INTRODUCTION.

The following pages constitute a further instalment of my description of the developmental phenomena of Lepidosiren paradoxa. I have devoted much time and

1 The first instalment, containing a description of the "External Features in Development," is to be found in 'Phil. Trans. Roy. Soc.,' B, vol. cxci, p. vol. 45, part 1.—new series.
labour to making a very detailed investigation of the stages in development here treated of, and I had originally intended in my description to go into something like corresponding detail. I have, however, altered my original intention in this respect, for various reasons: amongst others because in the interpretation of minute details of early development one is necessarily much influenced by preconceived ideas; and in the second place, because I find that these details vary to an extraordinary extent in different eggs—some of the variations being apparently due to variation in technical methods of investigation, but many being certainly true individual variations. So potent are these disturbing factors that I doubt very much whether a description going into very minute detail must not necessarily be to a great extent misleading, and so do harm. I therefore propose to limit myself in regard to the early stages of development to the endeavour to give an adequately complete general description of the phenomena observed, with only so much detail as may seem necessary to make the description clear.

The investigation of a holoblastic egg 7 mm. in diameter and packed with yolk involves great technical difficulties, as the whole of each egg has to be converted into thin sections. The full extent of these difficulties will only be appreciated by embryologists who have essayed a similar task. In order to help future workers I devote a few paragraphs to a general account of methods. Then follows an account of the phenomena observed, in which, as in my first paper, I reserve remarks of a general nature embodying views rather than facts for a concluding section, so that any reader may obtain the facts, which are naturally of greater importance, with a minimum of trouble.

299. As in that paper I naturally did not make precise statements regarding the interpretation to be put upon surface features without having assured myself first by the examination of sections that they were correct, it is unnecessary for Prof. Semon to feel the doubts about the behaviour of the blastopore in *Lepidosiren* which he expresses in his latest contribution on the development of *Ceratodus* (Semon, *Zoologische Forschungsreisen,* Band i, S. 327).
In conclusion I have to record the gratitude which I owe to my friend Mr. J. S. Budgett for the generous way in which he has placed his store of Protopterus embryos at my disposal. By his kindness I am able to interweave with my description, references to what takes place in the only other Dipnoest still surviving, and consequently to greatly increase its value.

**METHODS.**

The eggs and larvae on being brought in from the swamp were first studied alive. For permanent preservation two fluids were used—formalin and alcohol. Of the former solutions in water of from 5 per cent. to 10 per cent. were used, and I found formalin an admirable preservative for the early stages. It caused practically no shrinkage either of capsule or embryo. It further left the former transparent as in the fresh condition. The material of early stages fixed and preserved in formalin was found to be in admirable condition, both as to fixation and as to consistency for section work. This, however, only applies to the early and heavily yolk-laden stages.

The alcohol material was fixed in a variety of ways. Practically all the ordinary fixing agents were tried, but the best all-round results were obtained by corrosive sublimate and acetic acid, and Flemming's chrom-aceto-osmic solution (strong formula). Perenyi's solution proved to be unreliable.

For section cutting, after many weeks of failure, the following three stock methods were adopted:

1. Thick sections of early eggs, where the cell elements were very large, were cut with a "Jung" microtome after soaking for three days in thin celloidin, three days in thick celloidin, and thirty minutes in chloroform, followed by treatment with cedar oil until clear. The block was kept saturated with cedar oil, and the sections were transferred in order to a shallow tray containing the same fluid. The sections were then arranged upon strips of tissue-paper 3 inches by 1 inch within a space equal to the size of the cover-slip used. The paper strips with the sections lying on
them were now laid in a bath of absolute alcohol, to remove
the cedar oil, and then taken up and laid sections downwards
upon slides coated with a layer of dry collodion. A finger
was now passed lightly along the paper, giving a gentle
pressure, just sufficient to cause the celloidin of the sections
to adhere to the collodion on the slide. The slide was now
removed to 90 per cent. alcohol and the ordinary process of
staining carried out. In the subsequent dehydration pre-
vious to mounting a mixture of chloroform and absolute
alcohol was used for the final stage of the process.

II. To obtain thin sections of yolk-laden eggs, it was
necessary to embed in both celloidin and paraffin. The pre-
liminary embedding in celloidin was done as before. The
egg was taken from celloidin solution and dropped bodily
into chloroform for 15—30 minutes. At first I was in the
habit of transferring the celloidin block to cedar oil before
embedding in paraffin, but latterly I have frequently em-
bedded at once by the chloroform-paraffin method. It is of
great importance to keep the temperature of the water-bath
as low as possible, and also to diminish the length of time
during which the object is on the water-bath to the shortest
possible.

Sections were cut with a Cambridge rocking microtome,
and flattened with warm water on a slide coated with
glycerine and egg albumen. The water was drained off
and the slides put aside to dry in an atmosphere containing
vapour of alcohol and ether. It was found that drying in
an ordinary atmosphere over the water-bath caused the
celloidin-infiltrated section to dry, curl up, and break away
from the paraffin: this was avoided by drying in the manner
described. It is important, however, not to use an atmo-
sphere completely saturated with ether and alcohol vapour,
as this, by causing the celloidin to swell, may cause wrinkling
of the sections.

III. Older embryos were embedded in paraffin in the
ordinary way and cut with the rocking microtome.

Orientation.—For the accurate orientation of embryos
during the embedding process I use a special apparatus\(^1\) in which a pool of paraffin in contact with the block holder of the microtome is kept melted by a small loop of platinum, nickel, or other wire of high resistance and not easily oxidisable, heated by the current from one or two ordinary bichromate cells.

**Staining.**—After many trials of different staining fluids I adopted two stock methods.

I. Early eggs rich in yolk were stained in Grübner's "Safranin 0"—a saturated solution in absolute alcohol, diluted with an equal volume of distilled water. In regard to formalin eggs, difficulty was found in obtaining a good chromatin differential stain. This difficulty was completely got over by treating the eggs with corrosive sublimate solution for a couple of hours before transference to alcohol.

II. Later embryos were stained in Heidenhain's iron hematoxylin followed by faint staining with eosin. By this stain beautiful preparations were obtained showing minute nuclear detail to perfection.

**Mounting Medium.**—When sections of early eggs did not stain successfully they were mounted in colophonium, which on account of its lower refractive index shows up feebly stained structures better than Canada balsam.

**Reconstruction.**—In working out the organogeny of Lepidosiren I have found the following method of reconstruction from serial sections extremely useful. Sections 10\(\mu\) thick are drawn with the Abbe camera lucida at a magnification of 100 diameters upon finely ground sheets of glass 1 mm. in thickness. Sheets of glass bearing drawings of consecutive sections are then piled in position on top of one another, a fluid of the same refractive index as the glass being run in between adjacent sheets. The result of this is to convert the whole into a transparent block, in which the structures drawn are seen occupying space of three dimensions, forming a kind of model. Different organs are drawn in different colours, lead pencil or coloured crayons

\(^1\) Made for me by the Cambridge Scientific Instrument Company.
(not anilins) being used. It is best, I find, only to do one or two systems of organs at a time, the process being so rapid compared to ordinary modelling by Born's method that it can easily be repeated if necessary. When I first devised this method I used a chemical solution having the exact refractive index of the glass, but latterly I have used ordinary clove oil, which is near enough for practical purposes. With clove oil ordinary water-colour pigments may be used.\footnote{Mr. Budgett, who has been recently using my method of reconstruction, strongly recommends the use of moist water-colours.}

The above method is not meant to give a permanent model of the structures investigated, as does the Born method of reconstruction from sections; but, on the other hand, it involves far less expenditure of time, and is to be strongly recommended for purposes of research. The main principle of the method—the using sheets of glass or other transparent plates on which to draw the consecutive sections—has been used by other workers, e.g. Strasser and Dixon, and more recently by Vosmaer. I have not, however, come across any mention in literature of the two details upon which to my mind the chief beauty of the method rests, viz. the using sheets of ground glass to draw upon, and the subsequent rendering these transparent by an interposed fluid of high refractive index. The first of these details provides a particularly suitable surface upon which to draw; the second gives a perfect transparency to the mass of superimposed plates, quite unattainable where there are numerous alternating layers of substances differing so much in refractive index as do glass and air.

**Early Development of Lepidosiren.**

Segmentation and Origin of Segmentation Cavity.—A vertical section through a mature egg of Lepidosiren shows that the interior is filled with a mass of yolk granules, the protoplasmic substance between being so small in quantity as to be quite invisible. The yolk granules are rounded or occasionally subangular in form. Through the greater
part of the egg there are large granules, measuring, as a rule, between 0.15 mm. and 0.2 mm. in diameter, and of the characteristic salmon-pink colour, while the interstices between these are filled with smaller granules. There is no indication of a region of specially coarse-grained yolk in the centre of the egg, but towards the surface of the "animal" portion the large granules are absent, and there is present a superficial layer in which the yolk is entirely broken up into very minute particles, whose innumerable reflecting surfaces give to this part of the egg a snowy white appearance when seen by incident light. In the middle of this cap of fine-grained yolk lies the germinal vesicle, the details of whose structure I have not been able to make out satisfactorily.

As segmentation proceeds, the fine-grained yolk spreads downward towards the centre of the egg—the smaller blastomeres being distinguished by their fine-grained yolk from the larger lower blastomeres, where the yolk remains in large granules. Even in this latter region, however, the division planes become marked out by a septum of fine-grained yolk.

As mentioned in my former paper, the segmentation cavity begins to appear very early, in the form of chinks between the micromeres. In an egg of Stage 8 (Pl. 1, fig. 1) the cavity within the egg still remains in the form of such

1 Although the eggs laid by one female may be said to be on the whole more coarsely grained than those of another, yet there is much variation even amongst the eggs laid by a single female; e.g. in four eggs taken from one nest the large yolk granules averaged 0.018, 0.018, 0.020, and 0.022 mm. in diameter respectively; in three eggs taken from another nest the corresponding dimensions were 0.015, 0.015, and 0.02 mm.

2 This statement must be taken as true only in a general sense; every now and then one meets with a few coarse granules within the micromeres; while in the region of the macromeres irregular patches of comparatively fine-grained yolk frequently appear.

3 By "Stage x" I mean an egg whose external features have reached the stage of development represented by fig. x of my previous paper. At Prof. Lankester's suggestion I have had a figure (Text-fig. 1) prepared to illustrate the chief stages, and so to obviate the necessity of frequent reference to the plates of my previous paper.
The extent of these cavities varies considerably in different eggs of the same age, the blastomeres in some being more rounded, in others less rounded and more flattened against one another. The more rounded condition of the blastomeres in the former case does not appear to be associated with the nuclei being in a state of karyokinetic activity, as has been asserted to be the case in other forms.

Text-fig. 1, illustrating the course of development of the Dipneumona.—The stages are numbered in accordance with my earlier paper. Roman numerals indicate figures of Protopterus (after Budgett, 'Trans. Zool. Soc.,' vol. xvi). The remaining figures refer to Lepidosiren. In figs. 16—24 the embryo is for convenience shown spread out in one plane and viewed from the dorsal aspect. The magnification is slightly over two diameters. br. Rudiment of external gills. c.o. Cement organ. h.l. Rudiment of hind limb. p.n. Pronephros. 8. Egg during segmentation. 10. An early stage of invagination, the invagination groove stretching round about one third of the egg's circumference. 13. A later stage of invagination, the large yolk-cells being now for the most part covered in by small cells. xiii. Corresponding stage in Protopterus. 14. Egg at the close of invagination, showing the crescentic blastopore. 16. Dorsal view of an embryo in which the medullary folds have just become visible, diverging posteriorly to embrace the blastopore. 17. Later embryo where the folds have met behind the blastopore, and are approximated in the middle region of the embryo; the rudiment of the pronephros is now visible as a slight bulging on either side. 19. The medullary folds are nearly completely fused; the branchial rudiment is visible as a bulging in front of the pronephros; indications of the myotomes are seen between the pronephros and the neural rudiment. 22. The branchial rudiment has greatly increased in size, the optic rudiments are conspicuous, the pronephric ducts have grown considerably backwards. 24. Embryo in which the branchial rudiment has become completely segmented on the right-hand side; the central cavity of the neural rudiment has appeared as a dark shadow. 25. Side view of a slightly older embryo in its natural position on the egg; the rudiments of the four external gills now form distinct projections; the rudiment of the cement organ has appeared ventrally. xxxv. Corresponding embryo of Protopterus. 28. Larva three days after hatching. 31. Larva (thirteen days) in which the external gills have become pinnate, and the rudiments of the limbs have appeared (anterior hidden by external gills). 35. Larva with external gills at their maximum; the cement organ, now in course of atrophy, is seen beneath the throat. xxxv. Corresponding larva of Protopterus. 36. Young Lepidosiren with external gills in process of atrophy.
As already mentioned, the yolk in the micromeres is reduced to the condition of fine granules. These also become reduced in number, and the nucleus tends to be surrounded by an area of finely granular reticular protoplasm, almost free from yolk granules. The transition from the finely granular micromeres to the coarsely yolked macromeres is perfectly gradual.

Between Stages 8 and 9 there appears an irregular chink of larger size than the others amongst the lower micromeres (Pl. 1, fig. 2). This, the definite segmentation cavity, increases in size, spreading laterally, and at the same time approaching close to the upper surface of the egg, being eventually covered in by a roof of comparatively regular thickness throughout. This roof soon becomes composed of two regular layers of cells (figs. 3 and 4). As the segmentation cavity further increases in size these become flattened out, until the roof forms a thin translucent membrane through which in the entire egg the segmentation cavity appears as a dark shadow. The characters of the completed blastula may be sufficiently gathered from fig. 4.

The blastomeres on the floor and sides of the segmentation cavity are rounded, almost spherical in form, and project into the cavity. Usually, some of these spherical blastomeres appear to float quite free in the fluid of the segmentation cavity. This appearance does not of course prove that they are not really connected up to the other blastomeres by delicate protoplasmic strands; but such connecting threads if present are too delicate to be seen by ordinary observation.

Gastrulation.—The process of gastrulation in Lepidosiren may for convenience of description be divided into three periods, which I will call A, B, and C.

A. In this period, which marks the beginning of gastrulation, we have to do with a process of true invagination. The commencement of this process is indicated, as I have shown in my previous paper, by the appearance of a row of little depressions of the egg's surface arranged in a latitudinal direction a few degrees below the equator. These depres-
sions soon become joined up to form a continuous groove stretching through about one third of the circumference of the egg at this latitude (cf. Text-fig. 1, fig. 10). A section through the whole egg at this stage is given in fig. 6 (Pl. 2), and sections through the groove itself under a higher magnification in figs. 5 and 7 (Pl. 1).

In the cells lining the groove much of the yolk has passed into a state of fine subdivision, thus pointing to cell activity. From the open character of the groove during this stage it is obvious that we have to do with a process of true invagination. In some series of sections one can see very well (fig. 7) how the groove, although to the naked eye apparently coincident with the boundary between the cells with small and those with large yolk granules, lies really just within the region of the latter. The invagination in Lepidosiren is thus essentially a lower cell phenomenon.

The groove, as mentioned in my previous paper, gradually becomes more limited in extent by its lateral portions becoming flattened out. Had it extended at any period of ontogeny completely round the exposed area of large cells, we should have been able to speak of a yolk-plug. As it is probable that the disappearance of a yolk-plug bounded all round by an invagination groove is due to increase in size and richness of yolk in the egg, I had hoped to find it present in Protopterus. In this I have been disappointed, the condition in this respect being just as in Lepidosiren.

While the lateral parts of the groove flatten out and disappear, the middle part is deepening to form the archenteric cavity.

b. The exact method by which this takes place in its earlier stages forms a problem of considerable general interest, but at the same time one the final solution of which is attended with great difficulties.

The appearance of sections during this period is illustrated by fig. 8 (Pl. 2). The archenteric cavity runs obliquely inwards from the surface of the egg, and at its inner end turns upwards so as to run roughly parallel to the surface. The
whole cavity is slit-like in form and is eminently suggestive of having been formed by a process of splitting amongst the large yolk-cells, after the manner described by Robinson and Assheton in the case of the frog. Further evidence is afforded in this direction by the fact that many sections show the archenteric slit to end in a perfectly sharp edge (fig. 9), which may even appear to be prolonged by division planes along which the cells have not yet separated. Had I had to rely upon a small amount of material, I should almost certainly have described the archenteric formation during this stage as being carried on by a process of splitting. I have, however, examined now a very large number of series of sections, and I am disposed to think that the process is by no means one of simple splitting. In the first place, by looking through complete series of sections, one as a rule finds that, in certain sections, the archenteron terminates in a clear rounded end (fig. 10). It appears impossible to me to imagine that this can occur if the cavity is only extending by a splitting process. Further, it is usual to find that, round the tip of the archenteron, the cells have assumed a triangular shape in section, with their tips towards the archenteron, which strongly suggests the existence of a compressing force acting round the tip of the cavity, and of such a nature as would be caused by growth of either roof or floor of the cavity. On the whole, I conclude that increase of the archenteric cavity does take place mainly by true invagination during this stage also. The slit-like appearance in many sections may conceivably be an artefact due to the roof of the archenteron being squeezed down against its floor by the action of the fixing agent, or possibly the process of invagination may be aided by one of splitting. There seems nothing improbable and indeed little of importance in this, notwithstanding how much has been written on the subject. If it does occur it is only another example of a very common phenomenon in yolk eggs,—the formation by splitting of a cavity elsewhere formed by invagination.

As regards the probable cause of the invagination—beyond
the use of the vague phrase "differential growth"—nothing can be said. The absorption of the fluid in the segmentation cavity which is associated by Samassa with the invaginatory process of Amphioxus is excluded as an explanation of the phenomenon here, as the first obvious result of such absorption would be the collapse of the very thin and delicate roof of the segmentation cavity, and such collapse is conspicuously absent.

In transverse section the archenteron is seen to be, in this stage, a tube rounded in section—in other words, showing no signs of splitting laterally, and about '2 mm. in diameter, strikingly narrow in proportion to the diameter of the egg as compared with most holoblastic forms.

Towards the end of period B the archenteron approaches the margin of the segmentation cavity, and now we have very distinct evidence that the growth of the archenteron is not due to splitting, for the cells round its tip become pushed definitely into the segmentation cavity forming a rounded bulging into it (Pl. 2, fig. 8). As the process goes on the large-yolk cells become laid up against the original roof of the segmentation cavity, which, already two-layered, alters little in character and will later become definitive epiblast. The further stages in the obliteration of the segmentation cavity I will deal with later.

c. In the later stages of gastrulation we have certainly to do with a process of true invagination, the end of the archenteron being always quite smooth and rounded, with cuticular lining, and there being never any trace whatever of splitting (cf. Pl. 3, figs. 11 and 12). The precise character of this invagination could only be settled definitely by experiment upon the living egg, and such experiments, though attempted, proved absolutely fruitless on account of the tough egg capsule and the soft nature of the egg contents. From the study of sections 1 of the eggs I am disposed to believe that

1 In my account of the external features I pointed out that against the probability of such a backgrowth taking place, was the fact of the blastoporic lip not assuming the form of an arc of gradually diminishing radius with its
we have to do with an invagination of the large yolk-cells of the lower lip of the blastopore by the upper dorsal lip growing bodily down over them. The evidence upon which this belief rests is as follows:

(a) A sagittal section through an egg of this stage fixed in such a way as to avoid shrinkage of the capsule is shown in outline in Text-fig. 2. It is obvious that the general outline of the section suggests strongly that the dorsal lip of the concave side downwards. As a matter of fact this objection is done away with by the fact that in Protopterus frequently the lip does become concave downwards just as we should expect (cf. a forthcoming paper by Mr. Budgett in 'Trans. Zool. Soc. Lond.,' vol. xvi). The blastoporic lip becoming convex downwards in Lepidosiren I attribute now to the backgrowth being more active in the middle line than laterally.

TEXT-FIG. 2.—Camera outline of sagittal section through an egg in its capsule at a late stage of gastrulation. The lines O A, O B, and O C are drawn from the centre of the section so as to touch respectively the tip of the archenteron (O A), the edge of the small-celled area (O B), and the dorsal lip of the blastopore (O C).
blastopore is growing bodily downwards, wedging itself in between the capsule and the large yolk-cells, and causing as it does so the latter to invaginate into the floor of the archenteron.

(β) The frequency of mitotic figures in the region overlying the archenteron, and more especially in the dorsal lip, appear to indicate active growth of this region, and consequent backward migration of the blastoporic lip.

(γ) During the later stages of gastrulation I find that the angle between the lines O A and O B (passing from the centre of the section to the tip of the archenteron and to the margin of the small-celled area respectively) remains nearly constant, and the increase in the angle A O C corresponds fairly closely with the diminution in the angle C O B.

This seems to suggest that the line O C is gradually swinging through the arc between A and B. Otherwise we must believe that the lines O B and O A are swinging with equal velocity in a clockwise direction. It appears to me from study of my sections that this is not the case, the forward movement of the point B being very slow compared with the advance of the archenteric tip.

(δ) The cells of the ventral wall of the archenteron are continuous, without any visible change in character, with the large yolk-cells lying exposed on the outer surface of the egg below the blastopore.

On the whole, then, I believe that the evidence, such as it is, points to the view that the main factor of the increase in length of the archenteron during this last stage is the downgrowth of the blastoporic lip.

While these processes of formation of the archenteron have been going on the area of yolk-cells exposed has been gradually reduced, dorsally by the growth of the blastoporic lip, elsewhere by the gradual encroachment of the small-celled area. This spreading of the small cell margin over the yolk-cells is most rapid in the neighbourhood of the blastoporic lip, least so at the point opposite to this. In this latter region the superficial layer of small cells passes into a
thickened rim, which at first I called the growing edge of the epiblast. Further investigation showed, however, that the chief characteristic of this rim is not its growth, which is comparatively small, but the fact that it represents the mass of small cells on which the roof of the segmentation cavity rested at its margin. The thin two-layered epiblast, in fact, from this rim for a considerable distance is nothing else than the persistent roof of the segmentation cavity. This is shown to be the case by the fact that within a short distance of the rim one frequently finds the small blastomeres beneath the epiblast retaining their rounded form with chunks between, or we may even find the segmentation cavity still present as a continuous slit.

What spreading of small cells over the large yolk-cells does take place is brought about by addition to the margin of small cells cut off from the yolk. This is well shown by sections such as that in fig. 13, where there can be no question of true epibole or sliding of the small cell layer over the surface of the yolk-cells.¹

The slight extent of the movement over the yolk of the small-celled margin at the point opposite the blastopore rim is of importance as providing a nearly fixed point in the interpretation of sagittal sections. The evidence of these sections is, on the whole, that the dorsal roof of the archenteron is formed mainly by backgrowth of the dorsal lip, and as the medullary plate at its first appearance is practically coincident with the extent of the archenteron, Lepidosiren is brought into line with the Selachians, where almost the whole of what is commonly called the "embryo" is formed from a similar backgrowth.

The Segmentation Cavity.—I now return to the consideration of the segmentation cavity, which was left at a period when it was beginning to be encroached upon by the bulging wall of the archenteron. The further obliteration of the segmentation cavity, although it takes place

¹ By an error the word "epibole" was used in my former paper at one place (p. 322) when delamination was actually meant.
during the process of gastrulation, does not by any means keep time with the latter—a further support to my assertion that the former is not the direct cause of the latter.

What takes place may be said to be in general terms that the floor of the segmentation cavity is brought up against its roof. During this process, however, a transient phase occurs which is not without interest. While the cavity is still at its full development we notice a tendency for large-yolk blastomeres to become arranged round the segmentation cavity, and in close contact with its roof (cf. Pl. 2, fig. 8; or better, figure of Protopterus VIII); following this, the smaller blastomeres lying in and near the floor of the cavity push out processes, become irregular and angular in shape, and, attaching themselves to one another by their corners, form a loose and irregular sponge-work traversing the cavity completely (Pl. 3, fig. 11). As will be gathered from the figures, the segmentation cavity during this process, although broken up by the sponge-work, really extends through a much larger volume than it did before. As, however, gastrulation proceeds, the fluid filling the meshes of the sponge-work becomes absorbed, and the blastomeres resume their spherical or, as they become pressed closer together, polyhedral shape. We may still for a long time, however, observe chinks persisting here and there, especially laterally. The roof cells of the segmentation cavity remain all through the stages we are now describing sharply marked off from the large yolked elements which have been laid up against them.

Origin of the Mesoblast and Notochord.—Pl. 3, fig. 14, illustrates a section through an egg of Stage 12 and transverse to the axis of the medullary plate region. Lying over the archenteron and tapering off on each side is a mass of cells distinguished from the remainder of the inner cells by their smaller size, more finely granular yolk, and by their rounded form. Immediately over the archenteron these small cells are aggregated closely together, but laterally as a rule they are separated by wide chinks—the remains of the
segmentation cavity. At its outer edge this mass of small cells passes gradually into the large inner cells. The sum of small cells in question is the rudiment of notochord and mesoblast. It is perfectly continuous across the middle line, and is separated from the cavity of the archenteron by a definite archenteric roof composed of cells closely fitted together.

The cells of the notochordal-mesoblastic rudiment are the small blastomeres which are seen in earlier stages lying below the floor and round the edges of the segmentation cavity, or penetrating that cavity as a sponge-work.

A little later—in Stage 14 (cf. Text-fig. 1)—a transverse section (Pl. 3, fig. 15) exhibits very similar features, only now the mesoblastic cells are in close contact with one another, and the mesoblastic rudiment is found to be growing at its edges by delamination from the underlying large yolk-cells. The rate of this growth varies much. As a rule, in an egg of Stage 14 the mesoblast extends very little below the level of the archenteron on each side, though in one case I found that it had grown right round the ventral side of the egg. The process is in any case usually completed by Stage 18 or 20. For example, in an egg of Stage 18 I find that, although the actual splitting off of the mesoblast has taken place only to a level slightly below that of the archenteron, the superficial layer of yolk has become fine-grained all round the egg, and here and there a small mesoblast cell has separated off the large yolk-cells beneath. Such mesoblast cells are often split off far beyond the edge of the sheet of continuous mesoblast, so that when I speak of the mesoderm spreading over the hypoblast I must guard against giving the impression that the sheet is necessarily continuous up to a definite margin. Finally, in eggs of Stage 21 the stratum containing fine-grained yolk has been cut off the underlying hypoblast all over, as a definite layer, somewhat irregular in places, of rounded mesoblast cells.

Where the mesoblastic rudiment has in its early stages largely developed intercellular spaces, the boundary between it and the large yolk-cells is sharply marked very
early (except in the middle line and at its outer margin). Where the cells composing the rudiment are in close contact the line of demarcation may be for a time indistinct. But in any case by Stage 14 the mesoblastic rudiment on each side becomes separated definitely from the underlying hypoblast (except at its outer edge), and a little later (Pl. 4, fig. 16, and Text-fig. 3) it becomes separated in a similar way from the axial portion which will give rise to the notochord. This latter remains in the meantime attached to the hypoblast. It should be mentioned incidentally that the cells added to the edge of the mesoderm sheet tend to take on a rounded

**Text-fig. 3.—** Section through a complete egg of stage transverse to axis of embryo. *ent.* Enteron. *m.* Mesoblastic rudiment. *m.p.* Ectodermal thickening of medullary plate. *n.* Rudiment of notochord.
form as soon as they become separated from the hypoblast. It consequently often happens that, when the sheet is continuous up to its edge, this edge with its rounded cells is very sharply marked off from the hypoblast beyond. With only such sections to go by one might well believe that the sheet of mesoblast was quite independent of the hypoblast, and growing inwards over its surface from the blastoporic rim after the manner described for various forms by Lwoff, Brauer, and others. It is at once seen from the study of a complete series of stages, such as the above account is based upon, that any appearance of the kind is quite secondary, and that originally mesoblast and hypoblast rudiments are perfectly continuous. I will return to this question later on.

With the formation of the medullary keel the mesoderm sheet becomes thickened out to each side of it in the region where the myotomes are to be formed.

On account of the yolk-laden character of the mesoblastic rudiment it is difficult to make out when its segmentation begins. Distinct protovertebræ were first found in about Stage 17, where there were six present. They were squarish in section and were solid.

Cœlom.—The first parts of the cœlom to appear are myocoelic. In Stage 21 (Pl. 4, fig. 21) a cœlomic cavity is seen to have appeared in the centre of the myotome. This appears to arise by simple breaking down of the central cells, the cavity not having at first any sharply-marked outline, and irregular masses of yolk-laden protoplasm projecting into it. A little later (Stage 23) the outline is quite definite and the cavity is walled in by a single layer of regular columnar cells. From this the cœlom spreads outwards by definite splitting.

Early Development of Notochord.—The Notochordal rudiment was left (Pl. 4, fig. 16) at a stage in which it remains attached to the hypoblast on the separation of the mesoblast from it on each side. It forms a median dorsal ridge running along the middle line above the archenteric cavity.
yolk in the cells of this ridge is usually in a state of comparatively fine subdivision, though much coarser than that of the epiblast.

A set of division planes now become so arranged as to mark off the notochordal part of the ridge from the comparatively thin basal layer next the cavity of the archenteron (fig. 17, e. r.). The cells of this latter frequently, though by no means always, retain their yolk in a coarse-grained condition.

They are part of the definitive hypoblast, and form the roof of the enteron. The enteric roof is thus differentiated in situ from the cells of the archenteric roof, without any trace of ingrowth from the sides such as has been described by Lwoff, Brauer, and others.

The notochordal rudiment thus laid down retains for some time its comparatively undifferentiated condition (figs. 20 and 21), showing no obvious change beyond assuming a rounder, more definite outline as it separates off the hypoblast. About Stage 23 the separation is completed, and the
notochord, now circular in transverse section, develops a fine cuticular membrane which foreshadows the sheath, and in longitudinal section its cells are seen to be becoming flat and plate-like.

In due course the notochord becomes separated off from neighbouring structures by mesenchymatous tissue, partly directly cut off the subchordal region of the hypoblast (Text-fig. 4, h.m.), but for the most part arising by proliferation from the inner surface of the mesoderm at about the level of the nephric rudiment very much as in Selachians, except that there is no obvious trace of a segmental arrangement (Text-fig. 4, scl.). I propose to postpone further consideration of the mesenchyme till a later period.

Origin of the Central Nervous System.— Already in Stage 12, as has been mentioned (cf. Pl. 3, fig. 14), the epiblast has become somewhat thickened over the region of the archenteron, the thickening affecting the lower layer especially whose cells have become more regularly columnar. By Stage 14, when there runs forward from the blastopore a faint depression along the axis of the medullary plate, this thickening has become more marked, and in addition the deep layer of epiblast is becoming more than one-layered (cf. figs. 15 and 16). The medullary plate thickening of the epiblast, most marked along the mid-dorsal line, extends outwards for a considerable distance, gradually thinning away on either side. The axial portion of the medullary plate rapidly increases in thickness, forming a deep wedge-shaped keel, the rudiment of the neural cord. This medullary keel develops from before backwards, and in some eggs of Stage 14 it has already begun to be distinctly formed anteriorly. By Stage 16 (cf. Pl. 4, figs. 17 and 18), where the medullary groove is well formed but widely open, the keel has increased much in thickness, being about five cells thick posteriorly, and thickening out anteriorly to about three times as much. Just about the anterior limit of the archenteron the keel tapers off, first suddenly, then gradually, till the ordinary two-layered condition of the general ectoderm
is reached. The whole thickening of the keel is confined to the deep layer of the ectoderm—the outer layer passing unaffected over the floor of the groove. As the medullary folds approach one another the groove shallows out and disappears. Occasionally, in places, the folds come in contact before the groove has disappeared, so that for a short time they remain separated by a vertical chink (Pl. 4, fig. 19). As before suggested, this may be looked on as a last trace of a former method of formation of the spinal cord by involution, but any trace of cavity that is so enclosed in Lepidosiren is purely temporary and soon disappears. The keel is now (fig. 20) absolutely solid, and there is no indication of the formation of a central canal until about Stage 20 (fig. 21), when the cells of the interior of the neural rudiment are seen to begin to assume a regular arrangement and columnar form on each side of the median plane.

Along this plane the cells finally split apart, apparently by the secretion of fluid, the cavity in preserved specimens showing an abundant coagulum. The split appears somewhat irregularly, but by Stage 23 it has become continuous, forming a well-marked cavity in the region of the fourth ventricle, and stretching back from this through about three fourths of the extent of the neural rudiment. Anteriorly and posteriorly the neural rudiment still is solid.

**Note upon the Early Development of Protopterus.**

The egg of Protopterus is much smaller than that of Lepidosiren, measuring only about 3.5—4 mm. in diameter (Budgett). Corresponding with this the yolk granules are smaller, averaging about .015 mm. by .01 mm. They have also a characteristic difference in shape, being very frequently lenticular or fusiform. The blastula of Protopterus differs from that of Lepidosiren in the relatively greater depth and volume of the segmentation cavity, and in the greater relative extent of the micromeric region of the egg. The roof of the segmentation cavity is also thicker.

Gastrulation—The line of invagination appears nearer
the lower pole of the egg than in Lepidosiren, about 30° below the equator instead of about 10°. It is consequently visible from the beginning when the egg is viewed from the lower pole, forming part of the circumference of the small circle bounded by the edge of the small-celled area. The condition is exactly as in a typical Urodele or Anuran egg, only here the groove never extends round the whole circle to enclose a definite yolk-plug, but, as in Lepidosiren, shortens up, flattening out at each end. The examination of sections shows that here as in Lepidosiren the invagination groove is at its first appearance distinctly within the coarsely-yolked portion of the egg.

The general features of gastrulation closely resemble those in Lepidosiren, and it is therefore not necessary to describe them in detail. I give, however, figures illustrating three successive stages (Pl. 2, figs. vi, viii; and Pl. 3, fig. xii). By comparison of fig. vi with fig. viii, the vertical axis being marked by the position of the segmentation cavity, it will be readily seen how important a part is played by overgrowth of the blastopore lip. The orientation of the egg during these stages is rendered simpler than it is in Lepidosiren by the segmentation cavity retaining its original relations much longer.

At the close of gastrulation the appearance of the egg is practically identical with that of Lepidosiren. I notice, however, that very frequently an egg of Protopterus at this stage assumes an ellipsoidal form, with the blastopore either at one end or somewhat ventral to this. In Lepidosiren only pathological or unfertilised eggs assume an ellipsoidal shape.

As regards the further points of development treated of in this paper, there do not appear to be any noteworthy differences between what occurs in Protopterus and what has been described for Lepidosiren.

1 The Protopterus egg very frequently passes through a stage identical in appearance with the stage in the development of Triton figured by O. Heitwig in 'Jen. Zeits.,' Bd. xv, Taf. xii, fig. 1.
THE DEVELOPMENT OF LEPIDOSIREN PARADOXA.

Size of Nuclei during Early Stages of Development of Lepidosiren.—Owing to the small scale of the figures it is not possible to indicate the relative sizes of the nuclei in different parts of the egg. These bear, as one might expect, a rough relationship to the volume of the cell territories over which they preside; e. g. in two eggs of Stage 16 the nuclei of the ectoderm averaged 0:016 mm. and 0:014 mm. in diameter, those of the mesoderm 0:018 mm. and 0:016 mm., and those of the large yolk-cells 0:022 mm. and 0:021 mm. Again, in an egg of Stage 13 the nuclei in the region of the dorsal lip of the blastopore measured 0:015 mm., and those of the large yolk-cells 0:019 mm.

The measurements are in all cases the average of ten measurements of whole nuclei as seen in thick sections.

GENERAL REMARKS.

Segmentation.—In studying the segmentation of Lepidosiren I have been much struck by the readiness with which all trace of the division planes may be destroyed in the parts of the egg filled with large yolk-granules. The two commonest causes of this are, firstly, the use of a fixing agent of inferior penetrating power, the blastomeres running together into a continuous mass very soon after death if the fixing agent has not reached them; and secondly, the use of two thin sections. In cutting a section it would appear that the yolk-granules become very slightly displaced as they strike the edge of the knife, and if the section is very thin this is enough to completely obliterate the division planes. During segmentation in Lepidosiren thick sections will show an egg to be completely divided up into blastomeres, while in thinner sections the whole lower portion with coarsely-grained yolk seems to form a quite continuous unsegmented mass. The mass of uncleaved yolk figured by Semon in the middle of the Ceratodus egg, and upon which he bases the statement that this egg in its early stages of segmentation occupies a place intermediate between the telolecithal and centrolecithal types, may, I think, quite possibly be an artefact of this nature, due
to the fixing agent not having penetrated sufficiently rapidly; and it also seems by no means impossible that the lower part of the egg of Gymnophiona may be only apparently uncleaved for the same reason.

Segmentation Cavity.—The segmentation cavity of Lepidosiren arises in the normal fashion from intercellular chinks. Amia, whose segmentation otherwise so resembles that of Lepidosiren, is said to develop its segmentation cavity from intra-cellular spaces (Whitman and Eycleshymer). The mode of disappearance of the segmentation cavity, the blastomeres permeating it as a sponge-work, and then later rounding themselves off so as to leave the diminishing cavity in the form of chinks between them, resembles closely what occurs in Petromyzon as described by Nuel. It may quite possibly occur pretty generally, as in Lepidosiren this stage lasts such a short time that it might easily be missed.

The two-layered character of the roof of the cavity from an early stage is noteworthy. The roof, in fact, has taken on its definitive epiblastic character already in the blastula stage. In Ceratodus the roof is one-layered; and in other cases where it is two or three layers thick, it is usual for a one-layered condition to be passed through before it becomes definite epiblast (Petromyzon, Axolotl, Gymnophiona).

Blastoporic Lip Downgrowth.—In Amphioxus it has been shown that the blastopore occupies the hind end of the embryo. So it is with Lepidosiren, so that we may reasonably compare embryos of the two forms at the close of gastrulation.

It is commonly said that in a heavily yolked egg the macromeric part has become too bulky to allow of invagination. This is true only in a restricted sense, there not being room for the macromeric portion to be pushed bodily within the other as in Amphioxus. In such a form as Lepidosiren, however, new space is continually being provided by

1 'J. Morphol.,' vol. xii, p. 336.
2 'Arch. Biol.,' t. ii, p. 436.
the continued increase in area of the small-celled outer layer of the egg due to the backgrowth of the upper lip, and under this invagination goes on in the ordinary way. This is, it appears to me, the real significance of the backgrowth. It is a phenomenon directly associated with the increase in bulk of the macromeres. If this were true, we should find it become more and more pronounced as a developmental feature with increase in the quantity of yolk. This is, I think, what we do find, and we can also understand on this view why recent observers have failed to find such a process taking place in Amphioxus.

I do not propose to enter at length into the controversy which has raged over the parts played by invagination, splitting, downgrowth of dorsal lip, etc., in the gastrulation of vertebrates. Much of the evidence that has been brought seems to me unreliable, resting as it does on such characters as size of cells, size of yolk-granules, presence of pigment—characters which appear to me to be in great part merely the expression of greater or less metabolic activity for the time being, and which cannot therefore safely be used as criteria in treating of morphological questions.

Apart from these, the evidence afforded by the study of sections is of such a character that its interpretation is liable to be seriously affected by the observer's preconceived ideas. As regards observations on the living egg, many of the methods also seem open to the influence of very serious disturbing factors, either of a traumatic nature or of a simple physical character, such as movement of the egg as a whole, brought about by shifting of the centre of gravity due to the change in the relative extent and position of archenteric and segmentation cavities. The only really reliable method of investigation appears to be that of Kopsch, where the developing egg is submitted to prolonged photographic exposures, and the surface-cell movements worked out on the pictures so obtained.

My own conclusions with regard to the part played by backward movement of the blastopore lip agree closely with those reached by Kopsch for Amphibia, and my support of his views is strengthened by the fact that I had not seen his paper until I had finished my observations of the phenomenon in Lepidosiren.

As will have been gathered from the descriptive part of this paper, I am strongly of opinion that in Lepidosiren the main factor in the formation of the archenteron is a process of invagination. I am not at present, however, prepared to deny that during what I have called Stage B of gastrulation this process may not be aided to some extent by splitting.

Communication between Archenteron and Segmentation Cavity.—The view expressed by Kupffer in 1879,¹ that the enteron is formed by a fusion of the two originally separate cavities—archenteron and segmentation cavity—has recently been supported for the large eggs of Salamandra maculosa and Gymnophiona. It will be seen from figs. 8 and 11 how thin is the septum separating these cavities, and how easily they might be thrown into one by rupture of the intervening wall. In one or two eggs I have found this happen. I attribute it to the fixing fluid not having penetrated properly; but whether this be so, or whether it really existed in the living egg, it is in any case quite abnormal in Lepidosiren, and in all except these few exceptional cases the two cavities remain completely shut off.

Formation of Parts of Archenteric Roof from "Ectodermic" or "Animal" Cells.—In the preceding description I have made no statements regarding ingrowth of ectodermic or animal cells along the roof of the archenteron. Assertions that this occurs in other forms seem to me to be weakened by two fallacies. In the statement by Lwoff, Brauer, and others that the plate above the archenteron which gives rise to chorda and mesoderm is ecto-

dermic, there appears to me to lurk a confusion of ideas between the two pairs of antithetical terms—ectoderm and entoderm (or epiblast and hypoblast), and micromeres and macromeres (or animal cells and vegetable cells). The latter pair of terms are purely descriptive, and may be applied to blastomeres at once upon the evidence of an isolated observation. The former, on the other hand, are terms associated with definite theory; they are not to be applied on mere observations of sizes and shapes of cells, but involve the fate of the cells. It seems to me quite impossible to define a layer as hypoblastic except by asking one or other of the two questions: (1) Does it form the lining of an archenteric cavity? and (2) Does it become a certain part of the definitive epithelial lining of the gut? And if during the early stages of development a certain set of cells become invaginated along a considerable extent of the archenteric roof, this seems to me in itself amply sufficient reason for calling such cells hypoblastic quite apart from what their special characters of size, shape, content, and so on may be. There is no justification at all that I can see for calling the small fine yolked cells towards the upper pole of the egg epiblast, and on their extension in along the archenteric wall to found the statement that "ectoderm becomes invaginated." Because these cells behave as they do they are not ectoderm, but entoderm.

Further, a main character upon which these cells along the archenteric roof are relegated to the category of "ectoderm" or "animal cells" is the finely granular character of their yolk. Size of contained yolk granules is a form of evidence which must be used with the greatest caution, for wherever metabolism is active there the large yolk granules are broken down into fine granules to facilitate assimilation. All the yolk is destined to be so broken down eventually, and the fact of its having done so in some particular part of the egg earlier than elsewhere seems to indicate merely that metabolism is there more active. Consequently I can attach little weight to statements on the morphological nature of
particular cells based on the finely granular character of the yolk. I should attach much greater weight to the presence of large granules of yolk in cells, for when the yolk is in this form in a developing embryo it seems usually to indicate that it has remained so all through, it being at least very unusual for yolk to be secondarily built up again into large granules during embryonic development.

The finely granular character of the yolk frequently shown by the roof, as compared with that of the floor of the arch-enteron, I would look upon then as being merely a necessary accompaniment of the active growth of this region associated with the backgrowth of the blastopore lip.

What I have said regarding the unreliability of evidence of the morphological nature of cells from the finely granular character of their yolk contents applies equally well to the presence of black pigment in cells. I believe it to be one of the most general reactions to light stimulus for active but unspecialised cells to have their metabolism so affected as to cause the formation of this particular product. Examples are seen in the case of comparatively undifferentiated cells wandering into a position where they are subjected to light stimulus, e.g. to the surface of the body, or into the vicinity of a special light-collecting organ (e.g. Arthropod eye). Where pigment occurs in the smaller cells of a frog's egg it is, I think, to be correlated simply with the more active metabolism going on in these cells, and it is rather the absence of pigment in special cases which demands explanation; in many cases this may be due to natural selection—as in the case of eggs which are laid in a floating mass of white foam, where their being black would render them extremely conspicuous.

1 Which once produced may well be made use of as a protective agent for neighbouring tissues against the harmful influence of light rays.

2 With the criticisms in the foregoing paragraphs are to be associated those on similar lines of Houssay ('Arch. Zool. exp.', 2nd sér., t. viii) and Samassa ('Verh. Deutsch. Zool. Gesell.', Strasbourg, p. 139; also 'Arch. Entw. Mech.', Bd. ii and Bd. vii).
Formation of Secondary Enteric Roof.—Brauer has described in Gymnophiona the formation of the definitive enteric roof by a backgrowth of "vegetative cells" under the original archenteric roof. In Lepidosiren no such backgrowth takes place. It is to be noted, however, that there is much variation in the character of the yolk granules in the cells lining the archenteric roof immediately ventral to the chorda. Most usually these cells have fine granules, but very frequently, on the other hand, they are distinctly marked off from the chorda cells by their yolk remaining in much coarser granules (cf. Pl. 4, fig. 17). With only scanty material, in which the later stages happened to show this difference, one might well imagine it due to a secondary growth of large yolked cells in beneath the chorda rudiment. In view of this possibility of erroneous interpretation of sections I do not feel absolutely convinced that such a backward growth of large yolk-cells under the original cells of the archenteric roof as has been described by Brauer and also by Lwoff actually takes place.

In regard to Brauer's observations I might add that in my personal opinion the large-grained character of the cells figured by him as growing backwards makes it unlikely that they are multiplying with the activity which would be necessary on his view.

In regard to Brauer's fig. 59, where the large yolked cells extend right to the blastopore, it is of importance to note that the author expressly states that it is not a median section. In Lepidosiren it is only the median part of the archenteric roof that is fine-grained.

In Ceratodus Semon has described the roof of the enteron as being formed by an ingrowth from each side under the chorda rudiment. There is no such ingrowth in Lepidosiren. Where it does occur it may be looked on as a cænogenetic modification bearing the same relation to the method of chorda formation in Amphioxus, as the method of separation of the neural rudiment from the

ectoderm in *Amphioxus* does to the method occurring more usually by the formation of a neural groove. The method of chorda formation found in *Lepidosiren* may be compared, on the other hand, with the modification of the development of the neural rudiment occurring in Teleosts.

**Growth of Epiblast.**—In that the epiblast grows at its edge by delamination, *Lepidosiren* agrees with what has been found in various Amphibia (Houssay, Robinson and Assheton, Grönroos), but differs from what has been found by Brauer in *Gymnophiona*.

**Mesoblast Formation.**—In regard to the development of mesoblast there are two features of special interest. The first of these is the fact which cannot, I think, be doubted by anyone in *Lepidosiren*—that the so-called "gastral" mesoderm is formed directly out of the smaller blastomeres on each side of the archenteron. These masses are connected across the middle line, and the common rudiment of mesoderm and chorda is quite continuous. To go further than this and say, as has been done by others, that the notochord is derived from mesoderm, is quite unwarranted. I do not see any possible phylogenetic explanation of this phase in the formation of the mesoderm.

The later phase in its development which is of interest is that in which we see the mesoderm as a sheet upon each side, segmented or not according to its age, free at its inner thicker edge next the chorda, and thinning away to become continuous with the large yolk-cells or primitive hypoblast at its outer edge, where it continues to grow by delamination. Here we have a condition of things upon which I think a ray of light is thrown if we regard it as a fleeting reminiscence of the primitive method of mesoderm development in the Chordata.

The phenomena, in fact, in *Lepidosiren*, closely paralleled by those in *Petromyzon*, suggest a scheme of the steps by which the method of mesoderm formation in the higher vertebrates may have been derived from that found in *Amphioxus*, differing somewhat from that due to Hertwig.
Figs. 101 and 102 in Hertwig's 'Lehrbuch' are sufficient to illustrate his view of the derivation of the mesodermal rudiments in the higher vertebrates from the enterocellic pouches of Amphioxus. This view, as is well known, rests mainly on Hertwig's observations on the development of Triton, in which he found pouches of the archenteric cavity projecting on each side of the notochord into the mesoblastic rudiment, which pouches he interpreted as vestiges of the original communications between the archenteron and the cavity of enterocellic mesoderm pouches like those of Amphioxus. These observations of Hertwig appear to have failed to find adequate confirmation, and it seems to me that a scheme such as that represented below fits in better with the general facts of vertebrate development. Such a scheme, as will be seen, agrees in general principle with the theory suggested by Lankester and developed especially by O. Hertwig, that the mesodermal rudiments on each side of the vertebrate embryo represent the walls of the enterocellic pouches of Amphioxus; it differs from the Hertwig development of the theory in the detail that it regards the continuity often found in vertebrate embryos between mesoderm and hypoblast on each side of the notochord (and necessarily also the similar continuity between mesoderm rudiment and notochord) as being a secondary fusion rather than as representing the original connection of mesodermic diverticulum with wall of the archenteron.

The adjoining figures (Text-fig. 5) represent transverse sections through the embryos of Amphioxus, Petromyzon, Lepidosiren, and chick. In the case of the last three I have, for convenience, represented only a small portion of the whole section. As will be seen, the condition in Petromyzon agrees very closely with that in Amphioxus, and is immediately derivable from it by reduction in the size of the archenteric cavity.

The disappearance of the cavity of the enteric diverticulum
seems to me of no special weight; it is merely an additional example of a very common phenomenon, of the fact that hollow organs, formed primitively by involution of a cell-

layer, tend, where the cells are burdened with yolk, to arise from a solid rudiment, and to develop their cavity secondarily.
Passing on to Lepidosiren, the difference between it and Petromyzon is seen to be quite insignificant, consisting, in fact, only of difference in relative dimensions.

Finally, the condition of the mesoblast in one of the higher Vertebrata, as indicated in fig. D, seems to me to hang on equally well to the earlier members of the series. What difference there is is merely difference in shape and relative size. I hold, then, that in the series of Vertebrata there exist passing phases in the development of the mesoblast which may be readily linked on to one another, and that the existence of these phases may be accounted for by regarding them as reminiscences of phylogenetic stages in the modification of the process of mesoblast development.¹

Conclusion.—In general the phenomena described in this paper fully bear out what I referred to in my earlier communication—the extreme resemblance with corresponding features in the Urodela. As regards external features during the earlier periods of development this likeness is perhaps slightly less marked in Lepidosiren than in Protopterus, but as regards internal features of segmentation and gastrulation the most remarkable resemblance is seen.

The resemblance with Petromyzon is equally striking, and that with Ganoids only slightly less so. I do not feel it necessary to go into detail in this matter; it will only be necessary for the reader to turn to such figures as Houssay's² pl. xi, fig. 26 (transverse section of Axolotl, showing early stage of mesoblast); Calberla's³ fig. 7 (similar section through Petromyzon); Eycleshymer's⁴ pl. xx, fig. 8 (external view during early invagination of

¹ It will be noticed that, on the above hypothesis, the growth of the mesoblast at its outer side by continued delamination from the hypoblast would correspond to a continued deepening of the groove between mesoblastic and chordal rudiments of Amphioxus, and is therefore easily understood. Were Hertwig's scheme the true one this growth of the mesoblast would be quite incomprehensible.
² 'Arch. Zool. exp.,' 2nd série, tome viii.
³ 'Morph. Jahrb.' iii, Taf. xii.
⁴ 'J. Morphol.,' vol. x.
egg of Rana palustris), or Dean's\(^1\) pl. iv, fig. 62 (Acipenser, longitudinal section of egg during gastrulation), to see the remarkable unity which runs through these different types. Of the figures which I happen to have mentioned, the first three might have been used to illustrate the corresponding stages of Lepidosiren or Protopterus almost as well as the figures which I have given.

Looking at the broad facts in these three groups, and comparing them with what occur in other vertebrates, one cannot but be struck with the fact that in the only two groups in which it is almost certain that we have to do with poorly-yolked eggs in forms descended from richly-yolked ones, viz. the Teleostei and the higher Mammalia, we find that in neither has the process of gastrulation reverted to its original character. Rather by its profound modification from the normal type it bears witness to the changes which have taken place in its history. This being so, the comparatively simple type of gastrulation similar in Petromyzonts, Amphibia, Dipnoi, and Ganoids cannot but weigh strongly as evidence against the view propounded by Rabl, that any of these groups are descended from ancestors with large, richly-yolked meroblastic eggs.

There is one point which I should like, in conclusion, to draw attention to, and that is the shunting forwards in development of the rudiments of organ systems to an earlier period than that to which they normally belong. Thus by Stage 14, when gastrulation is just completed, the study of sections teaches us that the embryo is already a complicated triploblastic organism, with definite mesoblast and chorda.

I have obtained the small results recorded above only by prolonged work upon a most extensive material preserved with the greatest care and by the most approved methods. I have been greatly impressed by the variability observed amongst embryos of similar stages in development, much of it probably natural, much of it certainly due to differences

\(^1\) 'J. Morphol.,' vol. xi,
in methods of preservation, section cutting, etc.; so much so that my final description varies in many important respects from the rough draft made on a preliminary study of a few embryos. My experience convinces me of the futility of trying to give a fair description of the embryology of any type unless one has a very large material to go upon. Much of the discussion, involving often flat contradiction of distinguished observers' statements, which is constantly taking place appears to me to have a very probable cause in the small amount of material which has been made use of.

**Summary of the More Important New Facts.**

1. The segmentation cavity arises in Lepidosiren from intercellular chinks.
2. The roof of the segmentation cavity early becomes two-layered, and assumes the character of epiblast.
3. Gastrulation takes place, for the most part, by a true invaginatory process.
4. Spreading of small cells over large takes place by delamination, there being no true epibole.
5. The disappearance of the segmentation cavity is inaugurated by its penetration by a sponge-work of small blastomeres from its floor and sides.
6. The notochordal and mesodermal rudiments are at first quite continuous across the middle line.
7. The notochordal rudiment remains attached to the hypoblast for some time after the mesoderm has separated off on each side.
8. The enteric roof is formed in situ directly from the archenteric roof.
9. The mesoderm grows outwards on each side by delamination from the large yolk-cells.
10. The myoccele arises by breaking down of cells in the middle of the myotome.
11. Later on the myotome wall is composed of a single layer of regular columnar cells.
12. The first-formed mesenchyme arises from sclerotomic outgrowths, assisted by proliferation to a slight extent from the subchordal hypoblast.

13. The solid neural keel arises by thickening of the deep layer of the epiblast.

14. The egg of Protopterus closely resembles in its early development that of Lepidosiren.

15. The roof of the segmentation cavity is, however, thicker.

16. And the invagination groove appears about 20° nearer the lower pole of the blastula.

17. The early development of Lepidosiren and Protopterus shows an extraordinarily close resemblance to that of Amphibia Urodela; a close resemblance to that of Petroemyzon; and an only slightly less close resemblance to that of Ganoids.

EXPLANATION OF PLATES 1—4,
Illustrating Mr. Graham Kerr's paper on "The Development of Lepidosiren paradoxa," Part II.

As already pointed out, by "Stage n" I mean the stage represented by fig. n of my previous paper on the "External Features in Development." For the convenience of readers of the present paper I have copied and selected a number of these figures in Text-fig. 1 (p. 8). This will, I hope, obviate the necessity on the part of the reader of having to frequently refer to a separate publication.

Fig. 1.—Vertical section of egg of Stage 8, showing chinks between the micromeres. IV B 631.

Fig. 2.—Vertical section through egg, showing the commencing formation of the definite segmentation cavity, s. c. VII A 151.

Fig. 3.—Vertical section through the upper part of an egg slightly older than the last. The segmentation cavity has here begun to spread laterally. XXIV A 21.
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Fig. 4.—Vertical section through an egg, showing the segmentation cavity at its full development. V 201.

Fig. 5.—Vertical section through the groove of invagination just after its first appearance. (Stage 10) y 511.

Fig. 6.—Sagittal section through a very slightly more advanced egg. a. Spherical blastomeres round floor of segmentation cavity. i.g. Invagination groove. s.c. Segmentation cavity. V 371.

Fig. v.—Similar section through egg of Protopterus. D 192.

Fig. 7.—Part of the section drawn in Fig. 6 under a higher power, showing the groove to lie within the region of coarsely-yolked elements.

Fig. 8.—Sagittal section of an egg during a later stage of gastrulation, showing folding up of coarsely-yolked elements against the roof of the segmentation cavity. VI C 222.

Fig. vi.—Corresponding section through egg of Protopterus. A 411.

Figs. 9 and 10.—Small portions of two sagittal sections through an egg of similar age to the last. The sections show the tip of the archenteron; the section drawn in Fig. 9 favouring the idea of "splitting," that shown in Fig. 10 negating it. VI b 111 and VI b 201.

Fig. 11.—Sagittal section through an egg of Stage 12 to show the penetration of the segmentation cavity by a continuous sponge-work preparatory to its obliteration. 3* E 232.

Fig. 12.—Sagittal section through an egg of Stage 13 in which the segmentation cavity has become completely obliterated. xx D 262.

Fig. xii.—Corresponding section through egg of Protopterus. C 263.

Fig. 13.—Portion of a similar section to that in Fig. 11, to show the characters of the small cell margin. 3* D 211.

Fig. 14.—Stage 12. R 441.

Fig. 15.—Stage 14. 7* 551.

Fig. 16.—Stage 14. XXXVII C 542.

Fig. 17.—Stage 16. XXXVII E 531.

Fig. 18.—Stage 17. XXXIII 632.

Fig. 20.—Stage 21. XXXIV B 531.

Fig. 21.—Stage 21. XXXIV C 432.

These figures form a series meant to illustrate the gradual differentiation of the mesodermal, notochordal, and other rudiments. c.c. Indication of split to form central canal. e. Epiblast. ent. Enteron. e.r. Elements of enteric roof, here with coarsely-grained yolk. h. Hypoblast. m. Mesoblast. m.e. Thickened ectoderm of medullary plate. m.g.

Fig. 19.—Transverse section through neural rudiment.  Stage 18.

n.c. Involution of outer surface of ectoderm to form a vestigial neural canal.