Zoology. — *"On gastrulation and the covering of the yolk in the teleostean egg."* By Dr. J. Boeke. (Communicated by Prof. A. A. W. Hubrecht).

(Communicated in the meeting of January 26, 1907).

1. Generally the process of gastrulation in teleosts is described by the greater part of the embryologists as a folding in of the margin of the blastoderm and the forming, partly by this process of folding and partly by delamination, of a mass of cells that contains the elements both of the chorda and mesoderm and of the entoderm. Only Waclaw Berent, M. v. Kowalewski (in his paper of 1885), F. B. Sumner and myself have described a more or less independent origin of mesoderm and chorda on one side and the entoderm on the other side. Sumner called the mass of cells lying at the posterior end of the embryo, from which the entoderm originates, prostomal thickening; I kept the same name for them and regarded these cells as being derived from the periblast.

The large pelagic eggs of Muraenoids, which I could collect in large quantities at Naples, offer an extraordinarily good object for the study of these processes, much better than the eggs of Salmonides, studied chiefly by French and German authors [1]). The formation of chorda and mesodermic plates out of the folded portion of the blastoderm, and of the entoderm out of the "prostomal thickening", the mass of cells that lie at the hind-end of the embryo and are connected with the superficial layer and with the periblast, is clearly to be seen from the beginning of the foimation of the embryo until the closure of the yolk-blastopore (confirmed by Sumner in his paper of 1904) and after a renewed careful study of these eggs [2]) I can only confirm entirely and in full the conclusions arrived at in my former paper [3]) and the observations described there at some length.

But in accordance with the new and better definition of gastrula-

---

[1]) Neither Henneguy, nor Kopsch or Jablonowski, to take a few examples, did see anything of these differentiations. Sumner gives however of Salvelinus very clear figures and descriptions. (Arch. f. Entwickelungsmech. Bd 17. 1903).

[2]) During the last 2 or 3 years Muraenoid-eggs seemed to have disappeared entirely from the Gulf of Naples. Now (summer 1906) I found them again in sufficient quantities. When comparing the different eggs with each other, it seemed to me that they belong to a still larger number of different species than I concluded in my former paper (9), and that there must be distinguished at least 10 different species of Muraenoid eggs in the Gulf of Naples. Dr. Sanzo at Messina came to the same conclusion

[3]) Petrus Camper, Vol. 2, page 135—210 1902.

tion in vertebrates, given by HUBRECHT and KEIBEL and confirmed by a number of other embryologists, this process in the teleostean egg too must be revised and more sharply defined.

In my former paper I was led to divide the process of gastrulation into two "phases", one by which the gut-entoderm is formed and one by which chorda and mesoderm are differentiated. But now I think the line must be drawn still sharper and the second phase must be separated entirely from the process of gastrulation sensu strictiori.

According to the definition given by HUBRECHT gastrulation is a process by which a gut-entoderm is differentiated from an ectodermic layer, and thus the germ consists of two distinct layers. The process of formation of chorda and mesodermic plates, which follows directly on the process of gastrulation proper (notogenese HUBRECHT) is a secondary complication of the process, characteristic of the vertebrate embryo.

The most primitive mode of formation of the entoderm, according to HUBRECHT, is by delamination and not by invagination. But after all it is chiefly the outcome, the formation of the two germ-layers, that is of interest. As soon as these two layers are formed and may be distinctly separated from each other, the process of gastrulation is finished.

This is for example in amphioxus already the case at that stage of development, in which the gastrula is cap-shaped, the two layers (ectoderm and entoderm) are lying close against each other, the segmentation-cavity has disappeared, but the blastopore still extends over the entire breadth of the original blastula-vesicle. All the following processes until the closure of the blastopore ("Rückenmund" of HUBRECHT) are notogenesis and lead to the formation of the back (chorda) and of the mesodermic plates and to the closure of the gastrula-mouth.

When we study again the teleostean gastrulation-process from this point of view, we come to the conclusion, that in this case the process of gastrulation is ended as soon as the prostomal thickening has been formed, viz. at the beginning of the covering of the yolk. At that moment the "Anlage" of the entoderm is clearly differentiated, and the ectodermal cells begin to invaginate to form the chorda and mesodermic plates; the concentration of the cells towards the median line begins, the long and slender embryo is formed out of the broad and short embryonic shield. The blastula-cavity, in the cases in which it is developed, has disappeared as such; all the following processes, the longitudinal growth of the embryo, the covering of the yolk by the blastoderm ring, the closure of the yolk blastopore, belong to the notogenesis and we are no more entitled to reckon these processes

55*

to gastrulation proper as we are to do that of the covering of the yolk by the entoderm in sauropsids. During this longitudinal growth of the embryo new cells are produced by the prostomal thickening and pushed inwards to form the entoderm, but this may not be called gastrulation any more. The period of development, during which the yolk is being covered by the blastodermring, differs much in different embryos. In muraenoids at the time the yolk-blastopore is closed the embryo possesses from 5 to 10 pairs of primitive segments; the issuing larvae possess 58 to 75 segments. In salmonidae at the closure of the yolk-blastopore of the 57 to 60 segments 18 to 28 are differentiated. The other organs too are developed to a greater or lesser degree. To use the term gastrulation for the processes during this whole period of development leads us into difficulties.

THE first question we have to answer, when we study closer the process of gastrulation in teleosts, is: at what time does the process of gastrulation begin in the large meroblastic eggs?

Recently BRACHET[1]) has called attention to a process, which he calls "clivage gastruléen", and which he describes for the eggs of Rana fusca as the formation of a circular groove at the base of the segmentation-cavity around the yolk-mass, before there is to be seen a trace of a blastopore (Rusconic groove) at the outside of the egg: "immédiatement[2]) avant que la gastrulation ne commence, la cavité de segmentation, sphérique ou à peu près, occupe l'hémisphère supérieur de l'oeuf (de Rana fusca).... Bientôt, sur tout le pourtour du plancher de la cavité de segmentation, une fente se produit par clivage; cette fente 's enfonce entre les cellules de la zône marginale et les divise en deux couches: l'une, superficielle, prolonge directement la voûte de la cavité de segmentation, mais est formé par des cellules plus volumineuses et plus claires qu' au pôle supérieur; l'autre, profonde, fait corps avec les éléments du plancher. C'est ce clivage, que j'ai appelé "clivage gastruléen", c'est lui, qui caractérise la première phase de la gastrulation, parce qu'il amène, en dessous de l'équateur de l'oeuf, la formation d'un feuillet enveloppant et d'une masse cellulaire enveloppée, d'un ectoblaste et d'un endoblaste." And farther on: "lorsque ce clivage est achevé, il est clair, qu'a sa limite inférieure, l'ectoblaste et l'endoblaste se continuent l'un dans l'autre, comme le faisaient antérieurement la voûte et le plancher de la cavité de segmentation."

This line of continuity BRACHET calls "blastopore virtuel"; after a short time this virtual blastopore is converted into a real blastopore

---

[1]) Archives de Biologie Tome 19 1902 and Anatom. Anzeiger. Bd. 27 1905,
[2]) Anat. Anzeiger Bd. 27, p. 215.

by the formation of the groove that leads to the formation of the archenteric cavity. This groove is formed by delamination; until now there is no trace of invagination. This begins in what BRACHET calls the second phase of the gastrulation process, which leads to the formation of the archenteric cavity in its entire width, and is synchronic with the process of notogenesis, of the formation of the back of the embryo; "quand les lèvres blastoporales se soulèvent, quand de virtuelles elles deviennent réelles, c'est que le blastopore va commencer à se fermer, c'est que le dos de l'embryon va commencer à se former" (l.c. 1902, p. 225).

BRACHET is right here. Also there, where be draws a sharp line between the entirely embryogenic blastoporus of the holoblastic eggs and the blastoporus of the meroblastic eggs with a large amount of yolk, which is divided into two parts, an embryogenic blastoporus and a yolk-blastoporus.

But when he reckons these processes, which occur in the selachian and teleostean egg during the covering of the large mass of yolk and the closure of the blastopore, still to gastrulation, when he calls the entire process of covering of the yolk "clivage gastruléen", and calls the whole blastoderm ring "blastopore virtuel", he goes too far, and forgets the significance of the phenomena, occurring at the end of segmentation and during the formation of the periblast.

For the answer to the question, at what time does the gastrulation in the teleostean egg begin, his analysis of the phenomena of this process in the amphibian egg is extremely interesting.

The segmentation of the teleostean eggs is not regular during all its phases. When we combine the very accurate observations of KOPSCH on this account, we see, that in the segmenting blastoderm at a definite moment, about that of the 10th division of the embryonic cells, there occurs an important alteration.

Until the end of the 10th cell-division (in Belone) the different cells divide wholly synchronic; in Torpedo RÜCKERT found synchronism until the 9th division. By the tenth division the yolk-sac entoblast is formed (in Gobius, Crenilabrus, Belone), the two nuclei of the marginal segments, resulting from this division, remaining in the undivided protoplasm; where this does not occur at the tenth division the deviation is very small (in Cristiceps argentatus it partly begins at the 9th division, in Trutta fario at the eleventh division). Synchronically with the differentiation of yolk-sac entoblast the superficial layer ("Deckschicht") is differentiated. At the end of the 10th division all at once the blastoderm alters its form: it gets higher, more hill-shaped and the diameter ʻis lessened; the mass of cells

concentrates, the superficial layer is still more clearly visible as a
definite enveloping layer of cells. It is just the synchronic differen-
tiation of the superficial layer, which shuts off the blastoderm from the
surrounding medium and is the only way by which the developing
cells may get the oxygenium from the perivitelline fluid, on one side,
and of the periblast, by means of which the blastoderm is nourished
by the yolk, on the other side, which seems to me to be important;
by this synchronic differentiation a new phase in the developmental
process is initiated, and the series of changes have begun that lead
to gastrulation.

Very soon the blastoderm-disc flattens, at first only because the
superficial layer contracts a little, and the blastoderm sinks a little
into the yolk-sphere (fig. 8) but after that because the blastodisc
itself spreads out, flattens (fig. 9). The cells come closer together,
and soon the unilateral thickening that forms the first outwardly
recognisable beginning of the building of the embryo, becomes visible.

During these changes it is of no account whether a blastula-cavity
is formed, or not. As I have described elsewhere, in different murae-
noids during this stage a distinct blastula-cavity is formed, which may
be seen in the living egg. Afterwards follows the flattening of the
blastodisc and the disappearance of the cavity as such. The closer
study of young stages of the eggs of muraena N°. 7 [1]) showed me
however, that in these eggs no blastula-cavity is formed, and that
in this case the blastoderm, that takes just the same conical shape
as the hollow blastoderm in the other muraenoid eggs, remains solid
and is built up of a mass of loosely arranged cells. The further
development is the same as in the other series (c.f. fig. 1—3 on
plate 1).

This flattening of the blastodisc, following on the stage just described,
coinciding with the concentration of the cells of the blastoderm
towards the side where in later stages the embryo is formed, and
coming before the invagination (and partial delamination) of the
blastoderm cells, that leads to the formation of the chorda and the
mesodermic plates, is already a part of the gastrulation process and
must be compared with the "clivage gastruléen" of the amphibian egg.

Immediately on this "clivage gastruléen" follows the formation of
the prostomal thickening (that is the "blastopore réel" of BRACHET),
there where the superficial layer or pavement layer is connected
with the periblast, out of the surperficial cells of the periblast [2]) (c.f.

---

[1]) Comp. PETRUS CAMPER, Vol. II p. 150.

[2]) SUMNER (l. c. page 145) saw evidences for this mode of origin in the egg of
Salvelinus, but not in that of Noturus or Schilbeodes. On these two forms I can-

fig. 4, 5 and 6 on plate 1). It seems probable, that at least in some cases entodermcells are formed by delamination from the periblast at some distance from the surface in front of the prostomal thickening (fig. 5e). So here, as in many vertebrates, the entoderm is formed by delamination. At the moment of the differentiation of the prostomal thickening (figs. 2, 4), there is still no trace of the invagination of the mesodermcells, only a thickening of the mass of cells lying just overhead of the cells of the prostomal thickening. Immediately afterwards however a distinct differentiation of the mesoderm becomes visible. At that stage the notogenesis begins and the gastrulation process is finished. The prostomal thickening is the ventral lip of the "blastopore réel" of the Amphibian egg. For the developmental processes following on this stage I can contain myself with referring to my former paper. That here only a small, not very prominent tail-knob is formed and no far-reaching projecting tail-folds appear, as in the selachean embryo, is caused by the relation of the pavement-layer to the blastoderm and the periblast, which influence the development of teleostean egg ("développement massif" of HENNEGUY).

- 2. To determine the direction of growth of the blastodermring during the covering of the yolk, I used in my former paper the oil-drops in the yolk of the muraenoid eggs as a point of orientation, on the contention that these oildrops maintain (in the muraenoid egg) a constant position in the yolk. On this basis I constructed a scheme of the mode of growth of the blastoderm in the yolk. [1]) Both SUMNER and KOPSCH. rejected this contention and the scheme, SUMNER because of the fact, that by inverting the egg of *Fundulus heteroclitus* in a compress, the oil-drops may be caused to rise through the yolk and assume a position antipodal to their original one, which shows, that here the oil-drop may not be regarded as a constant point of orientation in the egg. In this SUMNER is perfectly right. Not only in Fundulus, but in several marine pelagic eggs too the oil-drops may be seen travelling through the yolk by converting the egg or bringing the young larva (in some species) in an abnormal position. In the muraenoid egg however the case is entirely different. Here the structure of the periblast and of the

not judge, but I will only mention here, that the figures, drawn by the author, are taken of much too late stages of development, to be convincing. And after all, where the blastodermcells are so much alike, as is the case in most teleostean eggs, one positive result in a favourable case as is offered in the muraenoid egg, is more convincing than several negative results in less favourable eggs.

[1]) l. c. page 142.

yolk-mass, which I described at full length in my former paper, completely checks the displacement of the oil-drops. This is to be concluded already from the behaviour of the normal egg. So in the eggs of Muraena No. 4 a large number of rather large oil-drops are lying at about equal distances from each other at the surface of the yolk-mass. During the entire process of covering of the yolk, the distance of these oil-drops remains the same, they maintain their relative position absolutely, and only during the slight disfigurement of the yolk-sphere, caused by the contraction of the blastodermring during the circumgrowth of the yolk (fig. 4 on plate 2) the position of the oil-drops is changed a little, only to become the same as before, after the yolk has regained its spherical form. When these oil-drops were lying loose in the yolk or in the periblast, they would have crowded together at the upper pole of the egg, or at least their relative position would have undergone a change during the covering of the yolk. Only when the yolk-mass in the developing embryo becomes pear-shaped and very much elongated (l.c. plate 2, fig. 6, 7), the oildrops of course change their position. Even then, however, they remain scattered through the yolk.

Experiments also show the constant fixed position of the oil-drops in the muraenoid eggs. When we transfix the egg-capsule carefully with a fine needle, it is possible to lift one of the oil-drops or a small portion of the peripheral yolk out of the egg. The other oil-drops retain their normal position, and in most cases such eggs develop normally and give rise to normal embryos. When we operate very carefully under a low-power dissecting-microscope, it is possible to leave the oil-drop connected with the periblast by means of a thin protoplasmatic thread. When we do this in a very early stage of development, at the beginning of the gastrulation-process, we see that this oil-drop, which surely may be regarded as a fixed point on the surface of the egg, retains its position in relation to the other oil-drops, until it is cut off from the periblast by the growing blastodermring. In fig. 2a, 2b, 2c and 2d on plate 2 I have drawn from life several stages of this process in an egg of Muraena No. 1. During my stay at the Stazione Zoologica at Naples, in August and September 1906, I performed several of these experiments with different muraenoid eggs. They all led to the same result, and confirmed my former statements. And so I believe that my contention was right and that the scheme I figured is a true representation of the facts. Of course only in a general sense, for there are many individual variations (so for example the case figured in fig. 3 on plate 2). And after all, when we compare this scheme with that

given by Kopsch for the trout, we see that they do not differ so very much, and that the displacement of the hinder end of the embryo is almost the same. In the text of my former paper however I expressed myself rather ambiguously, and brought my view into a too close contact with that expressed by Oellacher. The figures however show that my scheme differs rather much from that of Oellacher.

'But I differ from Kopsch in his supposition that the h e a d-end of the developing embryo is a fixed point on the periphery of the egg. I find myself here quite in harmony with Sumner, who draws from the large series of his extremely careful and exact experiments the conclusion, that "the head end also grows, or at least moves, forward, though to a much smaller extent" (l. c. page 115), and says: "1 regard it as highly probable (see Exp. 1, 3, 34, 35, 36 and Fig. 32) that the primary head end grows — or is pushed — forward from an original position on the margin" (l. c. page 139).

From different experiments of the author we may draw the conclusion, that in many cases this forward growth of the head-end is rather extensive (exp. No. 6, 10, 11 (partly), 26, 35, fig 10), and experiment N°. 6 (table VIII) among others shows, that under circumstances the direction of growth may be entirely reversed, so that the tail-knob of the embryo remains at the same place, and the head-end bends round the surface of the yolk.

Kopsch too, in his paper: "Ueber die morphologische Bedeutung des Keimhautrandes und die Embryobildung bei der Forelle", describes an experiment with simular results in the trout.

So it is not unreasonable to suppose, that in the spherical egg of the Muraenoids during the covering of the yolk the head end of the embryo is moving forward, and to a certain extent follows the growing blastodermring, which is the case chiefly during the later stages of the covering of the yolkmass, as I showed in my scheme. During the first stages of development it is chiefly the tail-end of the embryo which travels backwards, (see the scheme in my former paper and fig. 1—3 or plate 2), and Kopsch is right to locate here the centre of growth of the embryo.

The conclusion of Sumner, that for some time prior to the closure of the blastopore, the ventral lip of the latter (former anterior margin of the blastoderm) travels much faster than the dorsal lip (l. e. p. 115) is quite in harmony with my results for the muraenoid egg described in my former paper. [1])

---

[1]) Petrus Camper, l. c. p. 196.

3. At the end of the covering of the yolk, at the closure of the blastopore, KUPFFER's vesicle is formed after the manner described at length in my former paper. By SWAEN and BRACHET [2]) and by SUMNER the narrow passage connecting this vesicle with the exterior, through the closing blastopore, is regarded as representing the neurenteric canal. I do not think they are in the right here. KUPFFER's vesicle is a ventral formation. Dorsally it is separated from the cells of the medulla by the cells of the prostomal thickening and the pavement layer. An open canalis neurentericus is not found even in these forms. KUPFFER himself called the vesicle allantois. HUBRECHT followed him in this. In my former paper I compared the vesicle with the allantois of amniota on physiological grounds, and I think it is a very good thought of HUBRECHT to take up the old name of KUPFFER and compare the vesicle with the allantois on morphological grounds.

DESCRIPTION OF FIGURES ON PLATE 1 AND 2.

Plate 1.

Figg. 1—4. Median sections through eggs of Muraena N⁰. 1 on different stages of gastrulation. In fig. 3 gastrulation is finished and notogenesis is begun. In fig. 2 the structure of the yolk is drawn. Enlargement 40 times. Fig. 4a, 5 and 6 give median sections through the developing prostomal thickening and adjoining parts, seen under a higher power.

Figg. 7—9. The flattening of the blastodisc at the beginning of gastrulation in eggs of Muraena N⁰. 7. Enlargement 40 times.

Plate 2.

All the figures on this plate are drawn from life as accurately as possible.

Fig. 1a—1e. Covering of the yolk in an egg of Muraena N. 1.

Fig. 2a—2d. Covering of the yolk and closure of the blastopore in an egg of Muraena N⁰. 1. By means of a fine needle one of the oil-drops is nearly severed from the surface of the yolk, remaining connected with the periblast only by means of a thin protoplasmatic thread. In fig. 2c this oil-drop is cut off from the surface of the egg by the travelling blastodermring and is lying close against the egg-capsule EK. In fig. 2d (closure of the blastopore) this oil-drop is no more drawn in the figure.

Fig. 3. Unusually fargoing dislocation of the hinder end of an embryo during the covering of the yolk. The head end lies approximately at the former centre of the blastodisc.

Fig. 4. Compression of the yolk-sphere by the growing blastodermring in an egg of Muraena N⁰. 4. The oil-drops only temporarily changed their relative distances a little.

OD = oildrop.
pv = prostomal thickening
per = periblast.
Bl = blastoderm.
D   = pavement layer
e   = entoderm

Leiden, 17 January 1907.
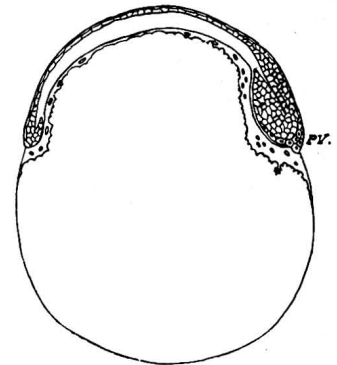
[2]) Archives de Biologie T. 20. 1904. page 601.
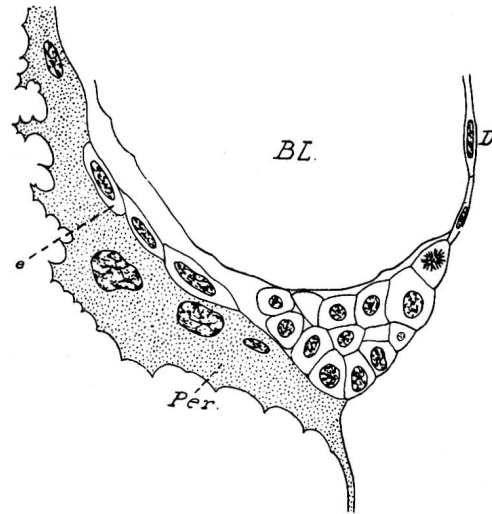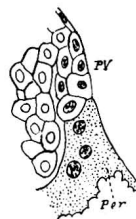
PLATE 1.
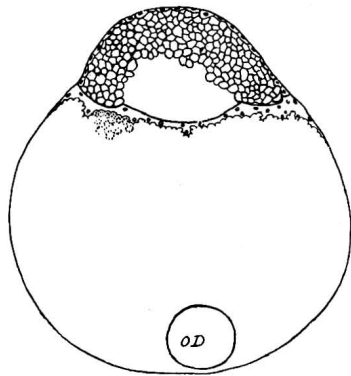
Fig. 1.
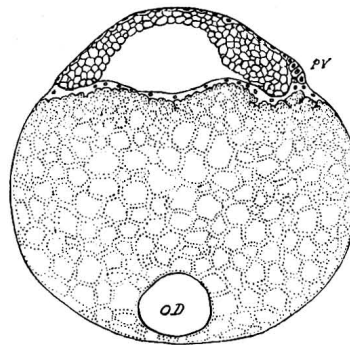
Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.
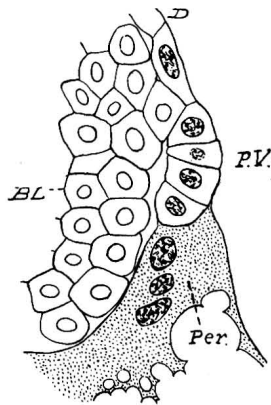
Fig. 6.
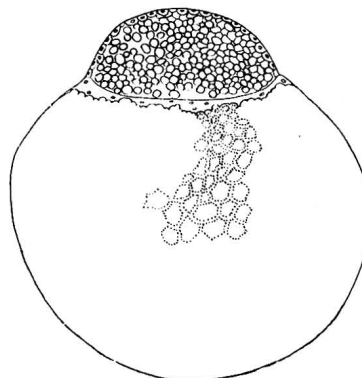
Fig. 7.

Fig. 8.

Fig. 9.

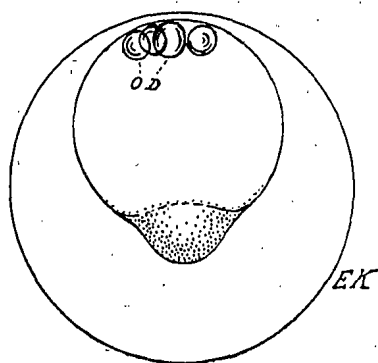*J. Boeke del.*

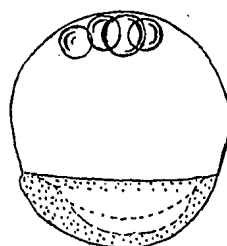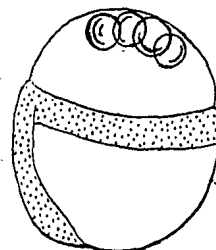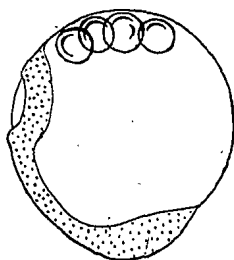J. BOEKE. On Gastrulation and the covering of the yolk in the teleostean egg.
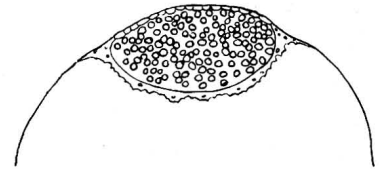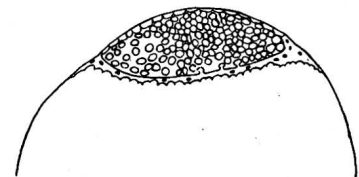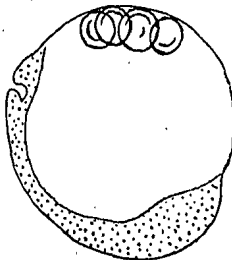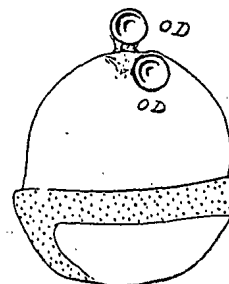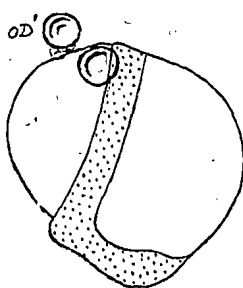
PLATE 2.

Fig. 1a.

Fig. 1b.

Fig. 1c.

Fig. 1d.

Fig. 1e.

Fig. 2a.

Fig. 2b.
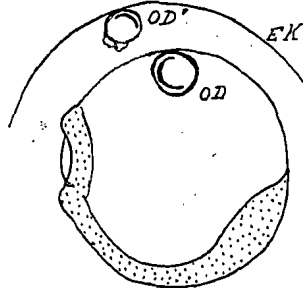
Fig. 2c.
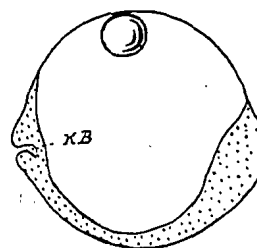
Fig. 2d.

Fig. 3.

Fig. 4.

J. Boeke del.