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CONTRIBUTIONS ON THE ONTOGENY OF OIKOPLEURA DIOICA

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Translated into English. Original is written in Germany.

INTRODUCTION

Our current knowledge of the reproduction and development of the appendicularians is extremely scant. While the number of investigators studying the embryology of the ascidians – ever since KOWALESKY derived from [the study of their embryology] their close relationship to *Amphioxus* – has been rather large, the ontogeny of the group that used to be generally regarded, and still is by most, as the most original of the tunicates has remained almost entirely unknown.

In 1903, GOLDSCHMIDT gave a brief and, in my opinion, not quite accurate description of the larva of *Oikopleura dioica* Fol discovered by him at Rovigno (Istria) [in Croatia] in the upwelling. That is the extent of our knowledge.

At the end of September last year, while studying plankton samples that had been taken in the harbor of Helder [The Netherlands], I noticed that *Oikopleura dioica*, which, with the exception of the winter months, occurs regularly in plankton near Helder, was in the process of reproduction. Several times I watched under the microscope how a larva hatched from an egg, and that caught my attention and brought it to the subject [of the work described here]. Several times I followed closely its rapid development, and one day, when a large number of eggs were present in the plankton, I added a little bit of formalin. Later examination yielded that the thus fixed sample, that had been taken on September 28, 1909, in the morning under calm weather conditions, was very rich in eggs in various stages of development, ranging from early cleavage stages to the typically bent larva ready to hatch, but that it contained almost no hatched larvae. Over the following weeks I examined plankton daily, that had been sampled under various weather conditions, during high and low tides, at different times during the day. Although I regularly found a limited number of larvae and individual eggs as in previous samples, they never again were numerous enough to make fixing and sorting through the sample worthwhile. After a few weeks they [*Oikopleura dioica*] disappeared completely from the plankton, including the adult animals. It is peculiar, that while GOLDSCHMIDT found the larva near Rovigno during the second half of March, I found the same stages near Helder during the second half of September. Next spring I will pay attention to whether *Oikopleura* might be reproducing at that time as well. During the summer that definitely is not the case.

I examined the fixed sample in small quantities on a slide under the microscope, at medium magnification.

I isolated the eggs I found with a pointed needle and used a fine pipette to transfer them into glycerin onto another microscopic slide. Soon they became more translucent than the living eggs that are strongly refractive. In this way I was able to isolate and examine a few hundred eggs, and I was unexpectedly lucky in that I found over time a complete series of subsequent stages of egg cleavage in addition to all transitional stages with dividing cells. Thus, eventually I was able to follow the egg cleavage without any gaps up to the moment where the cells of the endoderm are completely covered by those of the ectoderm [i.e., gastrulation]. I even succeeded in finding a still undivided egg, in which, however, the first mitotic spindle was already preformed.

As LOHMANN recently showed in a paper on the vertical distribution of the appendicularians in the Strait of Messina, the population of *Oikopleura dioica* is densest not at the surface but at a depth of 10 to 30 meters. "This completely aberrant behavior is rather strange and makes one wonder, whether *Oikopleura dioica*, which is distinguished from all other appendicularians by its separation of the sexes and its mandatory coast-dwelling, is not somehow tied in its existence to the sea-bottom. After GOLDTSCHMIDT's finding of the unusual larva is urgently required that the ontogeny of this species is not investigated merely in a comparative-anatomical manner, but also biologically…" However, based on what I stated above I am rather certain that the entire development occurs in the plankton. Sure enough, I cannot report anything on the laying of eggs and fertilization. However, I assume that *Oikopleura* is tied to the coasts because the development of the eggs either requires a low salt content or is enhanced by it. The plankton in which they [the eggs] were found consisted of typical neritic species, mainly the following:

Silicoflagellata: *Distephanus speculum*

Diatomaceae: *Bacteriastrum varians*

Biddulphia mobiliensis, sinensis

Chaetoceras debile, densum, sociale, Willei

Eucampia zodiacus

Guinardia flaccida

Rhizosolenia delicatula, setigera, Shrubsolei, Stolterfothii

Thalassiosira gravida

Peridiniales: *Ceratium batavum, fusus.*

Protozoa: *Cyttarocylis serrata var*. (Van Breemen).

Bryozoa: *Cyphonautes*.

Copepoda: *Oithona nana*.

Tunicata: *Oikopleura dioica* (ripe)

A typical coastal plankton, that is. From the regular measurements of temperature and salt content, conducted three times daily, I find that on September 28, 1909, at 7 a.m., the temperature of the water in the harbor was 14.4 °C, and its specific weight was 1.0236, at 2 p.m. the figures were 13.8 °C and 1.0224, respectively. At the time the plankton was sampled (10 to 11 a.m.) the temperature will most likely have been about 14 °C and the specific weight 1.023, which corresponds to a salt content of 30.5 0/00.

CLEAVAGE OF THE EGG

The egg of *Oikopleura* is spherical and has a diameter of on average 0.088 mm, a noteworthy size for such a small animal. According to CONKLIN the eggs of *Mogula* are only slightly larger, namely 0.10 mm in diameter, but those of *Cynthia* and *Ciona* are 0.15 mm. Strangely the diameter [of the Oikopleura egg] decreases as development progresses. While eggs in the initial stages of cleavage had a diameter of up to 0.095 mm, it decreased to as low as 0.085 for those already containing the doubly bent embryo. For any given developmental stage the diameter may vary as well and be larger or smaller by a few microns. The egg, by the way, is strongly refractive but very light and translucent when brought into glycerin where at deeper settings bottom cells can be seen almost as clearly as those at the top. Differently colored or otherwise distinguished yolk substances like those CONKLIN found to be especially apparent in the eggs of *Cynthia partita* cannot be observed here. The egg is surrounded by a tender, thin, glass-light egg membrane which maintains the spherical shape during the cleavage process and eventually is torn and left by the larva. There were no test cells [**Translator's note: I am only aware of the term 'test cells' in a botanical context and do not know what exactly is meant here]**. The polar bodies appear to be lost early on; I never encountered them.

About the cleavage process in general it can be said that it is very similar to those of the ascidians and of *Amphioxus*, for it is just like those holoblastic and adequal, and progresses bilaterally symmetrically from the beginning.

1st *Division* – The first division splits the egg into two identical halves which are pushed together so tightly by the apparently elastic egg membrane that they are not able to assume a spherical shape each, but appear as two semi-spheres pushed together with a large contact area. I found this two-cell stage 3 times.

2nd *Division* – At stage 4, encountered by me 4 times, no differences in the blastomere sizes were discernible either. During interphase the four segments align themselves in such a way that two opposing ones touch each

other in a "refractive groove," **[Translator's Note: I have not been able to find this term anywhere else on the web or in dictionaries and am not entirely sure what is meant by it. Alternative translations might be "breaking groove," "refractive furrow," "breaking furrow"…]** while the two lateral segments are pushed away from the pole. At one of the poles this groove was perpendicular to that at the other pole and was just as long (Pl. I. Fig. 4). The four blastomeres continue to be pushed together by the egg membrane, and, therefore, have the shape of quarter-spheres.

In the ascidians and *Amphioxus*, at stage four, two of the neighboring segments are a little larger than the other two, so that at that stage already a bilateral symmetry becomes visible. Only in *Distaplia* magnilarva, a compound ascidian, DAVIDOFF was not able to determine any difference in size, either.

3rd *Division* – The subsequent division is somewhat unequal and leads to a stage 8, observed 5 times by me, which corresponds to the same stage in the ascidians and in *Amphioxus*. 4 somewhat smaller cells are separated from 4 somewhat larger cells. However, among the 4 smaller cells, 2 in turn are a little larger than the other two, and because the 4 original blastomeres were identical in size, it follows just from that that of the 4 larger cells of stage 8 one pair as well is smaller than the other. Of the 4 original blastomeres one pair has divided more strongly unequally than the other pair, in which the division deviates only minimally from an equal one. The equatorial furrow is of major morphological importance in *Oikopleura* as well, for it separates the endodermal primordium/anlage [**Translator's note: I am not sure whether one can differentiate between the two and will use primordium as the preferred translation from here on.]** and probably also the primordia of the mesoderm and the neural tube from that of the ectoderm. During the further development the 4 smaller cells come to represent the vegetative half, and the 4 larger ones the animal half of the egg; a somewhat deviant behavior, for in the ascidians and in *Amphioxus*, in which this stage is similarly composed, it is the larger cells, as usual, that provide the vegetative half, and the smaller ones that provide the animal half.

At stage 8, for the first time the bilateral symmetry becomes apparent, and it now is possible to orient the egg and to name the cells. In doing so I preferred the nomenclature hitherto generally used in cell lineage works on annelids and mollusks over that introduced by KOFOID and later used by CASTLE and CONKLIN for the ascidians. For in the [latter] system it is not always easy to determine the origins of a cell and its membership of a specific cell complex at first sight from its formula. Also, the number of succeeding cell generations in our case is not very large, so that the extensive exponents that the second system requires would only serve to make its use cumbersome. Further simplification is achieved by my following the example of CHABRYS and CONKLINS in naming the corresponding cells in the left and right halves of the embryo with the same letters.

This way, a mere total of two letters is required.

At stage 8 we thus want to name the endodermal cell of the anterior left quadrant A and the ectodermal cell of the same quadrant a, although the latter here is larger than the former. Similarly, we name the cells of the posterior left quadrant B and b. The cells from the right half are labeled with the same letters; to distinguish them, we will underline them (and later their exponents).

4th *Division* – The fourth division usually is the most characteristic of the different types of cleavage. The geometric regularity of the first cleavages is beginning to be influenced more strongly by differentiation, however, is not yet fully dominated by it. As a result of the interaction of these two factors [i.e., geometric regularity and differentiation] of which the former later will be completely replaced by the latter, but which at this stage still are in balance, configurations are created that make the characteristics of the cleavage patterns of the different classes of animals most apparent. And so it is that *Oikopleura*, the ascidians, and *Amphioxus* also begin to differ at this point.

In *Oikopleura* the smaller vegetal cells divide perpendicularly to the plane of symmetry, and the larger animal cells divide more parallel to it, or rather meridionally. This results in the fact that at stage 16 the 8 cells of the vegetative half form a plate comprising two rows of 4 cells each that are parallel to the median plane, with the plate bending at its distal ends around the animal half, which itself forms a similar plate, perpendicular to the first and bending at its distal ends around the vegetal half. One can illustrate this relationship by crossing one's hands horizontally in such a way that the palms touch and the right hand is on top, the left hand at the bottom. If one then bends both hands a little, the right hand would correspond to the vegetal, the left hand to the animal plate.

At first glance the blastomere configuration thus formed, unlike that of the ascidians, exhibits a surprising similarity to the same stage of the *Amphioxus* egg. The likening to the two crossed hands, by the way, was not first used by myself but by CERFONTAINE in describing stage 16 of *Amphioxus*. There, however, it is the vegetal cells which divide parallel to the plane of symmetry and the animal cells that divide perpendicularly to it. The similarity thus turns out to be rather the opposite and, relative to the ascidians, consists merely of the fact that in *Oikopleura* and *Amphioxus* the 4 vegetative cells are opposed to the animal ones in their direction of division, while in the ascidians, which in a way constitute a mediating form, one pair each of the animal and the vegetative half has maintained the direction of division of *Amphioxus*

(although the somewhat oblique plane of division already hints at a deviation leaning towards *Oikopleura*), but the other pair divides just in *Oikopleura*, i.e., perpendicularly to the same direction as in *Amphioxus*.

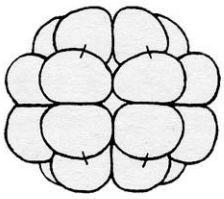


Fig. 1.
Stadium 16 bei *Amphioxus lanceolatus* (nach CERFONTAINE), vom animalen Pole.

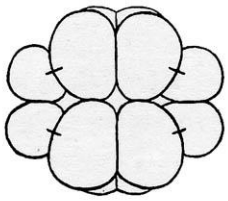


Fig. 2.
Dasselbe vom vegetativen Pole.

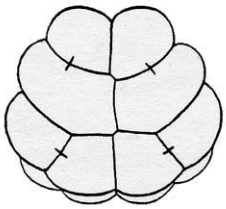


Fig. 3.
Stadium 16 bei *Cynthia partita* (nach CONKLIN), vom animalen Pole.

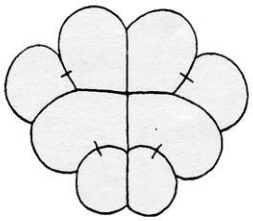


Fig. 4.
Dasselbe vom vegetativen Pole.

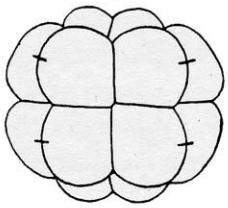


Fig. 5.
Stadium 16 bei *Oikopleura dioica*, vom animalen Pole.

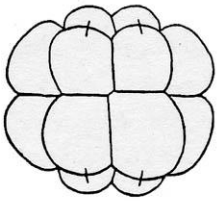


Fig. 6.
Dasselbe vom vegetativen Pole.

Fig. 1 – Stage 16 in *Amphioxus lanceolatus* (according to CERFONTAINE), seen from the animal pole.
Fig. 2 – The same seen from the vegetal pole.
Fig. 3 – Stage 16 in *Cynthia partita* (according to CONKLIN), seen from the animal pole.
Fig. 4 – The same seen from the vegetal pole.
Fig. 5 – Stage 16 in *Oikopleura dioica*, seen from the animal pole.
Fig. 6 – The same seen from the vegetal pole.

In other aspects as well *Oikopleura* is similar to the ascidians. For example, CONKLIN says about *Ciona*:

"All of the cell-division of this cleavage are approximately equal, except that of the posterior-dorsal cells, $B^{4.1}$ and $B^{\underline{4.1}}$ (B and <u>B</u> according to my nomenclature [**Translator's Note: The parentheses in this quote are insertions by Delsman]**). These cells divide very unequally, giving rise to two small posterior cells, $B^{5.2}$ and $B^{\underline{5.2}}$ (= B^1 and $B^{\underline{1}}$), which are the smallest in the entire egg.

Since the work of VAN BENEDEN and JULIN, these cells have been observed by all who have studied the ascidian cleavage, and they have served as the most important landmark in the orientation of the cleavage stages".

This description can be applied to *Oikopleura* just as is. Because of the very unequal division of the cells B and $\underline{B}$ now B^2 and $B^{\underline{2}}$ are the largest overall cells, B^1 and $B^{\underline{1}}$ the smallest. A^2 and $A^{\underline{2}}$ are only marginally larger than A^1 and $A^{\underline{1}}$, but they all are smaller than all of the cells of the animal half. Of those, a^2 and $a^{\underline{2}}$ are a little larger than a^1 and $a^{\underline{1}}$; b^2 and $b^{\underline{2}}$ are a little larger than b^1 and $b^{\underline{1}}$.

Although all of the divisions proceed strictly symmetrically, the symmetry of the thus formed cell complex is somewhat disturbed by a change in position of the blastomeres. This is reminiscent of spiral cleavage because the newly formed cells fit themselves into the spaces between the neighboring cells and thus a deviation from the original direction of division is created. At stage 16 the effect is still rather limited. However, as seen from the vegetative pole (Pl. I, Fig. 10) the cells A^1 and $A^{\underline{1}}$, and also B^1 and $B^{\underline{1}}$ exhibit, especially during the 8-16 division, a slight displacement to the right. In the subsequent divisions this displacement becomes more and more pronounced. The deviation always occurs in the same direction, so that in addition to the bilateral symmetry a certain asymmetry in the build of the egg results, which can be noticed as early as at stage 16 and which probably is directly related to the imperfect symmetry of the adult animal. For example the tail, as we will see blow, is preformed from the beginning in its twisted position that is characteristic for the adult appendicularian.

The fourth division as well is of great morphological importance. For more on that, see the description of the 6^{th} division. As we will see, gastrulation consists of the descendants of cells A^2 and $A^{\underline{2}}$ as well as B^2 and $B^{\underline{2}}$ being epibolically surrounded by all the other cells. We can see that the beginning of this epiboly is noticeable as early as at stage 16.

I observed a stage 16 a total of 14 times, and I found the 8-16 transition 4 times.

5^{th} *Division.* – The subsequent division does not involve all of the cells; it does not occur in the small B^1 and $B^{\underline{1}}$, and in B^2 and $B^{\underline{2}}$ it only happens after all other divisions have been completed. From here on, the daughter cell closest to the animal pole will be designated by the exponent 1, and the one closest to the vegetative pole will be designated by the exponent 2.

Thus, initially a stage 28 is formed, which I found only 1 time, and that apparently transitions immediately to stage 30.

Neither do the initial divisions up to stage 28 occur exactly at the same time. I found 6 occurrences of the transition 16-28. The first cells to divide are the ectodermal cells b^1, $b^{\underline{1}}$, b^2 and $b^{\underline{2}}$, followed by a^1, $a^{\underline{1}}$, a^2, $a^{\underline{2}}$, A^1 and $A^{\underline{1}}$, and even later the cells A^2 and $A^{\underline{2}}$ divide, and only after all of these divisions are complete do the large cells B^2 and $B^{\underline{2}}$ follow. Thus, the sequence of the divisions is not directly dependent on the relative size of the cells. I found 4 instances of stage 30, and 3 instances of the 28-30 transition.

As far as the directions of the divisions are concerned, the following rule can be stated: The 4 cells that abut at each of the two poles cleave perpendicularly to the previous division, the others, however, cleave once more in the same direction. This rule also applies to the 5th division of *Amphioxus*, thus, just like in the previous division, here, too, all planes of division are perpendicular to those of *Amphioxus*. For the ascidians, no similarly simple rule on the direction of division can be stated.

The division of the cells b^1 and $b^{\underline{1}}$ is a rather unequal one, the daughter cells $b^{1.1}$ and $b^{\underline{1.1}}$ are considerably larger than $b^{1.2}$ and $b^{\underline{1.2}}$. The other divisions are almost, if not perfectly, equal. The two cells of the animal half situated at the posterior pole of egg, $b^{1.2}$ and $b^{\underline{1.2}}$, at this stage exhibit two similar light-colored semispherical plasma humps [**Translator's Note: This appears not to be a standard term, merely a description.]** just like those observed by CONKLIN on the cells of the vegetative half, bordering on the equator at this point. They are fitted closely together and to the cells B^1 and $B^{\underline{1}}$, and both of them have two strongly refractive dots inside their interiors, similar to small nuclei, that become clearly visible later on.

The asymmetry that was hardly noticeable at stage 16 becomes more apparent during the 5th division. While the plane of contact, constituting the median plane, of the endodermal cells B^2 and B^2 and $A^{2.2}$ and $A^{\underline{2.2}}$ remains unchanged, at stage 28 a shifting of cells to the right at the anterior pole, and at the posterior pole of B^1 and $B^{\underline{1}}$ to the left is seen, so that the (broken) median line of the ectodermal half is completely oblique with respect to that of the endodermal half. In transition to stage 30 this shift also disturbs the thus far symmetrical position of the actual endodermal cells at the vegetatl pole. Cell $B^{2.1}$ is pushed to the front, and cell $A^{2.1}$, which formed one line with cells $A^{2.2}$, $A^{\underline{2.2}}$ and $A^{\underline{2.1}}$ at stage 28, performs a 90° turn around $A^{\underline{2.2}}$, next to which it was previously situated, so that it now becomes positioned in front of it (Fig. 16). At the same time, cells $A^{1.2}$ and $A^{\underline{1.2}}$ are being pushed outwards in the direction of the vegetative pole, thus partially covering up cells $A^{2.2}$ and $A^{\underline{2.2}}$. However, they are so light and transparent that they are easily overlooked in top view, and only the cells below them may be noticed. This shift then causes cells B^1 and $B^{\underline{1}}$ to be pushed apart from $b^{1.2}$ and $b^{\underline{1.2}}$. Between them, the two light plasma humps [of] $b^{1.2}$ and $b^{\underline{1.2}}$ are visible. Nevertheless, the plane of contact of cells $B^{2.2}$ and $B^{\underline{2.2}}$, $A^{2.2}$ and $A^{\underline{2.2}}$ continues to remain the original plane of symmetry, while the median plane of all other cells forms a considerable angle with it.

Figure 17 shows an optical cross-section of stage 30 parallel and close to the median plane. We will notice how the endodermal cells are pushing towards the center, while the ectodermal cells are spreading out more towards the surface. A blastocoel is never formed; a true blastula does not develop. We may refer to this stage as placula [**Translator's Note: The concept of a plakula/placula as a developmental stage appears to have been abandoned since 1910 – I cannot find this term in my dictionaries, and references on the web all are to the "placula hypothesis" by Bütschli.**]. Figure 7 in the text shows a cross-section of this stage. During the subsequent division the endodermal cells will be completely surrounded by the ectodermal cells; Fig. 17, as it were, shows the beginning of gastrulation.

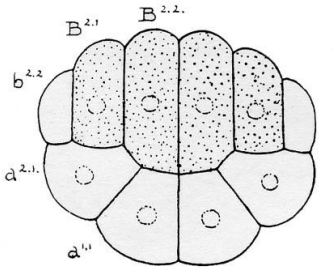


Fig. 7.
Optischer Querschnitt durch das Plakulastadium (Stadium 30).

Bemerkung: Die rechte und linke Hälfte dieser Figur liegen eigentlich nicht in einer Ebene, sondern bilden einen stumpfen Winkel mit einander infolge der beschriebenen Verschiebung der Zellen im Stadium 30 (vergl. Taf. 2 Fig. 16). Die zweite Hälfte wurde gezeichnet nachdem das Ei ein wenig umgerollt worden war.

Fig. 7 – Optical cross-section of the placula stage (stage 30).

Note: The right and left half of this figure actually do not lie on the same plane but form an obtuse angle with each other, due to the shifting of cells at stage 30 described above (cf. Pl. 2, Fig.16). The second half was drawn after turning the egg over a little.

It is a peculiar/characteristic [**Translator's Note: It is impossible to tell (due to the age of the publication) whether the intended meaning here is more in the sense of peculiar, strange, unusual, or rather in the sense of high characteristic of Oikopleura**.] phenomenon that at later cleavage stages the egg membrane is less and less filled by the egg, while at the same time the diameter of the [membrane], as shown above, decreases as well. Not only are the cells no longer flattened on their outer sides by the apparently flexibly tightened egg membrane as was the case during the earliest stages of cleavage, but there even is a space formed between them that is filled with a completely transparent jelly, causing the egg membrane to remain taut and to maintain its spherical shape. From this follows that the volume of the egg is significantly reduced during cleavage.

6th *Division.* – During the 6th division gastrulation takes place. This division, in which all cells participate, and which, regardless of the irregular arrangement of the cells, once again occurs bilaterally symmetrically, has three phases. At first, a stage 44 is formed, which immediately transitions to a stage 52, from which stage 60 is formed through the division of the final 8 cells.

Figure 18 shows the transition from stage 30 to stage 44. The surrounding of the eight ectodermal cells $B^{2.1}$, $B^{\underline{2.1}}$, $B^{2.2}$, $B^{\underline{2.2}}$, $A^{2.1}$, $A^{\underline{2.1}}$, $A^{2.2}$ and $A^{\underline{2.2}}$ by all the other cells has already been completed. When focusing at a deeper level, one can see them in their unchanged position on the inside. (Figs. 18, 21, 22). Just above the vegetal pole the cells growing around them meet and form a five-pointed star. There is no blastopore, neither is there a blastocoel or gastrocoel to be seen. The 8 endodermal cells, the 4 large ectodermal cells: $a^{1.1}$, $a^{\underline{1.1}}$, $a^{2.1}$, $a^{\underline{2.1}}$, and the 4 smaller cells of the vegetative half, $A^{1.1}$, $A^{\underline{1.1}}$, $A^{1.2}$ and $A^{\underline{1.2}}$ remain undivided.. The latter two form one of the arms of the five-pointed star.

The two arms opposite it also are formed by cells of the vegetative half, namely by the small cells B^{1} and $B^{\underline{1}}$. Those have changed their positions completely and have once more moved closer to each other while pushing towards the vegetative pole. They, as well, are in the process of dividing. The last two arms of the five-pointed star are made up of two cells formed from $b^{2.2}$ and $b^{\underline{2.2}}$, namely $b^{2.2.2}$ and $b^{\underline{2.2.2}}$.

This phase I was able to observe only once.

In the second phase then the four large ectodermal cells $a^{1.1}$, $a^{\underline{1.1}}$, $a^{2.1}$, $a^{\underline{2.1}}$ also divide in the direction of the small arrows shown in Fig. 19 (cf. Fig. 22), as well as the four smaller cells $A^{1.1}$, $A^{\underline{1.1}}$, $A^{1.2}$, $A^{\underline{1.2}}$, the latter [dividing] for the third time in the same direction. They form a double row of oblong cells, beginning at the blastopore and forming a shallow depression (Fig. 24). Because they later are overgrown by the surrounding cells and disappear from the surface I think it likely that they form the neural plate. Cell $A^{1.1.1}$ moves underneath cell $a^{1.2.2}$ which had already covered this half of cell $A^{1.1}$ during the first phase.

While cell $a^{\underline{2.2}}$ divides almost equally, the division of cell $a^{2.2}$ is decidedly unequal. A tiny cell $a^{2.2.2}$ is segregated. This apparently is related to the asymmetrical position of the neural plate.

The division of cells $b^{1.2}$ and $b^{\underline{1.2}}$as well is decidedly unequal; two very tiny cells $b^{1.2.2}$ and $b^{\underline{1.2.2}}$are formed, both of them bordering on the peculiar/characteristic plasma humps. I do not know whether the latter can be attributed the same status as cells. They both contain two small, strongly refractive bodies each.

The cells $B^{1.1}$ and $B^{\underline{1.1}}$, given off by B^{1} and $B^{\underline{1}}$, move underneath $b^{1.1.2}$, $b^{2.12}$, $b^{\underline{1.1.2}}$, and $b^{\underline{2.1.2}}$. Thus, $B^{1.1}$ and $B^{\underline{1.1}}$ disappear from the surface. They are likely to represent muscle cells, just like $B^{1.2}$ and $B^{\underline{1.2}}$, which later also will be overgrown by the surrounding cells. .

I found the 44-52 transition two times, and stage 52 seven times.

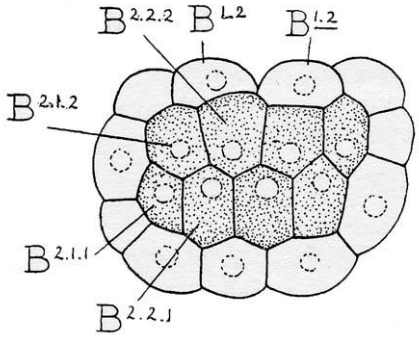


Fig. 8.
Optischer Querschnitt durch das Stadium 60.
Bemerkung: Aehnlich wie in Fig. 7 bilden auch hier rechte und linke Hälfte der Figur einen stumpfen Winkel.

Fig. 8 – Optical cross-section of stage 60. *Note*: Similarly to Fig. 7, the right and left half of this figure as well from an obtuse angle.

In the third and final phase, the 8 endodermal cells eventually divide, namely in dorsoventral direction, so that the plane of division coincides with the plane of the drawing in Figures 21 and 22. At first the 4 small cells (A) divide, followed immediately by the larger ones (B). The parts [**Translator's Note: It is unclear, to what parts this refers. It might refer to the products of cell division, but it is not clear**.] of each cell are identical.

The gastrula cavity, that in the ascidians turns into the archenteron, here is filled by endodermal cells dividing in dorsoventral direction. The causes for this deviating behavior, which, by the way, is not unique but also has been observed in the same way by DAVIDOFF in *Distaplia magnilarva*, a compound ascidian, without doubt have to be looked for in the peculiar/characteristic situation of gastrulation. The low number of cells as well as their shapes make a normal invagination and archenteron formation impossible.

I have been able to observe egg cleavage up to this point; the further development escapes observation. I found a sufficient number of further developed stages, but in whole specimen the fate of the cells no longer can be followed, and the formol fixing turned out to be insufficient for producing cuts. Thus, it cannot be determined with certainty which organs are formed by the various groups of cells.

In any case, however, it is apparent that the 1st through the 4th division are of great morphological importance, and that at stage 16 the most significant organ forming regions have been defined. The offspring of cells A^2, $A^{\underline{2}}$, B^2, $B^{\underline{2}}$ from the endoderm, cells A^1 and $A^{\underline{1}}$, which by way of continued division in the same direction eventually form an oblong, somewhat depressed cell plate originating at the blastopore, probably form the basis for the nervous system; cells B^1 and $B^{\underline{1}}$ probably are mesodermal. Because of their close relation to the neural primordium the assumption that cells A^2 and $A^{\underline{2}}$ form the chorda primordium is not without justification. This is especially true because we can see how the endoderm consists of cells of which one half is quite a bit larger than the other. At stage 30 and during the 6th division [those larger cells] are positioned around the extending neural primordium in the same bent manner, as at stage 16 the vegetative half of the egg is bent around the animal half and vice versa. The animal half appears to be of solely ectodermal nature.

THE EMBRYO

As GOLDSCHMIDT reports: “Cleavage, which leads to a spherical, somewhat flattened cell heap, occurs very quickly, within about two hours. Then, the inner processes escape observation until after another three hours one is able to discern that the pear-shaped, somewhat elongated embryo is separating into two segments by way of a circular cleavage furrow.” I encountered these stages as well in rather large numbers in my plankton sample, and also stages at which the embryo, doubly bent due to the longitudinal growth of the tail, is ready to hatch. But I found only a single, just hatched larva in my sample.

The separation of tail and body is not actually marked by a circular cleavage furrow,

but rather by a ventral constriction that becomes deeper and deeper, while at the same time the tail, inside which the chorda is visible as a row of cylindrical cells, similar to a roll of coins, grows longitudinally and bends inside the egg membrane. The proximal end of the chorda is still located inside the trunk (Fig. 27). To the left and right of the trunk we can see, if the embryo is in a suitable position, two cords of cells, the neural primordium and the caudal endoderm, as will become obvious later. Thus, the tail has already completed the 90° turn. However, if we now search the muscle primordia (Fig. 27) dorsal and ventral of the chorda we will not find them. Rather, the ectodermal cells seem to be situated immediately on top of the chorda. Only a cross-section will be able to reveal the true nature of things.

Thanks to the great transparency of the object it is not hard to obtain an optical cross-section of the tail. This yields the same image (Fig. 28) in any position. The tail is dorsoventrally flattened and, as noted, has already completed the 90° turn. At the center we see the circular chorda cell with its light plasma, and to its right another, even larger cell, while towards the left there is a tube with an extremely narrow or even absent lumen, formed by small cells, of which a cross-section shows 4. As development progresses it turns out that the row of cells on the right represents the tail endoderm, while the tube on the left will develop into the tail segment of the nervous system.

The cells of the tail endodermal cord not only have a somewhat larger cross-section, but they also are longer than the chorda cells, whose number later will be easily determined as 20. GOLDSCHMIDT in his Figure 2 obviously took these cells for muscle cells and counted 10 of them. I did not determine the number exactly, but it is much lower than that of the chorda cells. The chorda cells have the shape of thick round slices, about half as high as wide in diameter.

In the cross-section of the tail we see the flattened muscle cell[s] dorsal and ventral of the chorda cells. The ventral one appears to be larger than the dorsal one, but the latter are more longitudinally stretched out due to the bent tail (Fig. 27), which causes the former to be compressed. They form a simple dorsal and ventral row of ten cells each, exactly opposite of each other. The reagent induced dissolution of the tail muscles into ten muscle segments, lined up one after the other, as reported by LANGERHANS has, as is commonly known, been attributed by SEELINGER to its makeup of ten muscle cells, lined up one after another. Further development thus confirms this interpretation, if differently from what GOLDSCHMIDT had in mind when he determined the number of the tail endodermal cells to be ten.

(I myself am not absolutely convinced of the accuracy of that information, for the number of chorda cells, which is much more easily determined, is not given correctly in said Figure. And left of the chorda no such simple row of cells is even mentioned, there, the multicellular neural tube is found. Besides, it now has been shown that this item is of lesser importance.)

The cells of the ectoderm are peculiar/characteristic. The dorsal muscle cell [**Translator's Note – This almost certainly should refer to multiple cells, but is phrased in the singular in a way that in today's German no longer would be able to imply a plural.**] is barely encompassed by two ectodermal cells situated to either side of it, at times, the middle part even remains uncovered. Thus, when viewed from the side (Figure 27) one might mistake it [i.e., the muscle cells] for ectodermal cells. The two aforementioned rows of ectodermal cells that stretch across the entire length of the tail on the outside border on two rows of very small oblong cells with strongly refractive plasma that, in the cross-section, border on the tail endodermal and the neural cords. They are followed by two rows of larger ectodermal cells which are not able to encompass the ventral muscle cell, so that the latter remains completely uncovered.

From the preceding it becomes clear that, as noted above, the tail has already completed its 90° turn. All organ primordia are already in the same positions as in the adult animal. However, there usually still is a slight deviation of the tail endodermal cells in the ventral and of the neural cells in the dorsal direction noticeable.

If we now take a look at younger stages in which the segmentation of tail and trunk is just beginning, we still see roughly the same image. The tail endodermal and neural cells are not situated ventrally and dorsally, respectively, but are already in exactly the same position right and left of the chorda, while the muscle cell is found dorsally, barely covered by the two ectodermal cells, in short, just like in the larva that is ready to hatch. However, the two ectodermal cells outside the small, strongly refractive cells here border on the ectodermal cells of the body. As the segmentation of the tail progresses, this formation naturally is destroyed, and they position themselves around the ventral muscle cell. That way it is ascertained that the two rows of small cells will be in exactly lateral position after the embryo has left the egg and has extended itself. Because these rows extend on both sides across the entire length of the tail and join at its tip I think it likely that they play a role in the formation of the wide thin edge of the tail. Perhaps they can be traced back to, at least in part, the two cells $b^{1.2.2}$ and $\underline{b^{1.2.2}}$, which at stage 60 are characterized by their small size just like the two rows of cells in the embryo discussed here.

The chorda and the caudal endodermal cord are positioned closely together, with a wide plane of contact. Apparently, together they form the caudal gut segment at this stage.

The trunk of the embryo still forms a solid cell mass in which no specific organ primordia can be recognized.

The number of cells continues to be low; between the stage shown in Figures 23 and Figure 24 and stage 60 the number of cell divisions certainly is not high. If it were easier to obtain the eggs in larger quantity, hardly a more suitable object is imaginable from which to determine the origin of every single cell of the adult larva.

FURTHER DEVELOPMENT

There were no freshly hatched larvae contained in the plankton sample from which I obtained the material described in my observations above. I have followed the further development solely in living animals.

As the tail, growing longer and longer, bends back over the head, the still spherical egg membrane ruptures and the thick head comes out first. The larva sheds the fine egg membrane through the first weak twitches of its tail, and the membrane is left behind as a transparent tiny fleece. The larva – this name actually is not very appropriate in this instance – which has the shape of a frog larva, continues to be completely solid: there is neither a body cavity nor an intestinal cavity, no mouth and no anus. However, over the course of a few hours one can watch all organs beginning to form. Also, the little animal has begun, while still inside the egg, to excrete a thin jelly coat that also surrounds the tail and ends at the tip of the tail in a pointed, glass-light projection. Anyone who has studied the planktonic larvae of various marine invertebrates will especially notice the helplessness of the *Oikopleura* larvae that is caused by the complete lack of cilia. They are passively carried along by currents, and only from time to time do they twitch their tails restlessly. One is instinctively reminded of young fish larvae, while the larvae of *Amphioxus* are more strongly reminiscent of invertebrate larvae, for they have cilia and use them to propel themselves ahead in a helicoid motion.

Soon after leaving the egg, the borders of ectoderm and endoderm as well as the outline of the cerebral vesicle become clearly visible. Also, the segmentation of trunk and tail becomes more apparent. Inside the large, pear-shaped cerebral vesicle the cells move apart, so that a cavity is formed that soon increases in size. At the tip of a plasma stalk extending into the cavity a few small, strongly refractive otoliths are excreted.

In the meantime, the tail has considerably stretched out lengthwise, solely through the cells changing their form. For example, the chorda cells that previously were of disk-like shape by now have become longer and narrower, so that they now are longer than they are wide. And the height of the epidermis and muscle plates also has been greatly reduced by the cells' longitudinal stretching. The number of chorda cells now easily can be determined as 20; the two end cells are small and cap-shaped. The muscle cells as well are easily counted, they number ten. Viewed from the side they still appear to be forming the epidermis (Fig. 30). Apparently, the formation of muscular fibrils begins immediately after the larva leaves the egg, for its movements gradually become more energetic. Meanwhile, at the center of each of the borders between two chorda cells, and at first at the proximal end of the tail, a fine, strongly refractive little dot appears, that quickly increases in size and becomes a jelly-filled vacuole. GOLDTSCHMIDT took these vacuoles to be the nuclei of the chorda cells; he says: "Inside the chorda, the cell borders have disappeared, but the nuclei are still preserved for the most part. They begin dissolving from the posterior end." With this, he shows (in his Fig. 3) a larva, roughly at the same stage as my Figure 31, where the vacuoles at the proximal end of the tail have reached the size of nuclei, but have not yet appeared at the distal end. In my Fig. 31, the cell borders at this stage are still clearly visible. While KOWALEWSKY thought that in the ascidians the vacuoles were formed between two cells each, KLAATSCH claims their first appearance to be inside of those cells. I have to agree strongly with KOWALEWSKY; I have observed the initial appearance and gradual increase in size of the vacuoles several times in living animals. I never saw the fusion of the vacuoles.

The caudal segment of the nervous system, which is connected to the brain initially in a straight (Fig. 29) manner, with that connection later being bent when the abdomen begins to grow due to the development of the intestinal tract, begins to swell at the proximal end, while the remaining part grows thinner and thinner and exhibits irregular indentures. The proximal swelling constitutes the primordium of the tail ganglion.

The caudal endoderm has separated from the tip of the tail and is gradually retracted into the body, so that a cavity is formed to the right of the chorda. However, two cells of the endoderm remain inside this cavity. They are initially situated at the origin of the tail, but later move more in the posterior direction, where they can be found in the adult animal as well, compressed by the two muscle plates. These two cells, which have been noted before, also are remains of the tail endoderm (Fig. 31).

Let us now turn once again to the head segment. Gradually the individual parts of the intestinal tract differentiate: the branchial gut with one ventral and two sideways protrusions, the primordia of the endostyle and the two branchial ducts; the esophagus; the two parts of the stomach with their connecting orifice, and the hind-gut, which forms close to the ventral epidermis, but does not yet break through. The mouth as well has not been formed yet, and the entire intestinal tract, with the exception of the stomach, still is solid, without lumen.

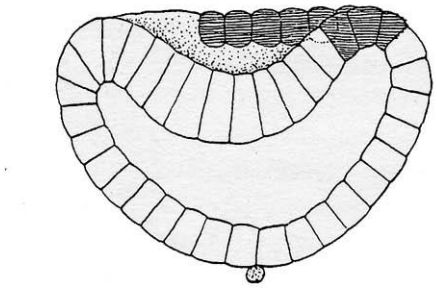
The ventral view (Fig. 31) shows the blind hind-gut in the middle and the, also blind, branchial duct protrusions on either side. More anterior is the endostyle protrusion, surrounded by a horseshoe-shape form, which apparently constitutes the dorsal row of cells of the endostyle.

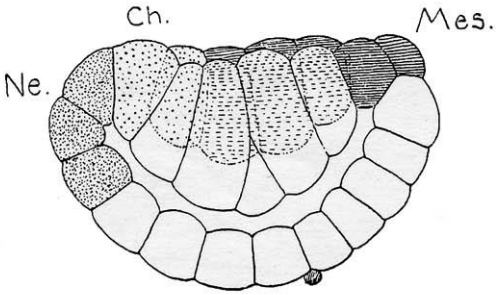
The anterior part of the esophagus pushes the ectodermal cells apart and pushes to the outside. Suddenly, now a lumen is formed by way of separation in the esophagus, in the branchial ducts, in the endostyle and the hind-gut. Thus, I cannot confirm GOLDSCHMIDT'S statement that the branchial gut is of ectodermal origin. Just as in the ascidians it is provided by the endoderm.

This is how far I observed the development in the living animal. The breaking through of the branchial ducts and the anus I have, unfortunately, not observed. However, I do not believe that GOLDSCHMIDT has done so, either; at the stage in his Figure 3 there is no mention of spiracles. There is, however, at that very spot the median protrusion of the endostyle, and perhaps he mistook this in a less clear specimen for a spiracle. After all, this figure is rather incomplete in all respects: the branchial gut is drawn as an ectodermal invagination and not connected to the stomach; the branchial ducts are not shown; the number of the jelly vacuoles, interpreted as nuclei by G., is too low, for the chorda consists of twenty cells; on the other hand, the number of epidermal cells and the cells taken by G. to be muscle cells of the tail is much too high. I do not think it likely that a larger part of the branchial duct, e.g., the entire outer branchial duct, was formed by the ectoderm, because the endodermal protrusions of the branchial gut are situated tightly against the ectoderm, as is the hind-gut. I am beginning to form the impression that the homologue of the ectodermal peribranchial space of the ascidians is missing here. However, I am not yet absolutely certain of that.

During the next season, I hope to be able to observe the breaking through of the branchial ducts and to be able to close other gaps in the observations reported here, as well. I did, however, consider the results obtained this far important enough to publish them now, and not wait until then to do so.

SUMMARY





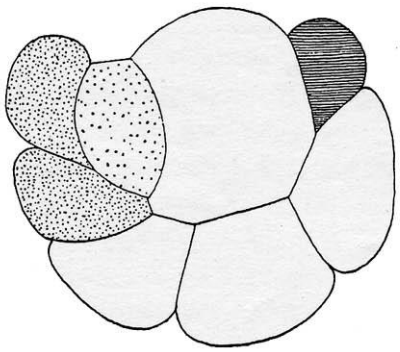


Fig. 9, 10. Schematischer Längsschnitt durch den Anfang der Gastrulation bei Amphioxus und Cynthia (nach CONKLIN).

Fig. 11. Optischer Längsschnitt durch den Anfang der Gastrulation bei Oikopleura.

In summary of the results obtained, we, therefore, can state that *Oikopleura* in its development is very close to the ascidians and *Amphioxus*. Because the appendicularians are rather generally considered to be the one group of tunicates that has remained closest to original type, the question lends itself whether this character might also be seen in their ontogeny, and whether the appendicularians in this respect might take an intermediate position between the ascidians and *Amphioxus*. The answer to this question cannot be anything but negative. Quite to the contrary, we see how the characteristics in which the ontogeny of the ascidians differs from that of *Amphioxus* here are even more pronounced. This is especially true, as CONKLIN remarks, for the number of cells and the degree of differentiation at any stage of development. In the ascidians, the number of cells is rather low, and the degree of differentiation high as compared to *Amphioxus*. For example, at the beginning of gastrulation *Amphioxus* has 512 cells, *Ciona* has 76 cells, but *Oikopleura* merely 30!

Regarding various aspects it is rather the ascidians that take an intermediate position between *Amphioxus* and *Oikopleura*. We saw this clearly, e.g., in the division most characteristic of the cleavage type, namely in the 4th division, at the blastula or placula (resp.) stage, during gastrulation and in the cross-section of the tail.

Figs. 9, 10 – Schematic longitudinal section of the beginning of gastrulation in *Amphioxus* and *Cynthia* (according to CONKLIN)

Fig. 11 – Optical longitudinal section of the beginning of gastrulation in *Oikopleura*.

The fact that the epiboly described and the formation of the gut by dorsoventral division of the endodermal cells does not constitute a primitive gastrulation, but rather had to be interpreted as a reduced invagination, is made yet clearer when one compares it to the same situation in the solitary ascidians, *Clavellina,* and the compound ascidians, where the latter probably can be derived from the former, with *Clavellina* forming a transition. As a rather telling example for the fact that, as HATSCHEK states, in the "phyletic modification of a *Tierform (Individuenkreis*) [**Translator's Note: These terms appear to have become obsolete; I was not able to find definitions anywhere. I suspect that 'species' is what is meant**.]it is never solely the final stage that is modified, but always the entire sequence from egg to final stage," DAVIDOFF now has shown "how the ontogeny within the group of the ascidians, from the solitary to the social to the compound forms, gradually deviates from the palingenetic modus ."

While in *Amphioxus* we find a large blastula and a typical invagination, the blastula of the ascidians has few cells, a narrow lumen and thick walls, as well as – just like in *Amphioxus* – a thickened vegetative half. Gastrulation is achieved by the bending inwards of the vegetative half until the rim of the cup forms a wide blastopore that then narrows. The more or less formed cleavage cavity sooner or later is reduced to a gap and eventually disappears completely.

In *Clavellina* the cleavage cavity is reduced to a gap or may be missing completely. The result of cleavage is a two-layered placula. Thus, true invagination no longer is possible. The subsequent gastrulation is mainly based on the arching and growing towards each other of the placula rims, due to unequal cell growth in the two germ layers. DAVIDOFF referred to this process as pseudoemboly.

In the compound ascidian investigated by DAVIDOFF, *Distaplia magnilarva*, finally, the shape of the placula is so stocky that not even a pseudoemboly occurs, but rather is replaced by a true epiboly. Shortly before gastrulation the endodermal cells divide dorsoventrally, so that they form a double layer and a true invagination is no longer possible.

We found a similar situation in *Oikopleura*, with the exception that here the dorsoventral division of the endodermal cells occurs shortly after gastrulation. So, in *Oikopleura* as well we appear to be dealing not with an original but an apomorphic situation. It does not fall within the frame of this paper to investigate further the question whether the similarity shown by both would justify the assumption of a closer relationship of compound ascidians and appendicularians.

NOTE WHILE IN PRESS

On April 13, 1910, I once again found individual eggs of *Oikopleura dioica* with doubly bent embryos in the harbor plankton. It thus turned out that *Oikopleura* in the North Sea, just like near Rovigno, also reproduce in spring.

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EXPLANTIONS OF THE PLATES

All figures in Plates I-III show *Oikopleura dioica* Fol.

Plate I

Fig. 1. Egg at stage 2.

Figs. 2 and 3. Egg at stage 4.

Figs. 4, 5 and 6. Egg at stage 4,with “refractive groove” at the poles.

Fig. 7. Egg at stage 8, seen from the right.

Fig. 8. The same, viewed from the vegetative pole.

Fig. 9. The same, viewed from the anterior.

Fig. 10. Egg at stage 16, seen from the vegetative Pole. The interrupted lines indicate the optical cross-section.

Fig. 11. The same, seen from the animal pole.

Fig. 12. Optical longitudinal section of the same.

Plate II

The letters in parentheses indicate the daughter cells of those cells currently undergoing division. Interrupted lines indicate the optical cross-section of the eggs.

Fig. 13. Egg at transitional stage 16-28, seen from the anterior pole.

Fig. 14. The same, seen from the posterior pole.

Fig. 15. Egg at stage 28.

Fig. 16. Egg at stage 30.

Fig. 17. Optical longitudinal section of stage 30.

Fig. 18. Egg at transitional stage 30-44, seen from the vegetal pole. Gastrulation complete. Interrupted lines indicate the endodermal cells visible at a deeper level of focus.

Fig. 19. The same, between the anterior and animal poles.

Fig. 20. The same, seen from the posterior pole.

Fig. 21. Stage 52, seen from the vegetal pole (cf. Fig. 18).

Fig. 22. The same, between the anterior and animal poles (cf. Fig. 19).

Fig. 23. The same, seen from the posterior pole (cf. Fig. 20).

Fig. 24. The same, seen from the anterior pole.

Plate III

Fig. 25. Embryo showing the beginning of trunk-tail separation.

Fig. 26. Optical section of the same, seen from the ventral side.

Fig. 27. Doubly-bent embryo, immediately prior to hatching. Optical longitudinal section.

Fig. 28. The same, seen from the ventral side. The optical cross-section of the tail is indicated.

Fig. 29. Newly hatched larva, with empty egg membrane on the side.

Fig. 30. More developed larva, from the side. Brachial gut, brachial ducts, esophagus and rectum without lumen. The mouth is breaking through. Jelly vacuoles appearing between chorda cells.

Fig. 31. Somewhat more developed larva, from the ventral side.

Fig. 32. Even more developed larva, with mouth orifice and intestinal lumen.

Anh. – Glass-light protrusion of the tail.
Ch. – Chorda cells
E. – Trunk endoderm
Eih. – Egg membrane
Ekt. – Ectodermal cells
End. – Primordium/anlage of the endostyle
Ent. – Tail endoderm
G. – Brain
Ga. – Tail ganglion
H. – Jelly coat
Kd. – Branchial gut
Km. – Branchial duct
LM. – Left half of stomach
M. – Site of mouth breaking through
Md. – Dorsal cell row of the endostyle
Mu. – Muscle cells
N. – Nerve cord
Nucl. – Nuclei of chorda cells
Oe. – Esophagus
Ot. - Otocyst
Py. – Pylorus
R. – Rectum
RM. – Right half of the stomach
Vac. – Vacuoles between the chorda cells
Verb. – Connecting orifice between the two stomach halves

Figures 1 to 29 were drawn at a magnification of about 600x. The magnification for the other figures was not determined.

Fig. 1.

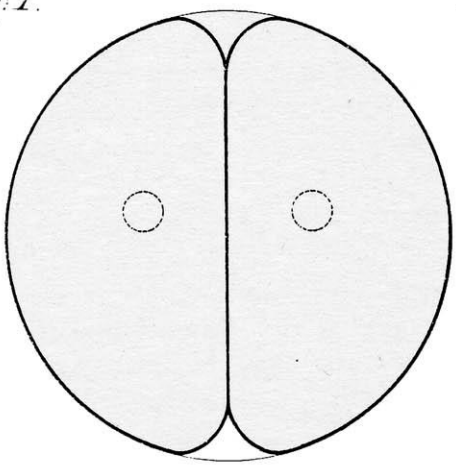
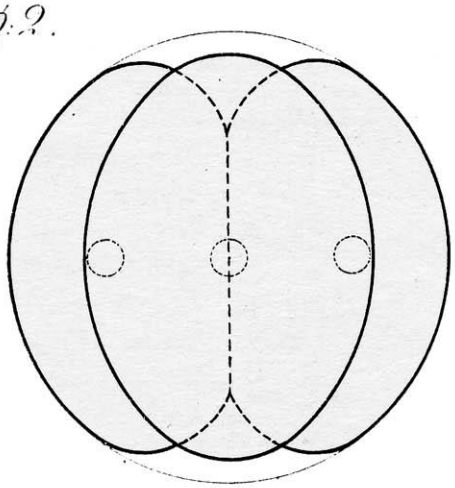


Fig. 2.



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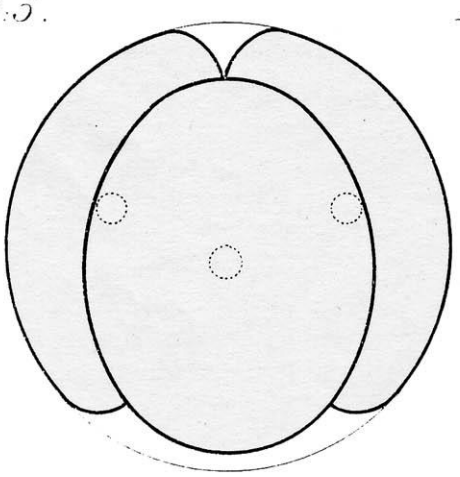


Fig. 6.

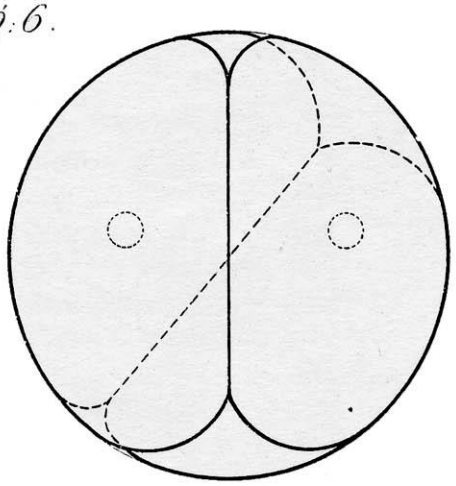


Fig. 9.

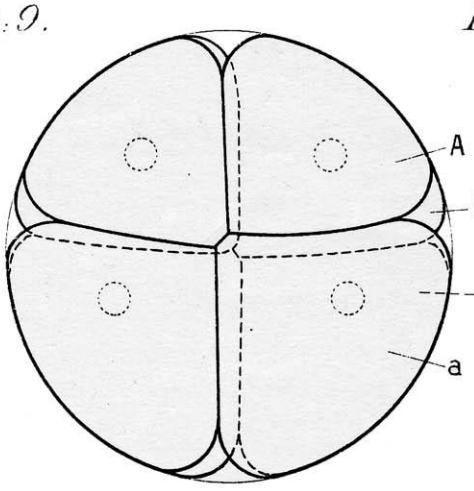


Fig. 10.

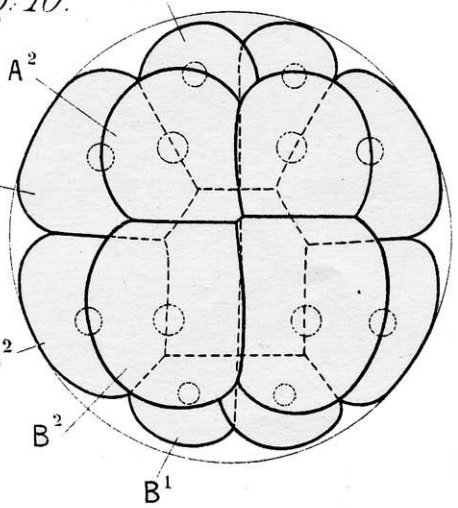


Fig. 3.

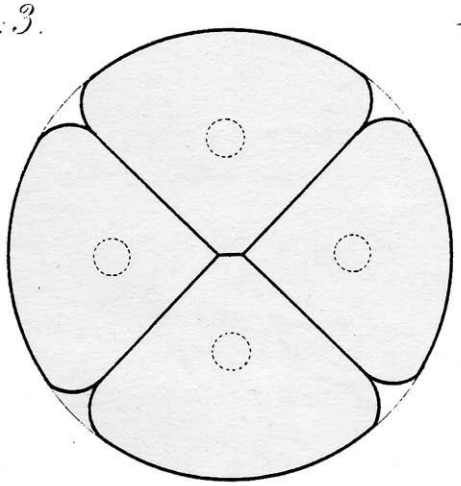


Fig. 4.

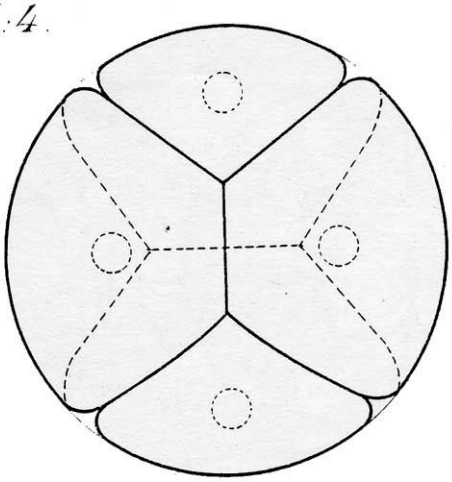
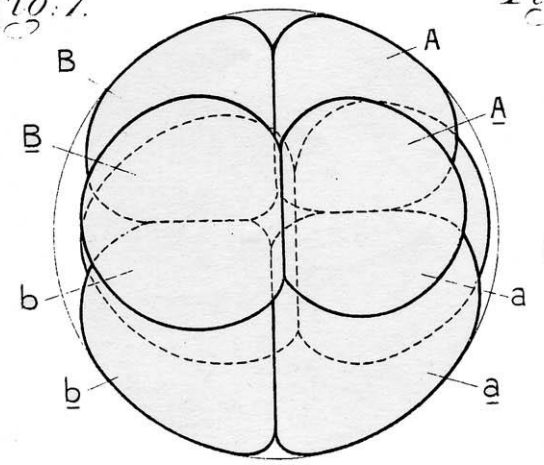


Fig. 7.



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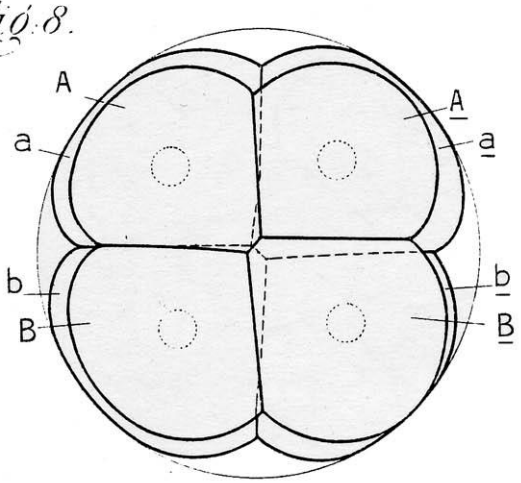

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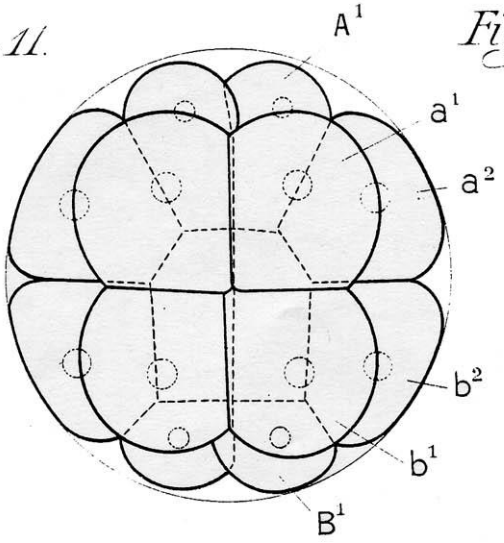

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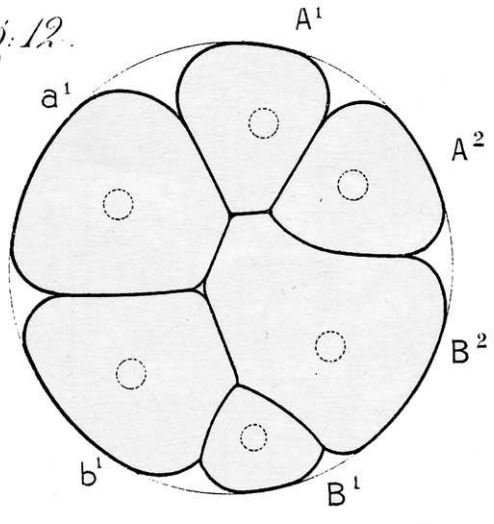

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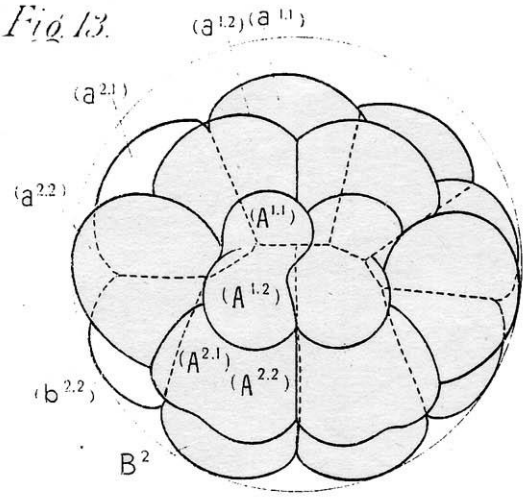

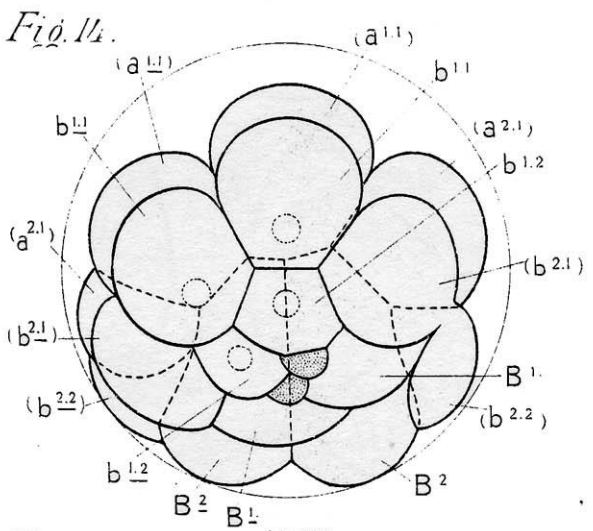

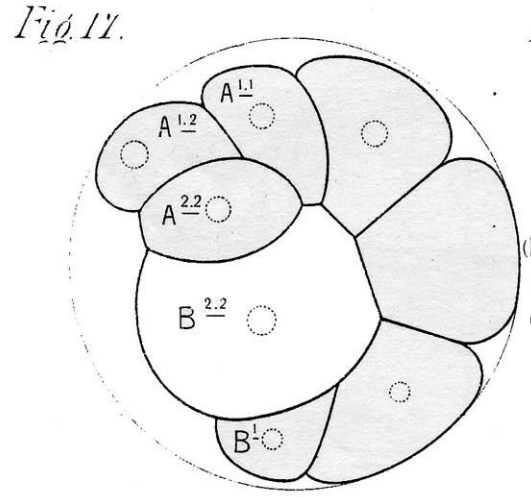

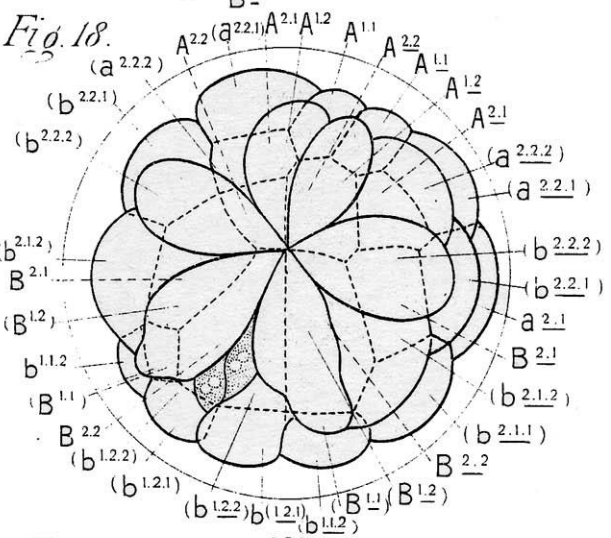

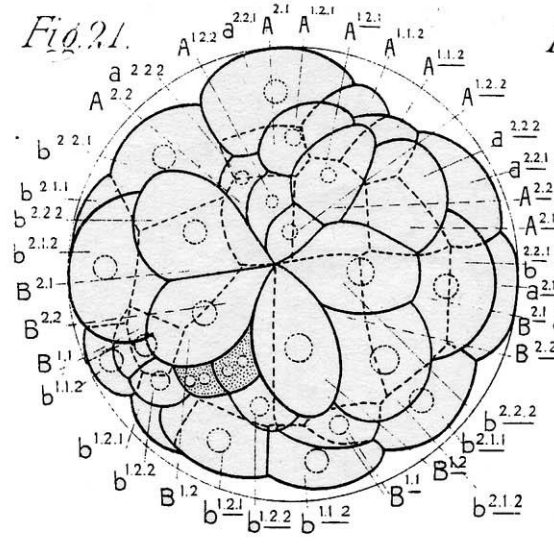

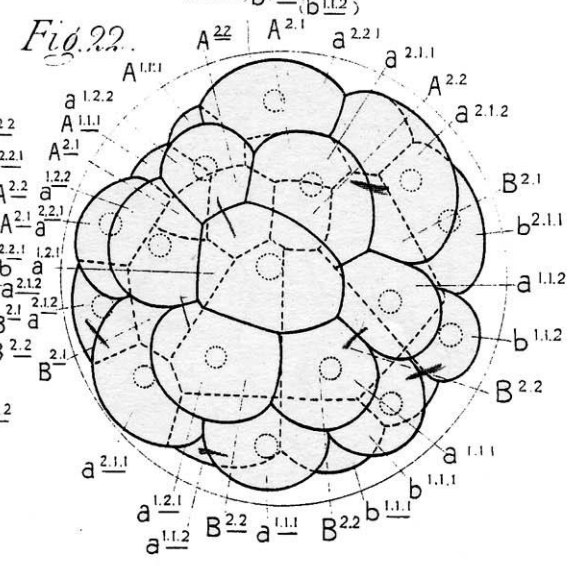

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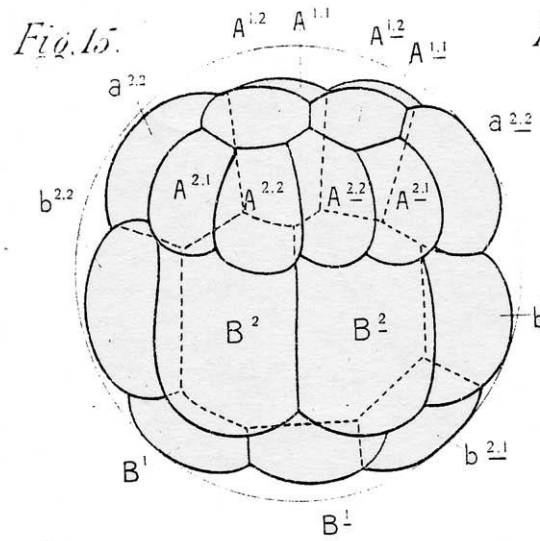

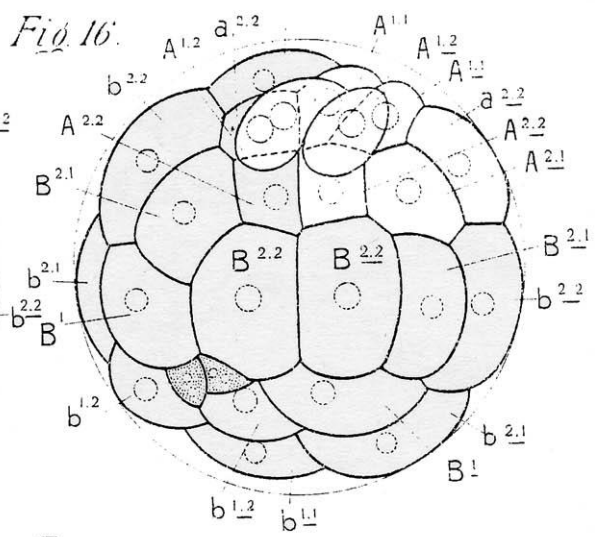

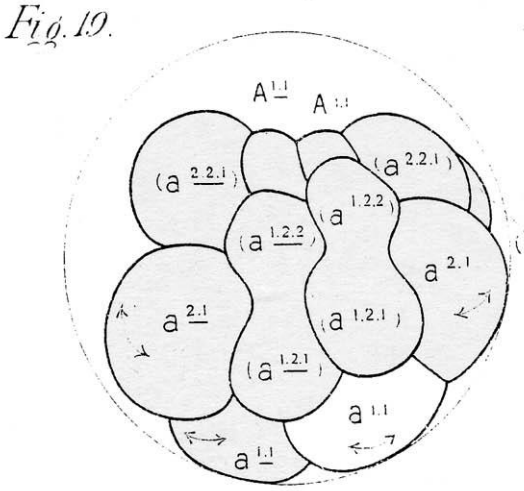

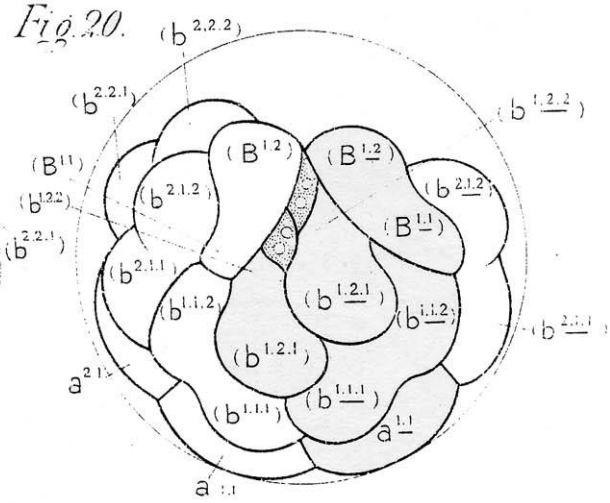

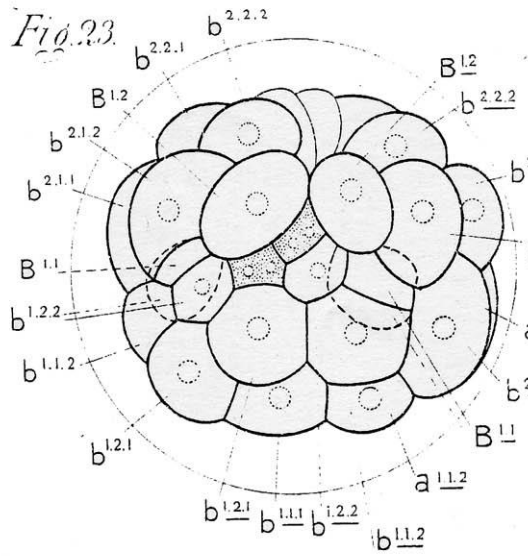

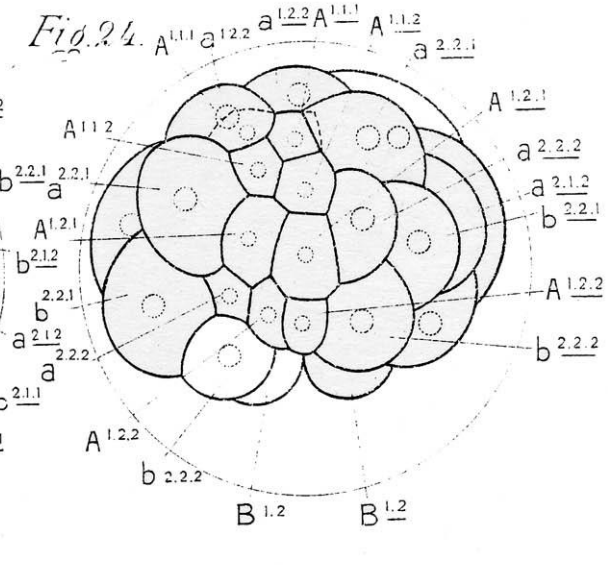

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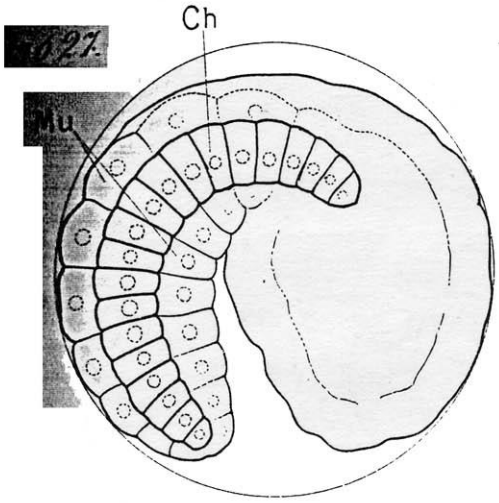

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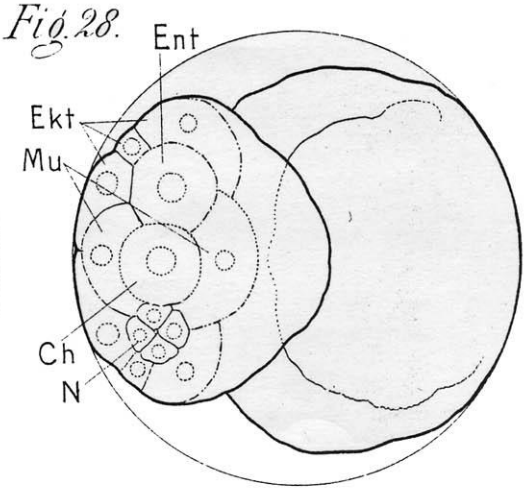
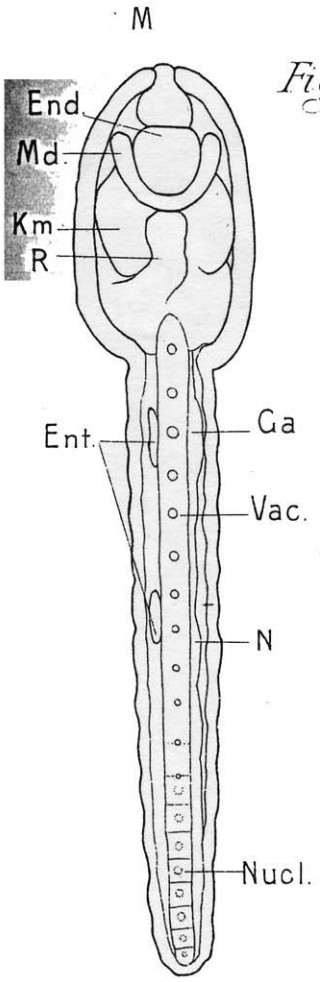


Fig. 28.



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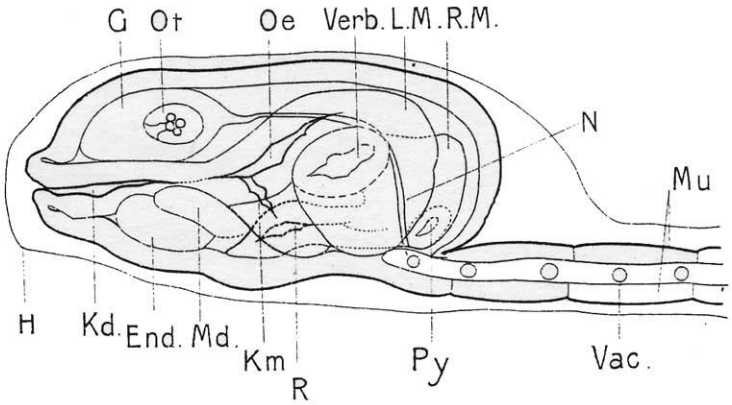

Fig. 32.

Fig. 25.

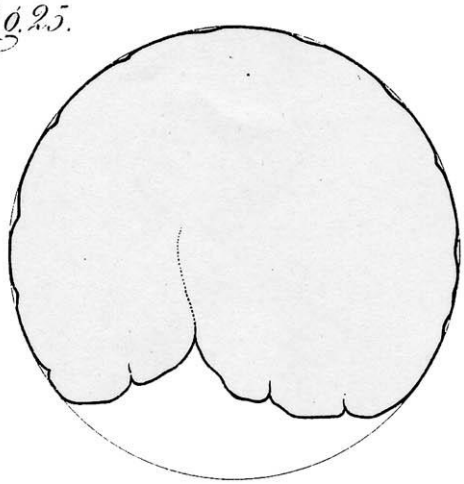

Fig. 26

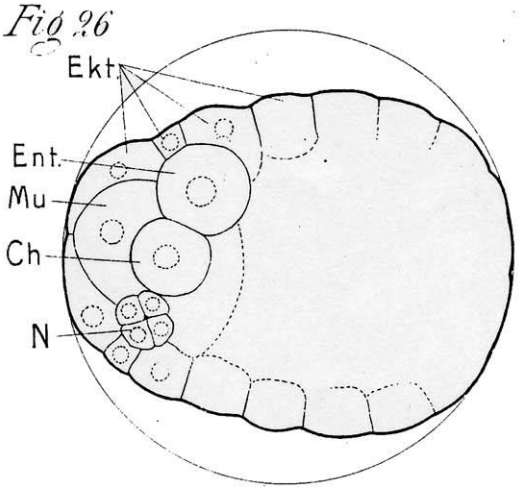

Fig. 29.

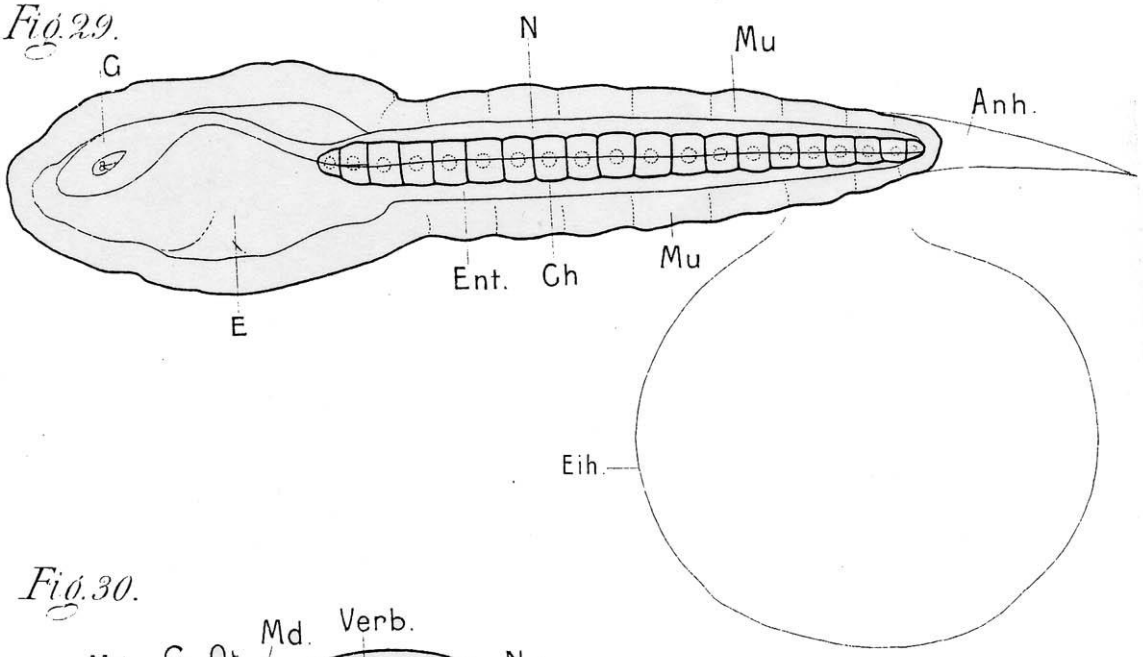

Fig. 30.