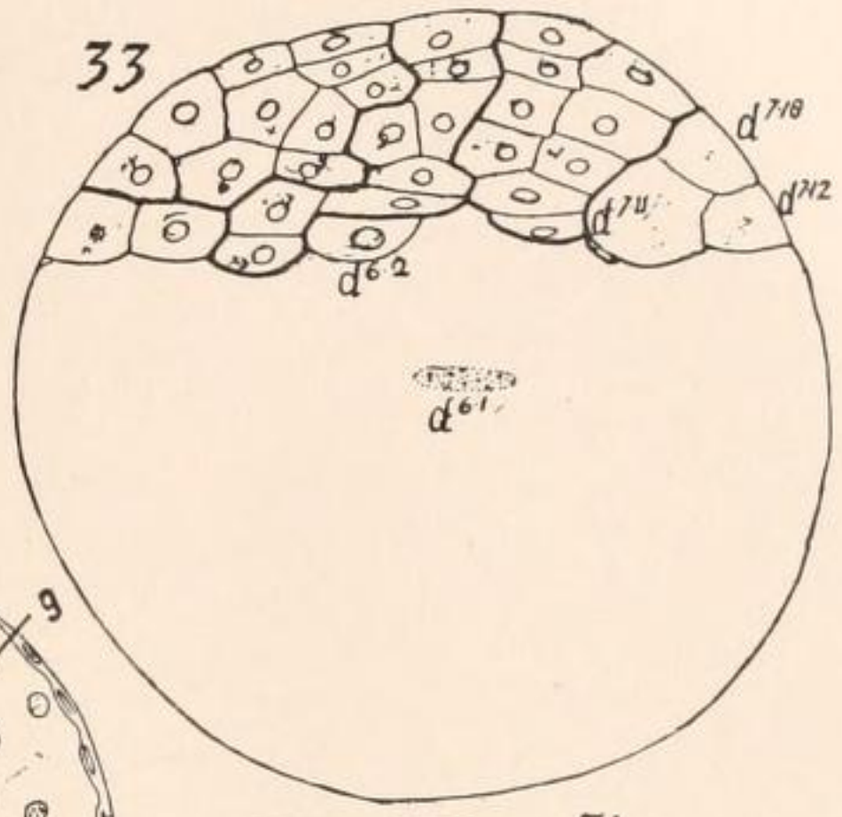


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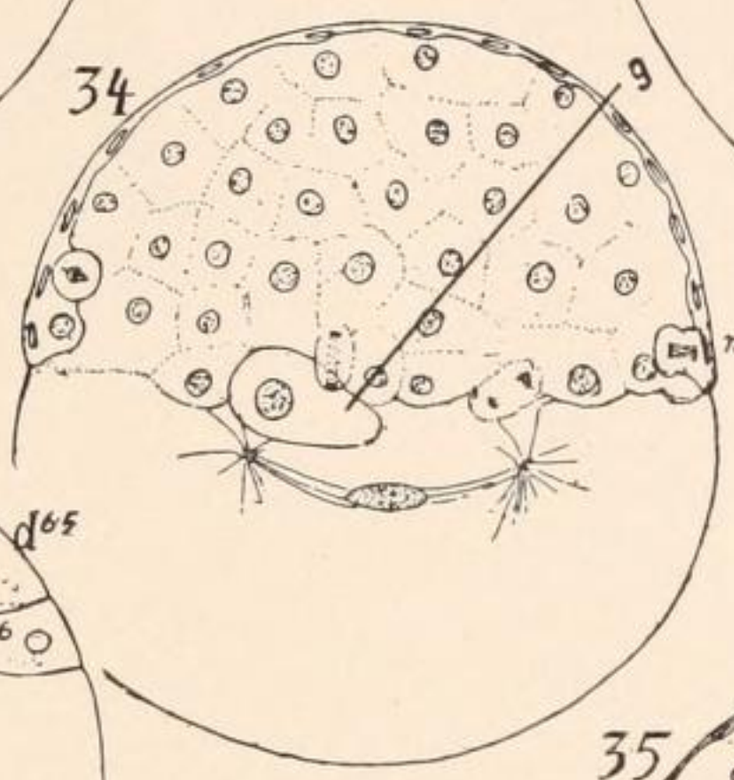


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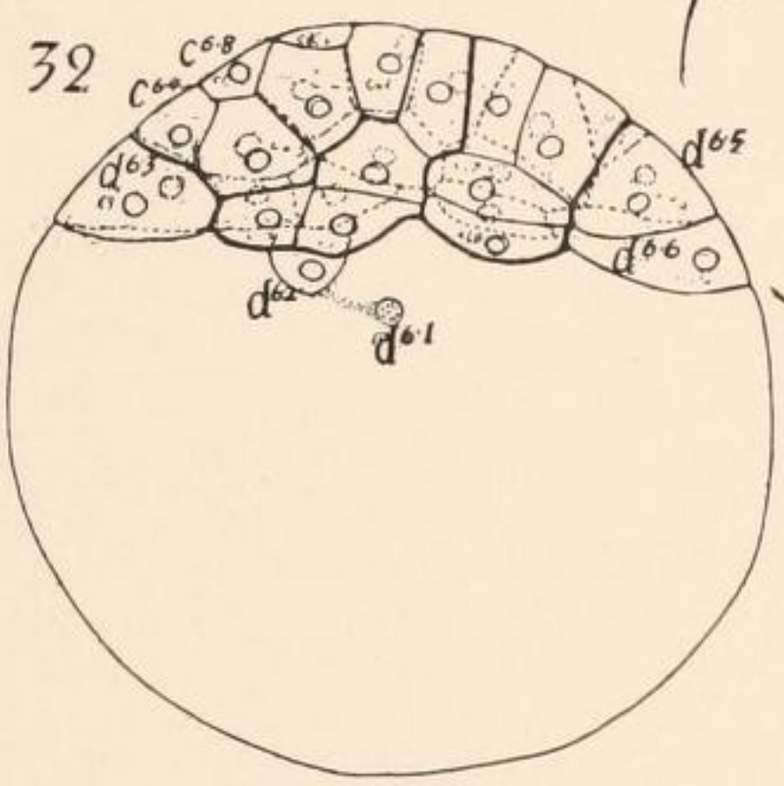


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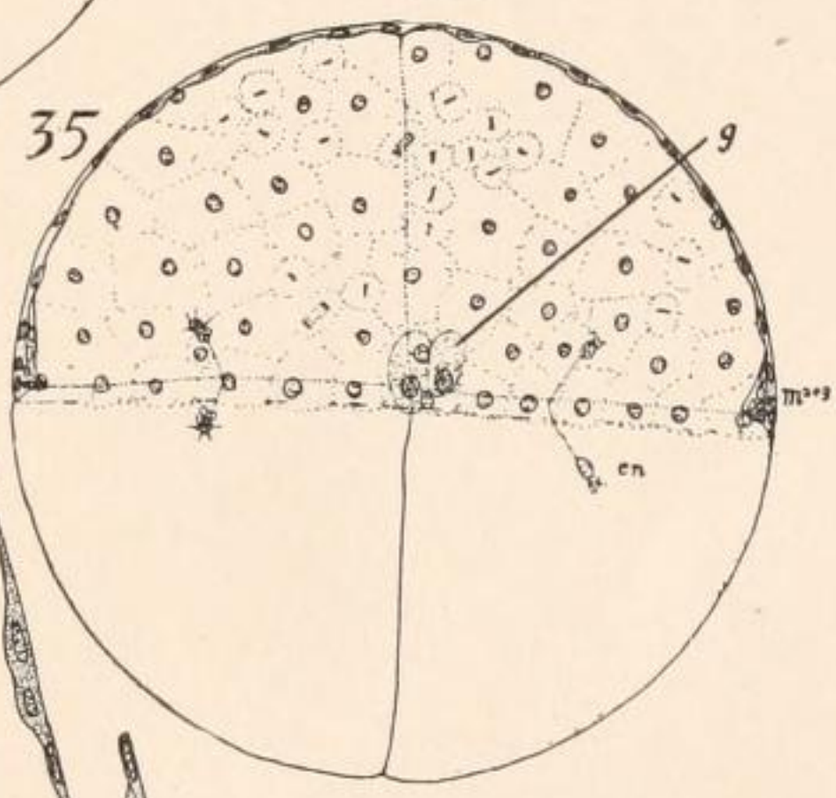


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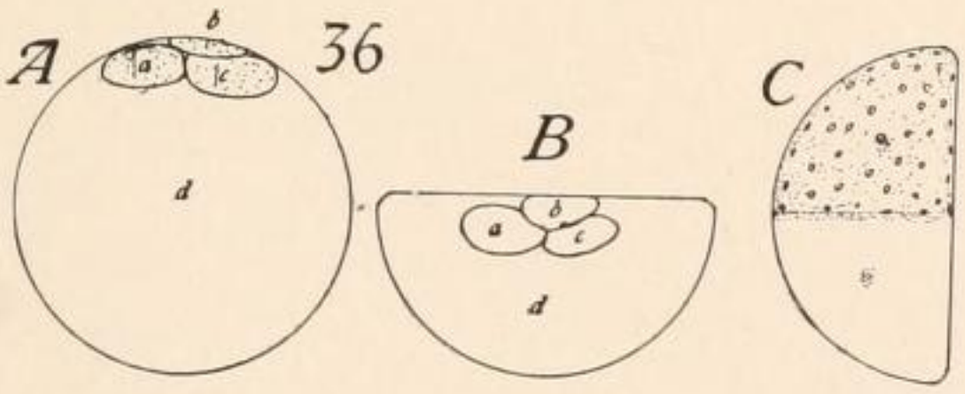


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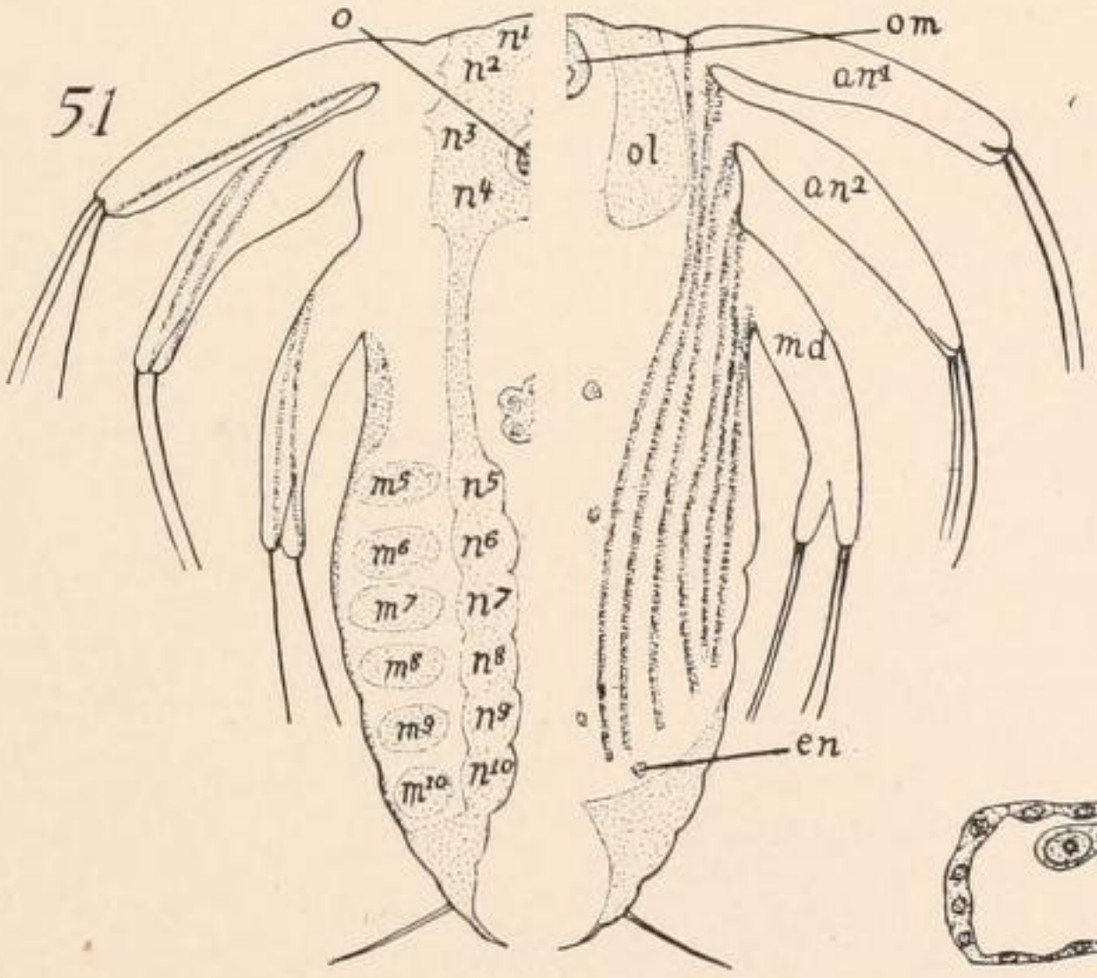


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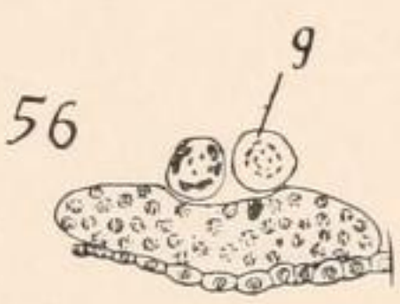


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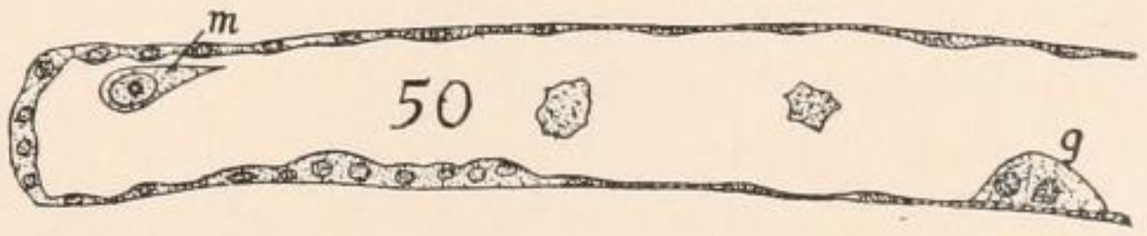

## PLATE IV.

(*Pandarus sinuatus Verrill. Figs. 37–39, viewed from the vegetal pole.*)

FIG. 37. Shows the metaphase of the first cleavage of the primary ectoblast cell. To the right below is a more highly magnified view of the spindle of the same, in the prophase. $g$ = primary germ cell.

FIG. 38. Shows the prophase of the second cleavage of the entoblast. The blastoderm is growing over the primary germ cell ($g$). Some mesoblast cells have been turned under the rim of the blastopore at the sides of the figure.

FIG. 39. About the same stage as Fig. 38, but the entoblast has not begun its second cleavage and the primary germ cell ($g$) is in the metaphase of division.

[In Figs. 40–42 half of the dorsal (animal) side of the egg is shown to the left and half of the ventral (vegetal) side to the right.]

FIG. 40. Just before closure of the blastopore ($b$). $X$ = small cell budding into the yolk. $An$ = mesoblastic rudiment of the first antenna. $An^2$ = mesoblastic rudiment of second antenna. $Md$ = mesoblastic rudiment of the mandible. The germ cells are shown (stippled) beneath the ectoderm — they have separated from one another.

FIG. 41. Stage *A*. Just after closure of the blastopore. The dorsal and ventral ectoderm is omitted save in the anterior and posterior portions. $m$, $m$ = mesoblast (muscle?) cells, just beneath the ectoderm. The germ cells have divided.

FIG. 42. Stage *A* a little later than 41. The eight stippled rods are the spindles of the entoblast. The other stippled areas are mesoblast.

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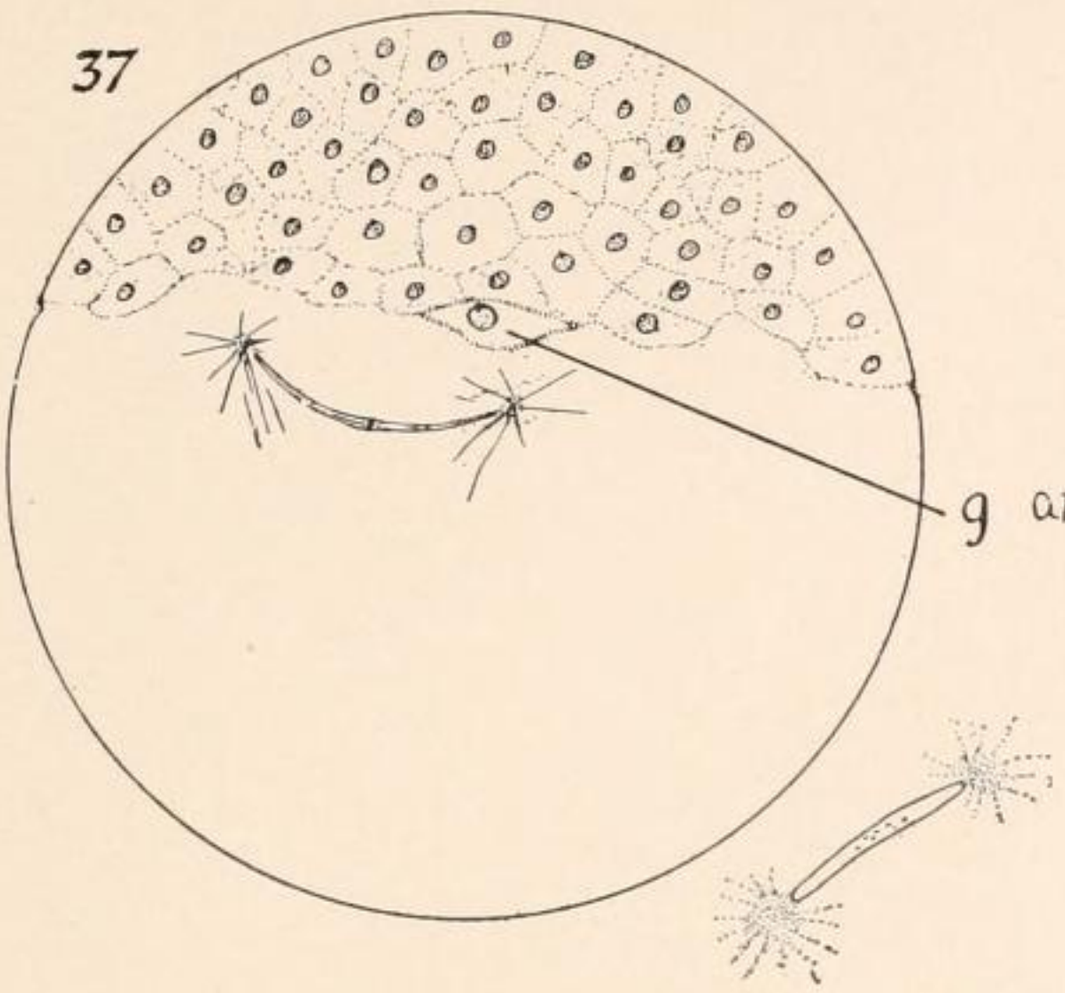


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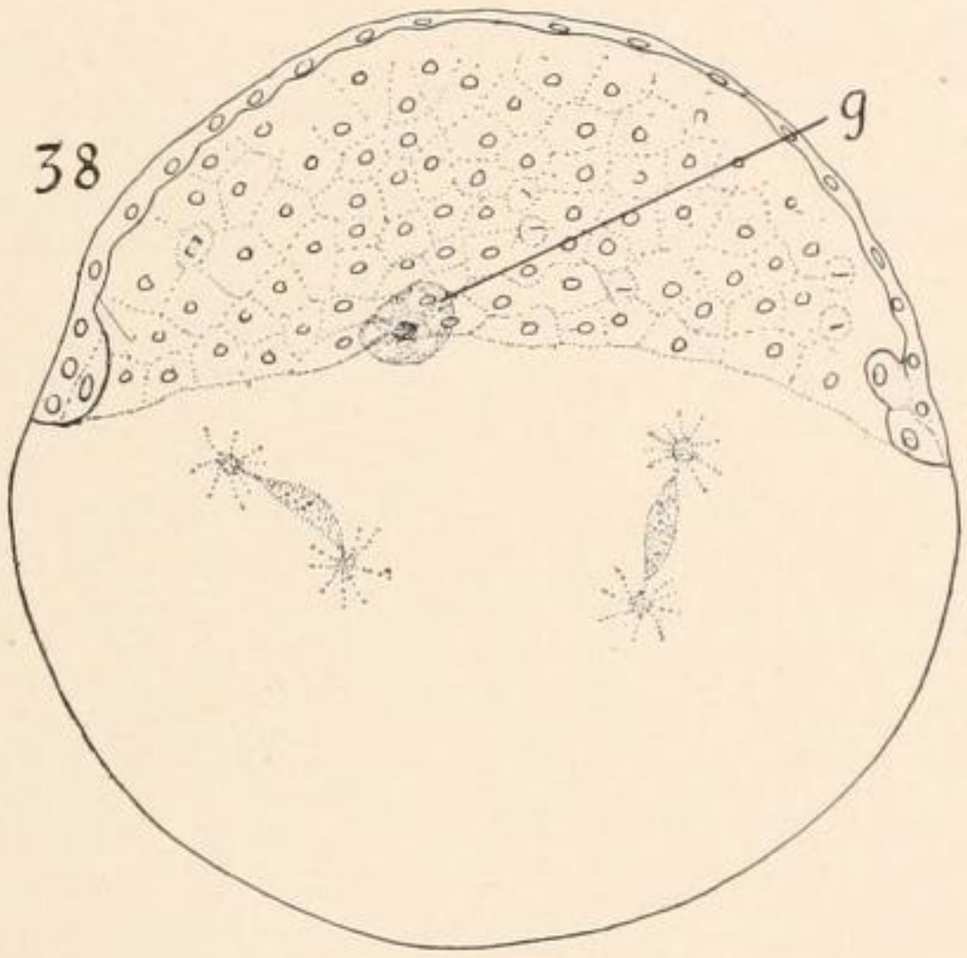


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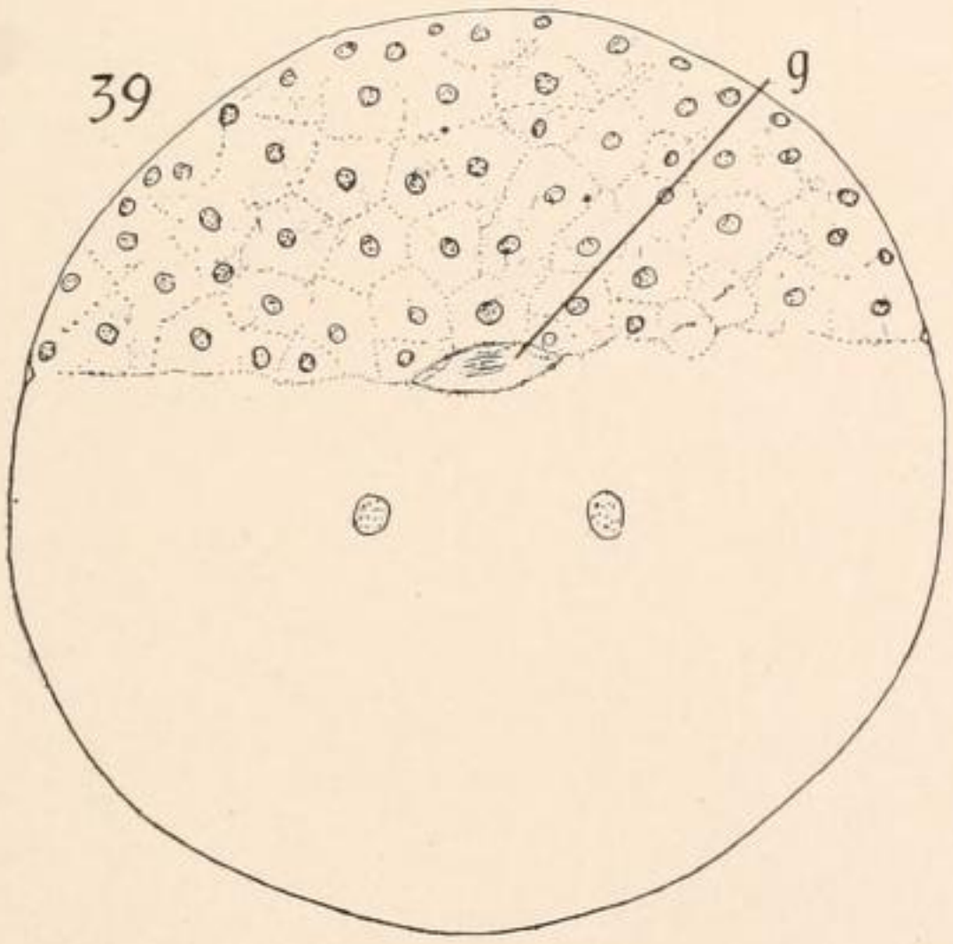


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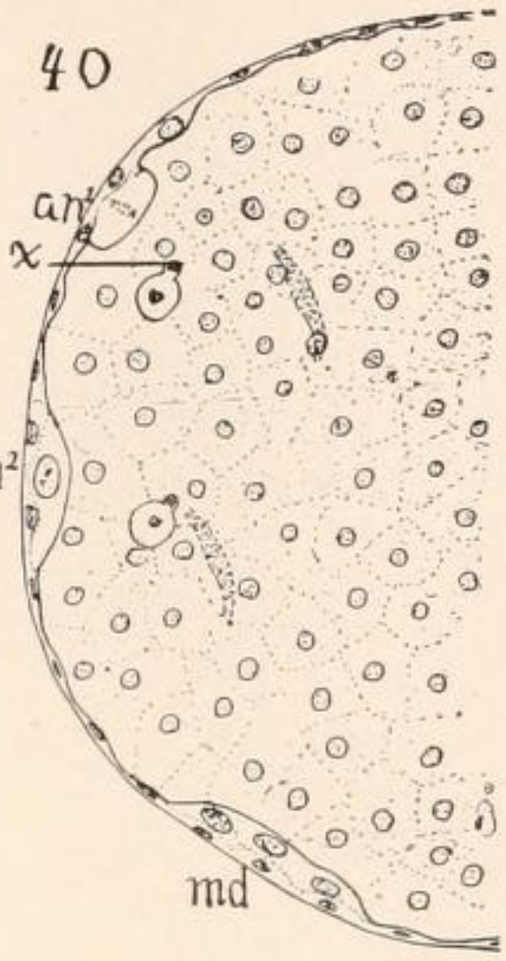


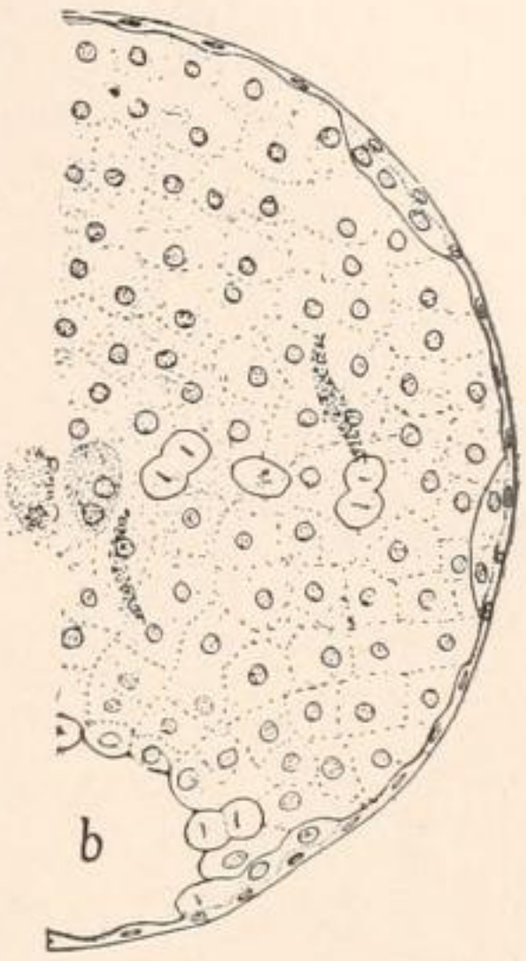


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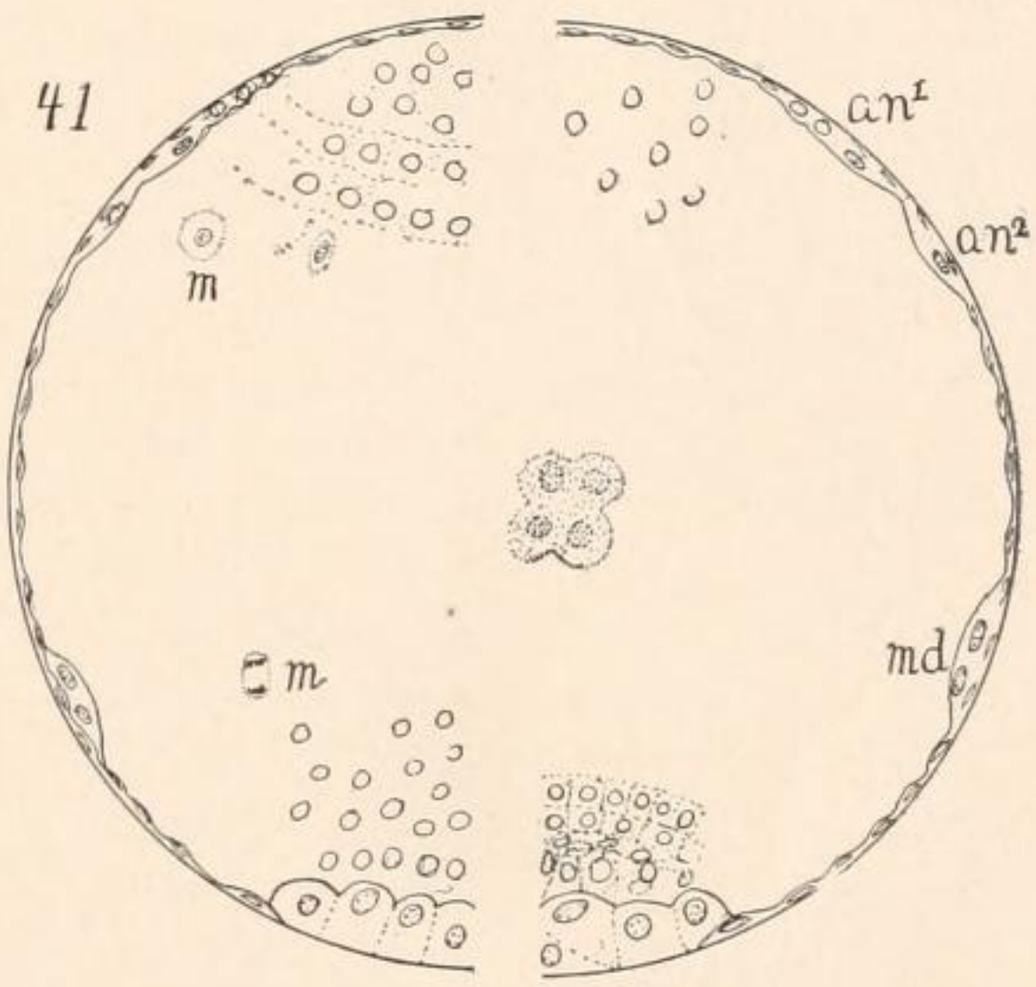


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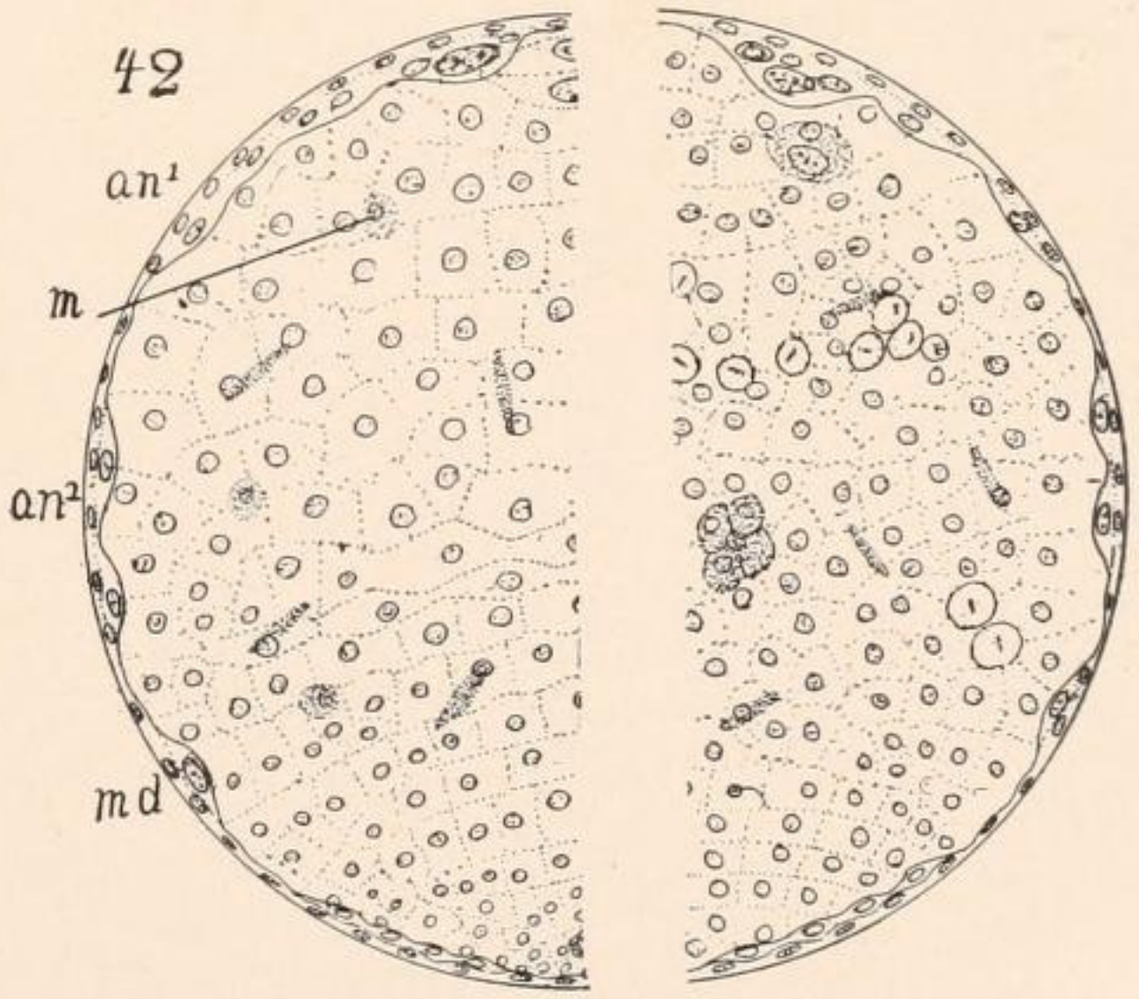

## PLATE V.

(*Pandarus sinuatus Verrill.*)

(One half of each figure shows the dorsal, the other half the ventral aspect. In Figs. 43 and 45 the ventral aspect is to the right and in the remaining figures to the left.)

FIG. 43. Stage *B*. The rudiments of the ganglia, $n^{1-4}$, are shown. The strippled rods are spindles of entoblast nuclei.

FIG. 44. Stage *C*. At *x* are minute (mesoblast?) cells in the yolk. Some muscles in the right side of the figure are beginning to elongate. Over the ganglion of the second antenna is a cluster of mesoblast cells of unknown history.

FIG. 45. Stage *D*. The appendages are beginning to bud out. The ganglia have become connected by thickened ectoderm (outlined by a dotted line). The muscle-cells are beginning to elongate radially, and above them the ectoderm cells are elongated in the opposite direction (some of them are represented by dotted outlines, *ec*). The entoblast is not represented, but a few small dark cells are shown in the yolk.

FIG. 46. Stage *D*, later than in Fig. 45. The ectoderm thickening to form the stomadæum is shown at *o*.

FIG. 47. The nauplius a short time before hatching.

FIG. 48. The nauplius just before hatching. In the left half of the figure the ganglia and mesoblastic rudiments of the post nauplius appendages are shown (stippled) in process of formation.

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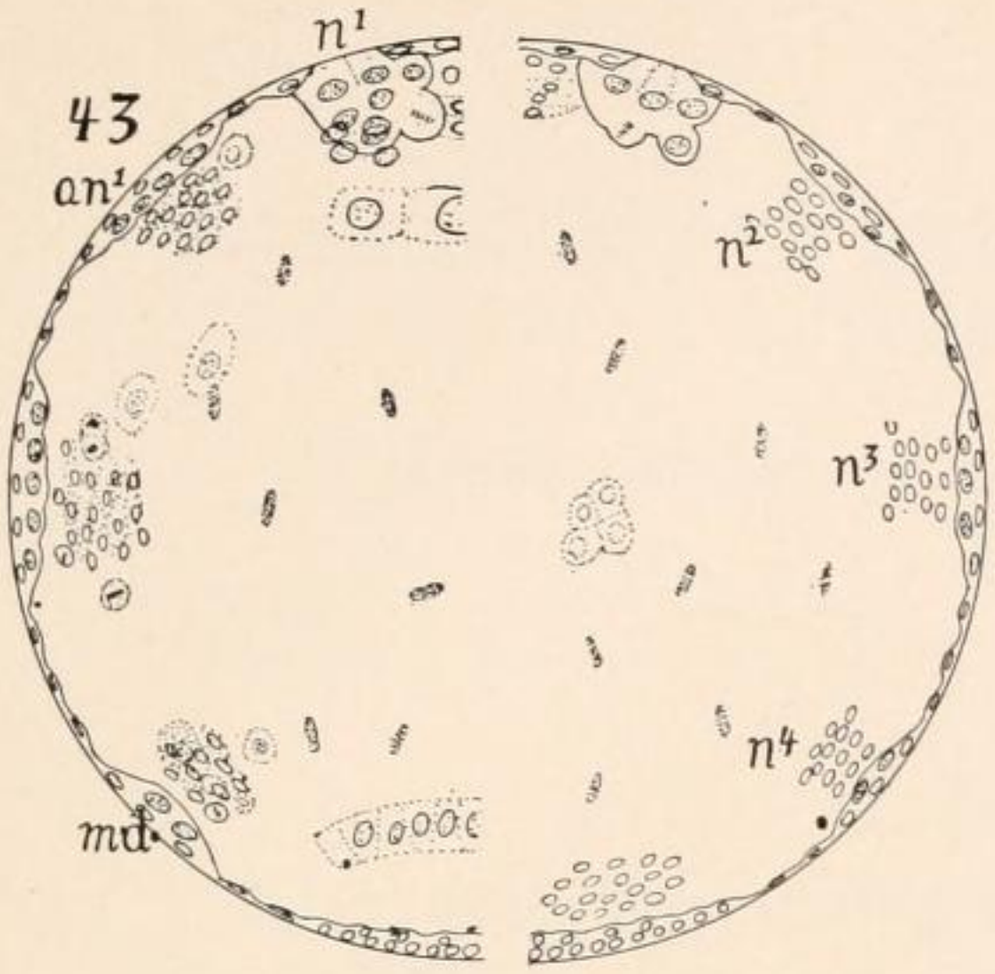


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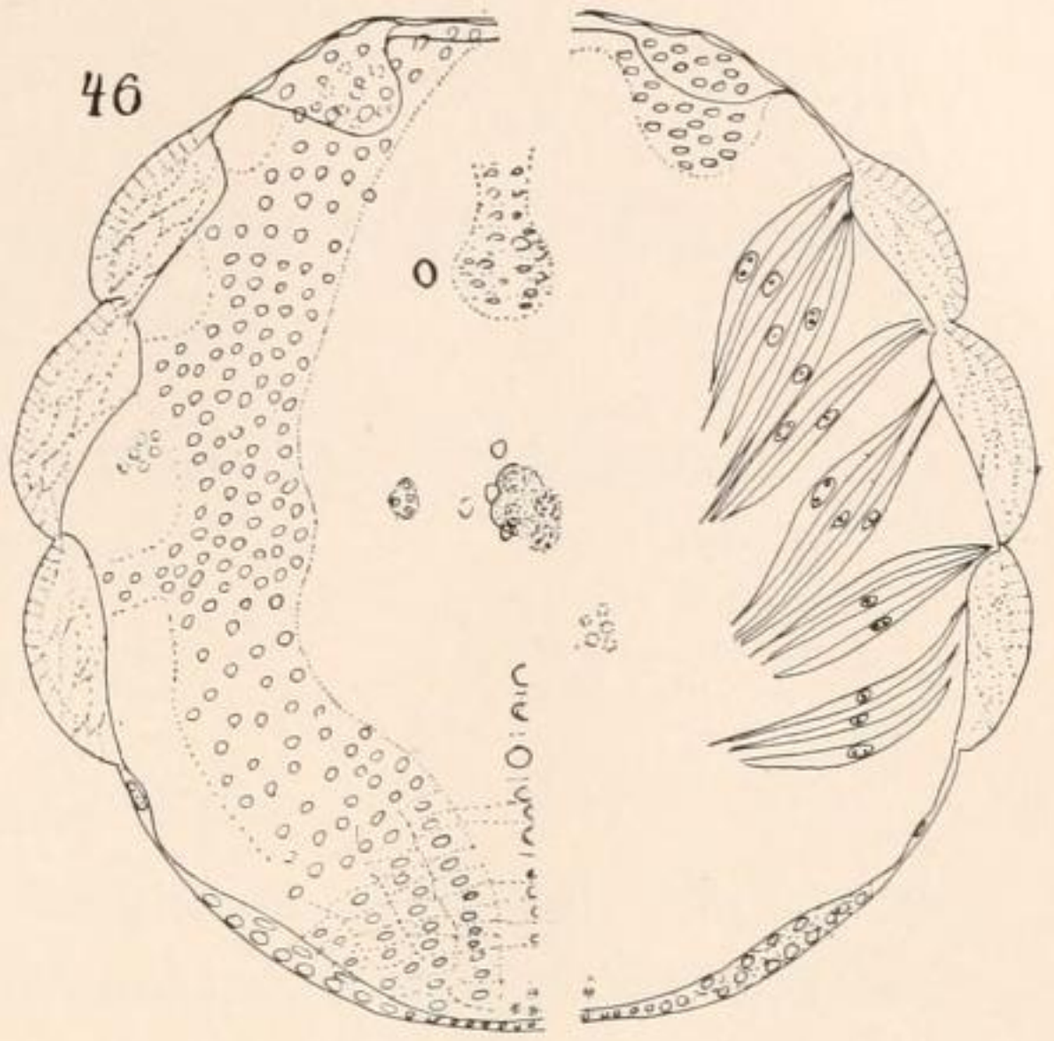


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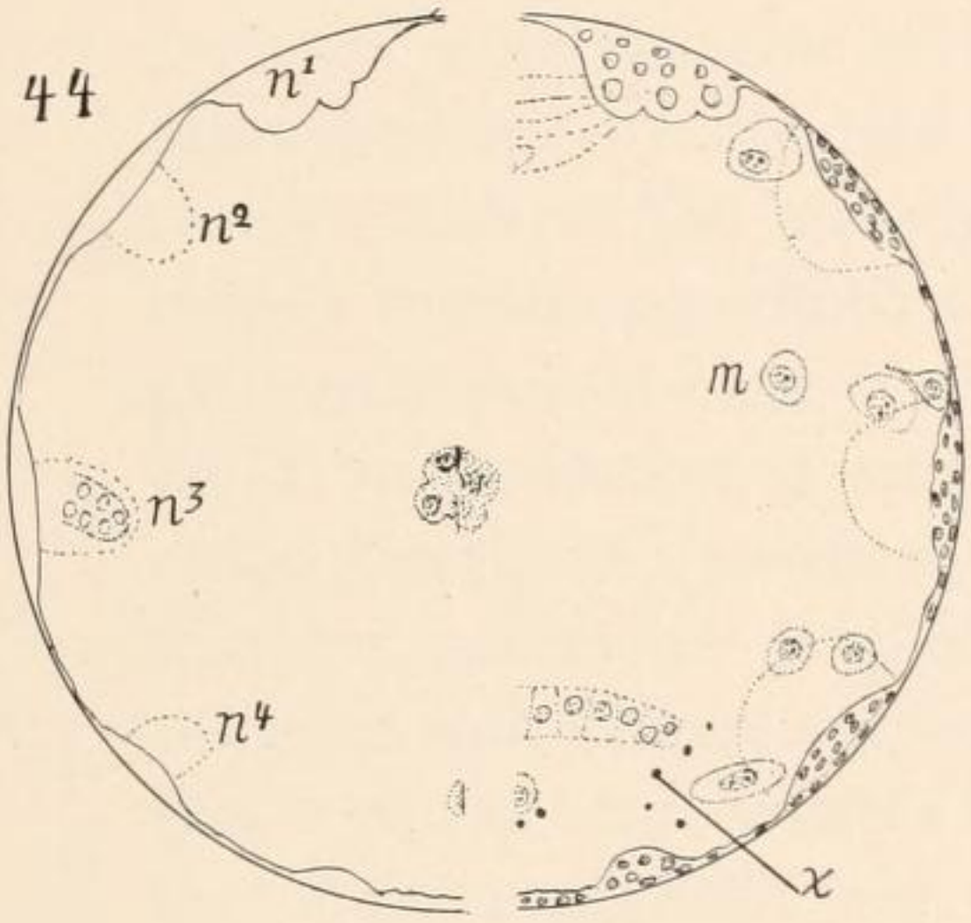


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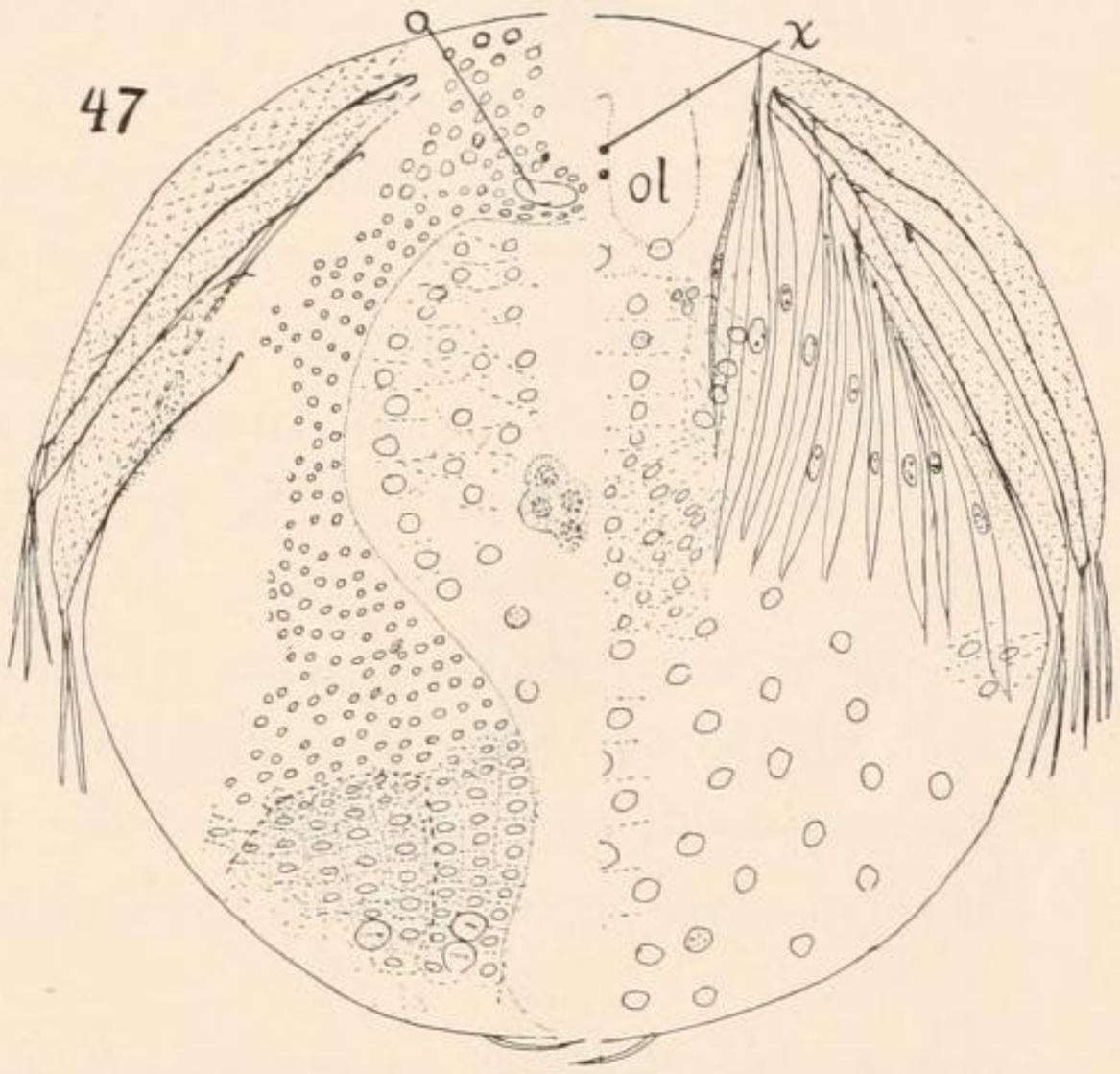


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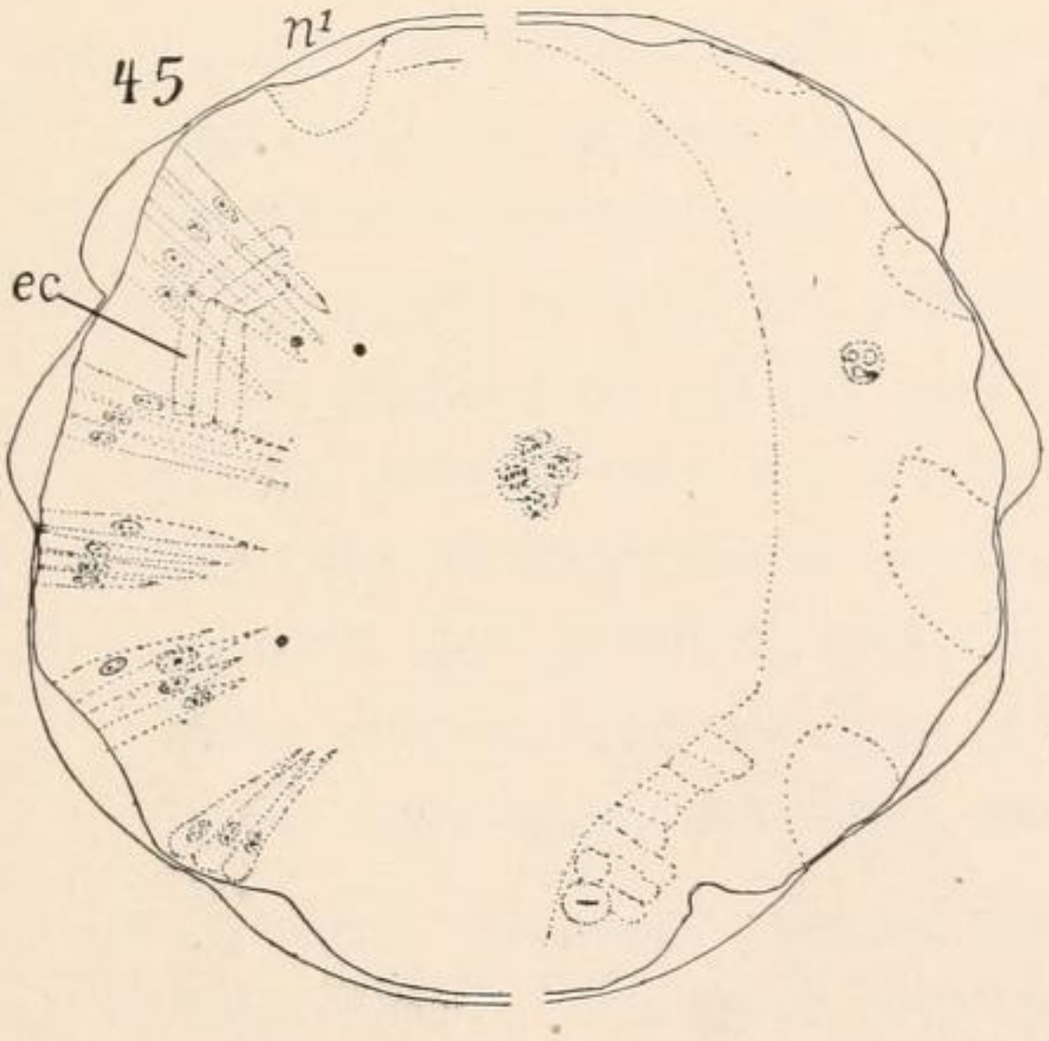


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