MEMOIRS.

A Preliminary Account *of the* Development *of the* Elasmobranch Fishes.[1] By F. M. Balfour, B.A., Trinity College, Cambridge. (With Plates XIII, XIV, and XV.)

During the spring of the present year I was studying at the Zoological Station, founded by Dr. Dohrn at Naples, and entirely through its agency was supplied with several hundred eggs of various species of Dog-fish (Selachii)—a far larger number than any naturalist has previously had an opportunity of studying. The majority of the eggs belonged to an oviparous species of *Mustelus,* but in addition to these I had a considerable number of eggs of two or three species of *Scyllium,* and some of the Torpedo. Moreover, since my return to England, Professor Huxley has most liberally given me several embryos of *Scyllium stellare* in a more advanced condition than I ever had at Naples, which have enabled me to fill up some lacunæ in my observations.

On many points my investigations are not yet finished, but I have already made out a number of facts which I venture to believe will add to our knowledge of vertebrate embryology; and since it is probable that some time will elapse before I am able to give a complete account of my investigations, I have thought it worth while preparing a preliminary paper in which I have briefly, but I hope in an intelligible manner, described some of the more interesting points in the development of the Elasmobranchii. The first-named species (*Mustelus* sp.?) was alone used for the early stages, for the later ones I have also employed the other species, whose eggs I have had; but as far as I have seen at present, the differences between the various species in early embryonic life are of no importance.

Without further preface I will pass on to my investigations.

The Egg-shell.

In the eggs of all the species of Dog-fishes which I have examined the yolk lies nearest that end of the quadrilateral

[1] Read in Section D, at the Meeting of the British Association at Belfast.

shell which has the shortest pair of strings for attachment This is probably due to the shape of the cavity of the shell, and is certainly not due to the presence of any structures similar to chalazæ.

The Yolk.

The yolk is not enclosed in any membrane comparable to the vitelline membrane of Birds, but lies freely in a viscid albumen which fills up the egg-capsule. It possesses considerable consistency, so that it can be removed into a basin, in spite of the absence of a vitelline membrane, without falling to pieces. This consistency is not merely a property of the yolk-sphere as a whole, but is shared by every individual part of it.

With the exception of some finely granular matter around the blastoderm, the yolk consists of rather small, elliptical, highly refracting bodies, whose shape is very characteristic and renders them easily recognizable. A number of striæ like those of muscle are generally visible on most of the spherules, which give them the appearance of being in the act of breaking up into a series of discs; but whether these striæ are normal, or produced by the action of water I have not determined.

Position of the Blastoderm.

The blastoderm is always situated, immediately after impregnation, near the pole of the yolk which lies close to the end of the egg-capsule. Its position varies a little in the different species and is not quite constant in different eggs of the same species. But this general situation is quite invariable.

Segmentation.

In a fresh specimen, in which segmentation has only just commenced, the blastoderm or germinal disc appears as a circular disc, distinctly marked off by a dark line from the rest of the yolk. This line, as is proved by sections, is the indication of a very shallow groove. The appearance of sharpness of distinction between the germ and the yolk is further intensified by their marked difference of colour, the germ itself being usually of a darker shade than the remainder of the yolk; while around its edge, and apparently sharply separated from it by the groove before mentioned, is a ring of a different shade which graduates at its outer border into the normal shade of the yolk.

These appearances are proved by transverse sections to be deceptive. There is no sharp line either at the sides or below separating the blastoderm from the yolk. In the passage between the fine granular matter of the germ to the coarser yolk-spheres every intermediate size of granule is present; and, though the space between the two is rather narrow, in no sense of the word can there be said to be any break or line between them.

This gradual passage stands in marked contrast with what we shall find to be the case at the close of the segmentation. In the youngest egg which I had, the germinal disc was already divided into four segments by two furrows at right angles. These furrows, however, did not reach its edge; and from my sections I have found that they were not cut off below by any horizontal furrow. So that the four segments were continuous below with the remainder of the germ without a break.

In the next youngest specimen which I had, there were already present eighteen segments, somewhat irregular in size, but which might roughly be divided into an outer ring of larger spheres, separated, as it were, by a circular furrow from an inner series of smaller segments. The furrows in this case reached quite to the edge of the germinal disc.

The remarks I made in reference to the earlier specimen about the separation of the germ from the yolk apply in every particular to the present one. The external limit of the blastoderm was not defined by a true furrow, and the segmentation furrows still ended below without meeting any horizontal furrows, so that the blastoderm was not yet separated by any line from the remainder of the yolk, and the segments of which it was composed were still only circumscribed upon five sides. In this particular the segmentation in these animals differs materially from that in the Bird, where the horizontal furrows appear very early.

In each segment a nucleus was generally to be seen in sections. I will, however, reserve my remarks upon the nature of the nuclei till I discuss the nuclei of the blastoderm as a whole.

For some little time the peripheral segments continue larger than the more central ones, but this difference of size becomes less and less marked, and before the segments have become too small to be seen with the simple microscope, their size appears to be uniform over the whole surface of the blastoderm.

In blastoderms somewhat older than the one last described the segments have already become completely separate masses, and each of them already possesses a distinct nucleus. They form a layer one or two segments deep. The limits of the blastoderm are not, however, defined by the already completed segments, but outside these new segments continue to be formed around nuclei which appear in the yolk. At this stage there is, therefore, no line of demarcation between the germ and the yolk, but the yolk is being bored into, so to speak, by a continuous process of fresh segmentation.

The further segmentation of the already existing spheres, and the formation of new ones from the yolk below and to the sides continues till the central cells acquire their final size, the peripheral ones being still large and undefined towards the yolk. These also soon reach the final size, and the blastoderm then becomes rounded off towards the yolk and sharply separated from it.

The Nuclei of the Yolk.

Intimately connected with the segmentation is the appearance and history of a number of nuclei which arise in the yolk surrounding the blastoderm.

When the horizontal furrows appear which first separate the blastoderm from the yolk, the separation does not occur along the line of passage from the fine to the coarse yolk, but in the former at some distance from this line.

The blastoderm thus rests upon a mass of finely granular material, from which, however, it is sharply separated. At this time there appear in this finely granular material a number of nuclei of a rather peculiar character.

They vary immensely in size—from that of an ordinary nucleus to a size greater than the largest blastoderm-cell.

In Pl. XIII, fig. 1, *n*, is shown their distribution in this finely granular matter and their variation in size. But whatever may be their size, they always possess the same characteristic structure. This is shown in Pl. XIII, figs. 1 and 2, *n*.

They are rather irregular in shape, with a tendency when small to be roundish, and are divided by a number of lines into distinct areas, in each of which a nucleolus is to be seen. The lines dividing them into these areas have a tendency (in the smaller specimens) to radiate from the centre, as shown in Pl. XIII, fig. 1.

These nuclei colour red with hæmatoxylin and carmine and brown with osmic acid, while the nucleoli or granules contained in the areas also colour *very intensely* with all the three above-named reagents.

With such a peculiar structure, in favorable specimens these nuclei are very easily recognised, and their distribution can be determined without difficulty. They are not present alone in the finely granular yolk, but also in the coarsely granular yolk adjoining it. They form very often a special row, sometimes still more markedly than in Pl. XIII, fig. 1, along the floor of the segmentation cavity. They are not, however, found alone in the yolk. All the blastoderm-cells in the earlier stages possess precisely similar nuclei! From the appearance of the first nucleus in a segmentation-sphere till a comparatively late period in development, every nucleus which can be distinctly seen is found to be of this character. In Pl. XIII, fig. 2, this is very distinctly shown.

(1) We have, then, nuclei of this very peculiar character scattered through the subgerminal granular matter, and also universally present in the cells of the blastoderm. (2) These nuclei are distributed in a special manner under the floor of the segmentation cavity on which new cells are continually appearing. Putting these two facts together, there would be the strongest presumption that these nuclei do actually become the nuclei of cells which enter the blastoderm, and such is actually the case. In my account of a segmentation I have, indeed, already mentioned this, and I will return to it, but before doing so will enter more fully into the distribution of these nuclei in the yolk.

They appear in small numbers around the blastoderm at the close of segmentation, and round each one of them there may at this time be seen in osmic acid specimens, and with high powers, a fine network similar to but finer than that represented in Pl. XIII, fig. 2, *n y*. This network cannot, as a general rule, be traced far into the yolk, but in some exceptionally thin specimens it may be seen in any part of the fine granular yolk around the blastoderm, the meshes of the network being, however, considerably coarser between than around the nuclei. This network may be seen in the fine granular material around the germ till the latest period of which I have yet cut sections of the blastoderm. In the later specimens, indeed, it is very much more distinctly seen than in the earlier, owing to the fact that in parts of the blastoderm, especially under the embryo, the yolk-granules have disappeared partly or entirely, leaving only this fine network with the nuclei in it.

A specimen of this kind is represented in Pl. XIII, fig. 2, *n y*, where the meshes of the network are seen to be finer immediately around the nuclei, and coarser in the intervals. The specimen further shows in the clearest manner that this network is *not* divided into areas, each representing a cell and each containing a nucleus. I do not know to what extent this network extends into the yolk. I have never yet seen the limits of it, though it is very common to see the coarsest yolk-granules lying in its meshes. Some of these are shown in Pl. XIII, fig. 2, *y k*.

This network of lines[1] (probably bubbles) is characteristic of many cells, especially ova. We are, therefore, forced to believe that the fine granular and probably coarser granular yolk of this meroblastic egg consists of an active organized basis with passive yolk-spheres imbedded in it. The organized basis is especially concentrated at the germinal pole of the egg, but becomes less and less in quantity, as compared with the yoke-spheres, the further we depart from this.

Admitting, as I think it is necessary to do, the organized condition of the whole yolk sphere, there are two possible views as to its nature. We may either take the view that it is one gigantic cell, the ovum, which has grown at the expense of the other cells of the egg-follicle, and that these cells in becoming absorbed have completely lost their individuality; or we may look upon the true formative yolk (as far as we can separate it from the remainder of the food yolk) as the remains of one cell (the primitive ovum), and the remainder of the yolk as a body formed from the coalescence of the other cells of the egg-follicle, which is adherent to, but has not coalesced with, the primitive ovum, the cells in this case not having completely lost their individuality; and to these cells, the nuclei, I have found, must be supposed to belong.

The former view I think, for many reasons, the most probable. The share of these nuclei in the segmentation, and the presence of similar nuclei in the cells of the germ, both support it, and are at the same time difficulties in the way of the other view. Leaving this question which cannot be discussed fully in a preliminary paper like the present one, I will pass on to another important question, viz.—

How do these nuclei originate? Are they formed by the division of the pre-existing nuclei, or by an independent formation. It must be admitted that many specimens are strongly in favour of the view that they increase by division. In the first place, they are often seen "two together;" examples of this will be seen in Pl. XIII, fig. 1. In the second place, I have found several specimens in which five or six appear close together, which look very much as if there had been an actual division into six nuclei. It is, however, possible in this case that the nuclei are really connected below and only

[1] The interpretation of this network is entirely due to Dr. Kleinenberg, who suggested it to me on my showing him a number of specimens exhibiting the nuclei and network.

appear separate, owing to the crenate form of the mass. Against this may be put the fact that the division of a nucleus is by no means so common as has been sometimes supposed, that in segmentation it has very rarely been observed that the nucleus of a sphere first divides,[1] and that then segmentation takes place, but segmentation generally occurs and then a new nucleus arises in each of the newly formed spheres. Such nuclei as I have described are rare; they have, however, been observed in the egg of a *Nephelis* (one of the Leeches), and have in that case been said to divide. Dr. Kleinenberg, however, by following a single egg through the whole course of its development, has satisfied himself that this is not the case, and that, further, these nuclei in Nephelis never form the nuclei of newly developing cells.

I must leave it an open question, and indeed one which can hardly be solved from sections, whether these nuclei arise freely or increase by division, but I am inclined to believe that both processes may possibly take place. In any case their division does not appear to determine the segmentation or segregation of the protoplasm around them.

As was mentioned in my account of the segmentation, these nuclei first appear during that process, and become the nuclei of the freshly formed segmentation spheres. At the close of segmentation a few of them are still to be seen around the blastoderm, but they are not very numerous.

From this period they rapidly increase in number, up to the commencement of the formation of the embryo as a body distinct from the germ. Though before this period they probably become the nuclei of veritable cells which enter the germ, it is not till this period, when the growth of the blastoderm becomes very rapid and it commences to spread over the yolk, that these new cells are formed in large numbers. I have many specimens of this age which show the formation of these new cells with great clearness. This is most distinctly to be seen immediately below the embryo, where the yolk-spherules are few in number. At the opposite end of the blastoderm I believe that more of these cells are formed, but, owing to the presence of numerous yolk-spherules, it is much more difficult to make certain of this.

[1] Kowalevsky ("Beitrage zur Entwickelungsgeschichte der Holothurien," 'Mémoirs de l'Ac. Imp. de St. Petersbourg,' vii ser., vol. xi, 1867) describes the division of nuclei during segmentation in the Holothurians, and other observers have described it elsewhere.

As to the final destination of these cells, my observations are not yet completed. Probably a large number of them are concerned in the formation of the vascular system, but I will give reasons later on for believing that some of them are concerned in the formation of the walls of the digestive canal and of other parts.

I will conclude my account of these nuclei by briefly summarizing the points I have arrived at in reference to them.

A portion, or more probably the whole, of the *yolk* of the Dog-fish consists of *organized material*, in which nuclei appear and increase either by *division* or by a process of *independent* formation, and a great number of these subsequently become the nuclei of cells formed around them, frequently at a distance from the germ, which then travel up and enter it.

The formation of cells in the yolk, apart from the general process of segmentation, has been recognised by many observers. Kupffer ('Archiv. für Micr. Anat.,' Bd. iv, 1868) and Owsjannikow ("Entwickelung der Coregonus," 'Bulletin der Akad. St. Petersburgh,' vol. xix) in osseous fishes,[1] Ray Lankester ('Annals and Mag. of Nat. History,' vol. xi, 1873, p. 81) in Cephalopoda, Götte ('Archiv für Micr. Anat.,' vol. x) in the chick, have all described a new formation of cells from the so-called food-yolk. The organized nature of the whole or part of this, previous to the formation of the cells from it, has not, however, as a rule, been distinctly recognised. In the majority of cases, as, for instance, in Loligo, the nucleus is not the first thing to be formed, but a plastide is first formed, in which a nucleus subsequently makes its appearance.

Formation of the Layers.

Leaving these nuclei, I will now pass on to the formation of the layers.

At the close of segmentation the surface of the blastoderm is composed of cells of a uniform size, which, however, are too small to be seen by the aid of the simple microscope.

The cells of this uppermost layer are somewhat columnar,

[1] Götte, at the end of a paper on "The Development of the Layers in the Chick" ('Archiv. für Micr. Anat.,' vol. x, 1873, p. 196), mentions that the so-called cells in Osseous fishes which Œllacher states to have migrated into the yolk, and which are clearly the same as those mentioned by Owsjannikow, are really not *cells*, but large *nuclei*. If this statement is correct the phenomena in Osseous fishes are precisely the same as those I have described in the Dog-fish.

and can be distinguished from the remainder of the cells of the blastoderm as a separate layer. This layer forms the epiblast; and the Dog-fish agree with Birds, Batrachians, and Osseous fish in the very early differentiation of it.

The remainder of the cells of the blastoderm form a mass, many cells deep, in which it is impossible as yet or till a very considerably later period to distinguish two layers. They may be called the *lower layer cells*. Some of them near the edge of this mass are still considerably larger than the rest, but they are, as a whole, of a fairly uniform size. Their nuclei are of the same character as the nuclei in the yolk.

There is one point to be noticed in the shape of the blastoderm as a whole. It is unsymmetrical, and a much larger number of its cells are found collected at one end than at the other. This absence of symmetry is found in all sections which are cut parallel to the long axis of the egg-capsule. The thicker end is the region where the embryo will subsequently appear.

This very early appearance of distinction in the blastoderm between the end at which the embryo will appear, and the non-embryonic end is important, especially as showing the affinity of the modes of development of Osseous fishes and the Elasmobranchii. Oellacher ('Zeitschrift für Wiss. Zoologie,' vol. xxxiii, 1873) has shown, and, though differing from him on many other points, on this point Götte ('Arch. für Micr. Anat.,' vol. ix, 1873) agrees with him, that a similar absence of symmetry by which the embryonic end of the germ -is marked off, occurs almost immediately after the end of segmentation in Osseous fishes. In the early stages of development there are a number of remarkable points of agreement between the Osseous fish and the Dog-fish, combined with a number of equally remarkable points of difference. Some of these I shall point out as I proceed with my description.

The embryonic end of the germ is always the one which points towards the pole of the yolk farthest removed from the egg-capsule.

The germ grows, but not very rapidly, and without otherwise undergoing any very appreciable change, for some time. The growth at these early periods appears to be particularly slow, especially when compared with the rapid manner in which some of the later stages of the development are passed through.

The next important change which occurs is the formation of the so-called "segmentation cavity."

This forms a very marked feature throughout the early stages. It appears, however, to have somewhat different relations to the blastoderm than the homologous structure in other vertebrates. In its earliest stage which I have observed, it appears as a small cavity in the centre of the lower layer cells. This grows rapidly, and its roof becomes composed of epiblast and only a thin lining of "*lower layer*" cells, while its floor is formed by the yolk (Pl. XIII, fig. 3, *s c*). In the next and third stage (Pl. XIII, fig. 4, *s c*) its floor is formed by a thin layer of cells, its roof remaining as before. It has, however, become a less conspicuous formation than it was; and in the last (fourth) stage in which it can be distinguished it is very inconspicuous, and almost filled up by cells.

What I have called the second stage corresponds to a period in which no trace of the embryo is to be seen. In the third stage the embryonic end of the blastoderm projects outwards to form a structure which I shall speak of as the "embryonic rim," and in the fourth and last stage a distinct medullary groove is formed. For a considerable period during the second stage the segmentation cavity remains of about the same size; during the third stage it begins to be encroached upon and becomes smaller, both absolutely, and relatively to the increased size of the germ.

The segmentation cavity of the Dog-fish most nearly agrees with that of Osseous fishes in its mode of formation and relation to the embryo.

Dog-fish resemble Osseous fish in the fact that their embryos are entirely formed from a portion of the germ which does not form part of the roof of the segmentation cavity, so that the cells forming the roof of the segmentation cavity take *no share* at any time in the formation of their embryos. They further agree with Osseous fish (always supposing that the descriptions of Oellacher, loc. cit., and Götte, 'Archiv für Micr. Anat.,' Bd. ix, are correct) in the floor of the segmentation cavity being formed at one period by yolk. Together with these points of similarity there are some important differences.

(1) The segmentation cavity in the Osseous fish from the first arises as a cavity between the yolk and the blastoderm, and its floor is never at any period covered with cells. In the Dog-fish, as we have said above, both in the earlier and later periods the floor is covered with cells.

(2) The roof in the Dog-fish is *invariably* formed by the epiblast and a row of flattened lower layer cells.

According to both Götte and Oellacher the roof of the segmentation cavity in Osseous fishes is in the earlier stages formed *alone* of the two layers which correspond with the single layer forming the epiblast in the Dog-fish. In Osseous fishes it is very difficult to distinguish the various layers, owing to the similarity of their component cells. In Dog-fish this is very easy, owing to the great distinctness of the epiblast, and it appears to me, on this account, very probable that the two above-named observers may be in error as to the constitution of its roof in the Osseous fish. With both the Bird and the Frog the segmentation cavity of the Dog-fish has some points of agreement, and some points of difference, but it would take me too far from my present subject to discuss them.

When the segmentation cavity is first formed, no great changes have taken place in the cells forming the blastoderm. The upper layer—the *epiblast*—is composed of a single layer of columnar cells, and the remainder of the cells of blastoderm, forming the lower layer, are of a fairly uniform size, and polygonal from mutual pressure. The whole edge of the blastoderm is thickened, but this thickening is especially marked at its embryonic end.

This thickened edge of the blastoderm is still more conspicuous in the next and second stage (Pl. XIII, fig. 3).

In the second stage the chief points of progress, in addition to the increased thickness of the edge of the blastoderm, are—

(1) The increased thickness and distinctness of the epiplast, caused by its cells becoming more columnar, though it remains as a one-cell-thick layer.

(2) The disappearance of the cells from the floor of the segmentation cavity.

The lower layer cells have undergone no important changes, and the blastoderm has increased very little if at all in size.

From Pl. XIII, fig. 3, it is seen that there is a far larger collection of cells at the embryonic than at the opposite end.

Passing over some rather unimportant stages, I will come to the next important one.

The general features of this (the third) stage in a surface view are—

(1) The increase in size of the blastoderm.

(2) The diminution in size of the segmentation cavity, both relatively and absolutely.

(3) The appearance of a portion of the blastoderm projecting beyond the rest over the yolk. This projecting rim extends for nearly half the circumference of the yolk, but is

most marked at the point where the embryo will shortly appear. I will call it the "embryonic rim."

These points are still better seen from sections than from surface views, and will be gathered at once from an inspection of Pl. XIII, fig. 4.

The epiblast has become still more columnar, and is markedly thicker in the region where the embryo will appear. But its most remarkable feature is that at the outer edge of the "embryonic rim" (*e r*) it turns round and becomes continuous with the lower layer cells. This feature is most important, and involves some peculiar modifications in the development. I will, however, reserve a discussion of its meaning till the next stage.

The only other important feature of this stage is the appearance of a layer of cells on the floor of the segmentation cavity.

Does this layer come from an ingrowth from the thickened edge of the blastoderm, or does it arise from the formation of new cells in the yolk?

It is almost impossible to answer this question with certainty. The following facts, however, make me believe that the newly formed cells do play an important part in the the formation of this layer.

(1) The presence at an earlier date of almost a row of nuclei under the floor of the segmentation cavity (Pl. XIII, fig. 1).

(2) The presence on the floor of the cavity of such large cells as those represented in fig. 1, *b d*, cells which are very different, as far as the size and granules are concerned, from the remainder of the cells of the blastoderm.

On the other hand, from this as well as other sections, I have satisfied myself that there is a distinct ingrowth of cells from the embryonic swelling. It is therefore most probable that both these processes, viz. a fresh formation and an ingrowth, have a share in the formation of the layer of cells on the floor of the segmentation cavity.

In the next stage we find the embryo rising up as a distinct body from the blastoderm, and I shall in future speak of the body, which now becomes distinct as the embryo. It corresponds with what Kupffer (loc. cit.) in his paper on the "Osseous Fishes" has called the "embryonic keel." This starting-point for speaking of the embryo as a distinct body is purely arbitrary and one merely of convenience. If I wished to fix more correctly upon a period which could be spoken of as marking the commencing formation of the embryo, I should select the time when structures first appear to mark out the portion of the germ from which the embryo becomes formed; this period would be in the Elasmobranchii, as in the Osseous fish, at the termination of segmentation, when the want of symmetry between the embryonic end of the germ and the opposite end first appears.

I described in the last stage the formation of the "embryonic rim." It is in the middle point of this, where it projects most, that the development of the embryo takes place. There appear two parallel folds extending from the edge of the blastoderm towards the centre, and cut off at their central end by another transverse fold. These three folds raise up, between them, a flat broadish ridge, "*the embryo*" (Pl. XIV, fig. 5). The head end of the embryo is the end nearest the centre of the blastoderm, the tail end being the one formed by its (the blastoderm's) edge.

Almost from its first appearance this ridge acquires a shallow groove—the medullary groove (Pl. XIV, fig. 5, *m g*)—along its middle line, where the epiblast and hypoblast are in absolute contact (*vide* fig. 6 *a*, 7 *a*, 7 *b*, &c.), and where the mesoblast (which is already formed by this stage) is totally absent. This groove ends abruptly a little before the front end of the embryo, and is deepest in the middle and wide and shallow behind.

On each side of it is a plate of mesoblast equivalent to the combined vertebral and lateral plates of the Chick. These, though they cannot be considered as entirely the cause of the medullary groove, may perhaps help to make it deeper. In the parts of the germ outside the embryo the mesoblast is again totally absent, or, more correctly, we might say that outside the embryo the *lower layer cells* do not become differentiated into hypoblast and mesoblast, and remain continuous only with the lower of the two layers into which the *lower layer cells* become differentiated in the body of embryo. This state of things is not really very different from what we find in the Chick. Here outside the embryo (*i. e.* in the opaque area) there is a layer of cells in which no differentiation into hypoblast and mesoblast takes place, but the layer remains continuous rather with the mesoblast than the hypoblast.

There is one peculiarity in the formation of the mesoblast which I wish to call attention to, *i. e.* its formation as two lateral masses, one on each side of the middle line, but not continuous across this line (*vide* figs. 6 *a* and 6 *b*, and 7 *a* and 7 *b*). Whether this remarkable condition is the most primitive, *i. e.* whether, when in the stage before this the mesoblast is first formed, it is only on each side of the middle line that the differentiation of the lower layer cells into hypoblast and mesoblast takes place, I do not certainly know, but it is undoubtedly a very early condition of the mesoblast. The condition of the mesoblast as two plates, one on each side of the neural canal, is precisely similar to its embryonic condition in many of the Vermes, *e.g. Euaxes* and *Lumbricus*. In these there are two plates of mesoblast, one on each side of the nervous cord, which are known as the *Germinal streaks* (Keimstreifen) (*vide* Kowalevsky Würmern u. Arthropoden; Mém. de l'Acad. Imp. St. Petersbourg, 1871).

From longitudinal sections I have found that the segmentation cavity has ceased by this stage to have any distinct existence, but that the whole space between the epiblast and the yolk is filled up with a mass of elongated cells, which probably are solely concerned in the formation of the vascular system. The thickened posterior edge of the blastoderm is still visible.

At the embryonic end of the blastoderm, as I pointed out in an earlier stage, the epiblast and the lower layer cells are perfectly continuous.

Where they join the epiblast, the *lower layer cells* become distinctly divided, and this division commenced even in the earlier stage, into two layers; a lower one, more directly continuous with the epiblast, consisting of cells somewhat resembling the epiblast-cells, and an upper one of more flattened cells (Pl. XIII, fig. 4, *m*). The first of these forms the hypoblast, and the latter the mesoblast. They are indicated by *hy* and *m* in the figures. The hypoblast, as I said before, remains continuous with the whole of the rest of lower layer cells of the blastoderm (*vide* fig. 7 *b*). This division into hypoblast and mesoblast commences at the earlier stage, but becomes much more marked during this one.

In describing the formation of the hypoblast and mesoblast in this way I have assumed that they are formed out of the large mass of lower layer cells which underlie the epiblast at the embryonic end of the blastoderm. But there is another and, in some ways, rather a tempting view, viz. to suppose that the epiblast, where it becomes continuous with the hypoblast, in reality becomes involuted, and that from this involuted epiblast are formed the whole mesoblast and hypoblast.

In this case we would be compelled to suppose that the mass of lower layer cells which forms the embryonic swelling is used as food for the growth of the involuted epiblast, or else employed solely in the growth over the yolk of the non-embryonic portion of the blastoderm; but the latter possibility does not seem compatible with my sections.

I do not believe that it is possible, from the examination of sections alone, to decide which of these two views (viz. whether the epiblast is involuted, or whether it becomes merely continuous with the lower layer cells) is the true one. The question must be decided from other considerations.

The following ones have induced me to take the view that there is no involution, but that the mesoblast and hypoblast are formed from the lower layer cells.

(1) That it would be rather surprising to find the mass of lower layer cells which forms the "embryo swelling" playing no part in the formation of embryo.

(2) That the view that it is the lower layer cells from which the hypoblast and mesoblast are derived agrees with the mode of formation of these two layers in the Bird, and also in the Frog; since although, in the latter animal, there is an involution, this is not of the epiblast, but of the larger cells of lower pole of the yolk, which in part correspond with what I have called the lower layer cells in the Dog-fish.

If the view be accepted that it is from the lower layer cells that the hypoblast and mesoblast are formed, it becomes necessary to explain what the continuity of the hypoblast with the epiblast means.

The explanation of this is, I believe, the keystone to the whole position. The vertebrates may be divided as to their early development into two classes, viz. those with *holoblastic ova,* in which the digestive canal is formed by an *involution* with the presence of an "*anus of Rusconi.*"

This class includes "Amphioxus," the "Lamprey," the "Sturgeon," and "Batrachians."

The second class are those with *meroblastic ova* and no *anus of Rusconi,* and with an alimentary canal formed by the infolding of the sheet of hypoblast, the digestive canal remaining in communication with the food-yolk for the greater part of embryonic life by an umbilical canal.

This class includes the "Elasmobranchii," "Osseous fish," "Reptiles," and "Aves."

The mode of formation of the alimentary canal in the first class is clearly the more primitive; and it is equally clear that its mode of formation in the second class is an adaptation due to the presence of the large quantity of food-yolk.

In the Dog-fish I believe that we can see, to a certain extent, how the change from the one to the other of these modes of development of the alimentary canal took place.

In all the members of the first class, viz. "*Amphioxus*," the "Lamprey," the "Sturgeon," and the "Batrachians," the epiblast becomes continuous with the hypoblast at the so-called "anus of Rusconi," and the alimentary canal, potentially in all and actually in the Sturgeon (*vide* Kowalevsky, Owsjannikow, and Wagner, 'Bulletin der Acad. d. St. Petersbourg,' vol. xiv, 1870, "Entwicklung der Störe"), communicates freely at its extreme hind end with the neural canal. The same is the case in the Dog-fish. In these, when the folding in to form the alimentary canal on the one hand, and the neural on the other, takes place, the two foldings unite at the corner, where the epiblast and hypoblast are in continuity, and place the two tubes, the neural and alimentary, in free communication with each other.[1]

There is, however, nothing corresponding with the "anus of Rusconi," which merely indicates the position of the involution of the digestive canal, and subsequently completely closes up, though it nearly coincides in position with the true anus in the Batrachians, &c.

This remarkable point of similarity between the Dog-fish's development and the normal mode of development in the first class (the holoblastic) of vertebrates, renders it quite clear that the continuity of the epiblast and hypoblast in the Dog-fish is really the remnant of a more primitive condition, when the alimentary canal was formed by an involution. Besides the continuity between neural and alimentary canals, we have other remnants of the primitive involution. Amongst these the most marked is the formation of the embryonic rim, which is nothing less than the commencement of an involution. Its form is due to the flattened, sheet-like condition of the germ. In the mode in which the alimentary canal is closed in front I shall show there are indications of the primitive mode of formation of the alimentary canal; and in certain peculiarities of the anus, which I shall speak of later, we have indications of the primitive anus of Rusconi; and finally, in the general growth of the epiblast (small cells of the upper pole of the Batrachian egg) over the yolk (lower pole of the Batrachian egg), we have an example of the manner in which the primitive involution, to form the alimentary canal, invariably disappears when the quantity of yolk in an egg becomes very great.

I believe that in the Dog-fish we have before our eyes

[1] This has been already made out by Kowalevsky, "Wurmern u. Arthropoden," loc. cit.

one of the steps by which a direct mode of formation comes to be substituted for an *in*direct one by involution. We find, in fact, in the Dog-fish, that the cells from which are derived the mesoblast and hypoblast come to occupy their final position in the primitive arrangement of the cells during segmentation, and not by a subsequent and secondary involution.

This change in the mode of formation of the alimentary canal is clearly a result of change of mechanical conditions from the presence of the large food-yolk.

Excellent parallels to it will be found amongst the Mollusca. In this class the presence or absence of food-yolk produces not very dissimilar changes to those which are produced amongst vertebrates from the same cause.

The continuity of the hypoblast and epiblast at the embryonic rim is a remnant which, having no meaning or function, except in reference to the earlier mode of development, is likely to become lost, and in Birds no trace of it is any longer to be found.

I will not in the present preliminary paper attempt hypothetically to trace the steps by which the involution gradually disappeared, though I do not think it would be very difficult to do so. Nor will I attempt to discuss the question whether the condition with a large amount of food-yolk (as seems more probable) was twice acquired—once by the Elasmobranchii and Osseous fishes, and once by Reptiles and Birds—or whether only once, the Reptiles and Birds being lineal descendants of the Dog-fish.

In reference to the former point, however, I may mention that the Batrachians are to a certain extent intermediate in condition between the *Amphioxus* and the Dog-fishes, since in them the yolk becomes divided during segmentation into lower layer cells and epiblast, but a modified involution is still retained, while the Dog-fish may be looked upon as intermediate between Birds and Batrachians, the continuity at the hind end between the epiblast and hypoblast being retained by them, though not the involution.

It may be convenient here to call attention to some of the similarities and some of the differences which I have not yet spoken of between the development of Osseous fish and the Dog-fish in the early stages. The points of similarity are—(1) The swollen edge of the blastoderm. (2) The embryo-swelling. (3) The embryo-keel. (4) The spreading of the blastodern over the yolk-sac from a point corresponding with the position of the embryo, and not with the centre of the germ. The growth is almost nothing at that point, and most rapid at the opposite pole of the blastoderm, being less and less rapid along points of the circumference in proportion to their proximity to the embryonic swelling. (5) The medullary groove.

In external appearance the early embryos of Dog-fish and Teleostei are very similar; some of my drawings could almost be substituted for those given by Oellacher. This similarity is especially marked at the first appearance of the medullary groove. In the Dog-fish the medullary groove becomes converted into the medullary canal in the same way as with Birds and all other vertebrates, except Osseous fishes, where it comes to nothing, and is, in fact, a rudimentary organ. But in spite of Oellacher's assertions to the contrary, I am convinced from the similarity of its position and appearance to the true medullary groove in the Dog-fish, that the groove which appears in Osseous fishes is the true medullary groove; although Oellacher appears to have conclusively proved that it does not become converted into the medullary canal. The chief difference between the Dog-fish and Osseous fish, in addition to the point of difference about the medullary groove, is that the epiblast is in the Dog-fish a single layer, not divided into nervous and epidermic layers as in Osseous fish, and this difference is the more important, since, throughout the whole period of development till after the commencement of the formation of the neural canal, the epiblast remains as a one-cell-deep layer of cells, and thus the possibility is excluded of any concealed division into a neural and epidermic layer, as has been supposed to be the case by Stricker and others in Birds.

Development of the Embryo.

After the embryo has become definitely established, for some time it grows rapidly in length, without externally undergoing other important changes, with the exception of the appearance of two swellings, one on each side of its tail.

These swellings, which I will call the *Caudal lobes* (figs. 8 and 9, *t s*), are also found in Osseous fishes, and have been called by Oellacher the *Embryonal saum.* They are caused by a thickening of mesoblast on each side of the hind end of the embryo, at the edge of the embryonic rim, and form a very conspicuous feature throughout the early stages of the development of the Dog-fish, and are still more marked in the Torpedo (Pl. XIV, fig. 9). Although from the surface the other changes which are visible are very insignificant, sections show that the *notochord* is commencing to be formed.

I pointed out that beneath the medullary groove the epiblast and hypoblast were not separated by any interposed mesoblast. Along the line (where the mesoblast is deficient) which forms the long axis of the embryo, a rod-like thickening of the hypoblast appears (Pl. XIV, figs. 7 *a* and 7 *b*, *ch* and *ch'*), first at the head end of the embryo, and gradually extending backwards. This is the rudiment of the notochord; it remains attached for some time to the hypoblast, and becomes separated from it first at the head end of the embryo, and the separation is then carried backwards. This thickening of the hypoblast projects up and comes in contact with the epiblast, and in the later stages with bad (especially chromic-acid) specimens the line of separation between the epiblast and the thickening may become a little obscured, and might possibly lead to the supposition that a structure similar to that which has been called the "*axis cord*" was present. In all my best (osmic-acid) specimens the line of junction is quite clear; and any one who is aware how easily two separate masses of cells may be made indistinguishably to fuse together from simple pressure will not be surprised to find the occasional obscurity of the line of junction between the epiblast and hypoblast. In the earlier stage of the thickening there is never in the osmic-acid preparations any appearance of fusion except in very badly prepared ones. Its mode of formation will be quite clear without further description from an inspection of Pl. XIV, figs. 7 *a* and 7 *b*, *ch* and *ch'*. Both are taken from one embryo. In fig. 7 *b*, the most anterior of the two, the notochord has become quite separated from the hypoblast. In fig. 7 *a*, *ch*, there is only a very marked thickening of hypoblast, which reaches up to the epiblast, but the thickening is still attached to the hypoblast. Had I had space to insert a drawing of a third section of the same embryo there would only have been a slight thickening of the hypoblast. In the earlier stage it will be seen, by referring to figs. 6 *a* and 6 *b*, that there is no sign of a thickening of the hypoblast. My numerous sections (all made from embryos hardened in osmic acid) showing these points are so clear that I do not think there can be any doubt whatever of the notochord being formed as a thickening of the hypoblast. Two interpretations of this seem possible.

I mentioned that the mesoblast appeared to be primitively formed as two independent sheets, *split off, so to speak, from the hypoblast*, one on each side of the middle line of the embryo. If we looked upon the notochord as a third *median sheet of mesoblast*, split off from the hypoblast somewhat later than the other two, we should avoid having to admit its hypoblastic origin.

Professor Huxley, to whom I have shown my specimens, strongly advocates this view.

The other possibility is that the notochord is primitively a true *hypoblastic* structure which has only by adaptation become an apparently *mesoblastic* one in the higher vertebrates. In favour of this view are the following considerations:

(1) That this is the undoubtedly natural interpretation of the sections. (2) That the notochord becomes separated from the hypoblast after the latter has acquired its typical structure, and differs in that respect from the two lateral sheets of mesoblast, which are formed coincidently with the hypoblast by a homogeneous mass of cells becoming differentiated into two distinct layers. (3) That the first mode of looking at the matter really proves too much, since it is clear that by the same method of reasoning we could prove the mesoblastic origin of any organ derived from the hypoblast and budded off into the mesoblast. We should merely have to assert that it was really a mass of mesoblast budded off from the hypoblast rather later than the remainder of the mesoblast. Still, it must be admitted that the first view I have suggested is a possible, not to say a probable one, though the mode of arguing by which it can be upheld may be rather dangerous if generally applied. We ought not, however, for that reason necessarily reject it in the present case. As Mr. Ray Lankester pointed out to me, if we accept the hypoblastic origin of the notochord, we should find a partial parallel to it in the endostyle of Tunicates, and it is perhaps interesting to note in reference to it that the notochord is the only *unsegmented* portion of the axial skeleton.

Whether the strong *à priori* arguments against the hypoblastic origin of the notochord are sufficient to counterbalance the natural interpretations of my sections, cannot, I think, be decided from the single case of the Dog-fish. It is to be hoped that more complete investigations of the Lamprey, &c., may throw further light upon the question.

Whichever view of the primitive origin of the notochord is the true one, its apparent origin is very instructive as illustrating the possible way in which an organ might come to change the layer to which it primarily belonged.

If the notochord is originally a mesoblastic structure, it is easy to be seen how, by becoming separated from the hypoblast a little later than is the case with the Dog-fish, its true mesoblastic origin would become lost; while if, on the other hand, it is primitively a hypoblastic structure, we see from higher vetebrates how, by becoming separated from the hypoblast rather earlier than in the Dog-fish, viz. at the same time as the rest of the mesoblast, its primitive derivation from the hypoblast has become concealed.

The view seemingly held by many embryologists of the present day, that an organ, when it was primitively derived from one layer, can never be apparently formed in another layer, appears to me both unreasonable on *à priori* grounds and also unsupported by facts.

I see no reason for doubting that the embryo in the earliest periods of development is as subject to the laws of natural selection as is the animal at any other period. Indeed, there appear to me grounds for the thinking that it is more so. The remarkable differences in allied species as to the amount of food-yolk, which always entail corresponding alterations in the development—the different modes of segmentation in allied species, such as are found in the Amphipoda and Isopoda—the suppression of many stages in freshwater species, which are retained in the allied marine species—are all instances of modifications due to natural selection affecting the earliest stages of development. If such points as these can be affected by natural selection I see no reason why the arrangement of individual cells (or rather primitive elements) should not also be modified; why, in fact, a mass of cells which was originally derived from one layer, but in the course of development became budded off from that layer and entered another layer, should not by a series of small steps cease ever to be attached to the original layer, but from the first moment it can be distinguished should be found as a separate mass in the second layer.

The change of layers will, of course, only take place where some economy is effected by it. The variations in the mode of development of the nervous system may probably be explained in this way.

If we admit that organs can undergo changes, as to the primitive layer from which they are derived, important consequences must follow.

It will, for instance, by no means be sufficient evidence of two organs not being homologous that they are not developed from the same layer. It renders the task of tracing out the homologies from development much more difficult than if the ordinary view of the invariable correspondence of the three layers throughout the animal kingdom be accepted. Although I do not believe that this correspondence is invariable or exact, I think that we both find and should expect to find that it is, roughly speaking, fairly so.

Thus, the muscles, internal skeleton, and connective tissue are always placed in the adult between the skin (epidermis) and the epithelium of the alimentary canal.

We should therefore expect to find them, and, as a matter of fact, we always do find them, developed from a middle layer when this is present.

The upper layer must always and does always form the epidermis, and similarly the lower layer or hypoblast must form a part of the epithelium of the alimentary canal. A full discussion of this question would, however, lead me too far away from my present subject.

The only other point of interest which I can touch on in this stage is the commencing closure of the alimentary canal in the region of the head. This is shown in Pl. XIII, fig. 6 *a*, 6 *b*, and Pl. XIV, 7 *b*, *n. a.* From these figures it can be seen that the closing does not take place as much by an infolding as by an ingrowth from the side walls of the alimentary canal towards the middle line. In this abnormal mode of closing of the alimentary canal we have again, I believe, an intermediate stage between the mode of formation of the alimentary canal in the Frog and the typical folding in which occurs in Birds. There is, however, another point in reference to it which is still more interesting. The cells to form the ingrowth from the bottom (ventral) wall of the alimentary canal are derived by a continuous fresh formation from the yolk, being formed around the nuclei spoken of above (*vide* p. 329). All my sections show this with more or less clearness, especially those a little later than fig. 6 *b*, in which the lower wall of the alimentary canal is nearly completed. This is the more interesting since, from the mode of formation of the alimentary canal in the Batrachians, &c., we might expect that the cells from the yolk would take a share in its formation in the Dog-fish. I have not as yet made out for certain the share which is taken by these freshly formed cells of the yolk in the formation of any other organ.

By the completion of its lower wall in the way described, the throat early becomes a closed tube, its closing taking place before any other important changes are visible in the embryo from surface views.

A considerable increase in length is attained before other changes than an increase in depth of the medullary groove and a more complete folding off of the embryo from the blastoderm take place. The first important change is the formation of the protovertebræ.

These are formed by the lateral plates of mesoblast, which I said were equivalent at once to the vertebral and lateral plates in the Bird, becoming split by transverse divisions into cubical masses.

At the time when this occurs, and, indeed, up till a considerably later period, the mesoblast is not split into somatopleure and splanchnopleure, and it is not divided into vertebral and lateral plates. The transverse lines of division of the protovertebræ do not, however, extend to the outer edge of the undivided lateral plates.

The differences between this mode of formation of the protovertebræ and that occurring in Birds are too obvious to require pointing out. I will speak of them more fully when I have given the whole history of the protovertebræ of the Dog-fish.

I will only now say that I have had in the early stages to investigate the formation of the protovertebræ entirely by means of sections, the objects being too opaque to be otherwise studied.

The next change of any importance is the commencement of the formation of the head. The region of the head first becomes distinguishable by the flattening out of the germ at its front end.

The flattened-out portion of the germ grows rapidly, and forms a spatula-like termination to the embryo (Pl. XIII, fig. 8).

In the region of the head the medullary groove is at first totally absent (*vide* section, Pl. XIV, fig. 8 *a*).

Indeed, as can be seen from fig. 8 *b*, the laminæ dorsales, so far from bending up at this stage, actually bend down in the opposite direction.

I am at present quite unable even to form a guess what this peculiar feature of the brain means. It, no doubt, has some meaning in reference to the vertebrate ancestry if we could only discover it. The peculiar spatula-like flattened condition of the head is also (*vide* loc. ant. cit.) apparently found in the Sturgeons; it must therefore almost undoubtedly be looked upon as not merely an accidental peculiarity.

While these changes have been taking place in the head not less important changes have occurred in the remainder of the body. In the first place the two caudal lobes have increased in size, and have become, as it were, pushed in together, leaving a groove between them (fig. 8, *t s*). They are very conspicuous objects, and, together with the spatula-like head, give the whole embryo an almost comical appearance. The medullary canal has by this time become completely closed in the region of the tail (figs. 8 and 8 *b*).

It is still widely open in the region of the back, and, though more nearly closed again in the neck, is, as I have said, flattened out to nothing in the head.

The groove[1] between the two caudal lobes must not be confused (as may easily be done) with the medullary groove, which by the time the former groove has become conspicuous is a completely closed canal.

The vertebral plates are not divided (*vide* fig. 7) into a somatopleuric and splanchnopleuric layer by this stage, except in the region of the head (*vide* fig. 8 *a*, *p p′*), where there is a distinct space between the two layers, which is undoubtedly homologous with the pleuro-peritoneal cavity of the hinder portion of the body.

It is probably the same cavity which Oellacher (loc. cit.) calls in Osseous fishes the pericardial cavity. In the Dogfish, at least, it has no connection with the pericardium. Of its subsequent history I shall say a few words when I come to speak of the later stages.

The embryo does not take more than twenty-four hours in passing from this stage, when the head is a flat plate, to the stage when the whole neural canal (including the region of the head) is closed in. The other changes, in addition to the closing in of the neural canal, are therefore somewhat insignificant. The folding off of the embryo from the germ has, however, progressed considerably, and a portion of the hind gut is closed in below. This is accomplished, not by a tail-fold, as in Birds, but by two lateral folds, which cause the sides of the body to meet and coalesce below. At the extreme hind end, where the epiblast is continuous with the hypoblast, the lateral folds turn round, so to speak, and become continuous with the medullary folds, so that when the various folds meet each other an uninterrupted canal is found passing round from the neural into the alimentary canal, and placing these two in communication at the tail end of the body. Since I have already mentioned this, and spoken of its significance, I will not dwell on it further here.

The cranial flexure commences coincidently with the closing in of the neural canal in region of the brain, and the division into fore, mid, and hind brain becomes visible at the same time as or even before the closing of the canal occurs. The embryo has now become more or less transparent, and proto-

[1] This groove is the only structure which it seems possible to compare with the so-called "primitive groove" of Birds. It is, however, doubtful whether they are really homologous.

vertebræ, of which about twenty are present, can *now* be seen in the fresh specimens. The heart, however, is not yet formed.

Up to this period, a period at which the embryo becomes very similar in external appearance to any other vertebrate embryo, I have followed in my description a chronological order. I shall now cease to do so, since it would be too long for a preliminary notice of this kind, but shall confine myself to the history of a few organs whose development is either more important or more peculiar than that of the others.

The Protovertebræ.

I have thought it worth while to give a short history of the development of the protovertebræ, firstly, because it is very easy to follow this in the Dog-fish, and, secondly, because I believe that the Dog-fish have more nearly retained the primitive condition of the protovertebræ than any other vertebrate whose embryology has hitherto been described with sufficient detail.

I intend to describe, at the same time, the development of the spinal nerves.

I left each lateral mass of mesoblast in my last stage as a plate which had not yet become split into a somatic and a splanchnic sheet (Pl. XIV, fig. 8 *a, v p*), but which had become cut by transverse lines (not, indeed, extending to the outer limit of the sheet, but as yet not cut off by longitudinal lines of cleavage) into segments, which I called protovertebræ.

This sheet of mesoblast is fairly thick at its proximal (upper) end, but thins off laterally to a sheet two cells deep, and its cells are so arranged as to foreshadow its subsequent splitting into somatic and splanchnic sheets. Its upper (proximal) end is at this stage level with the bottom of the neural canal, but soon begins to grow upwards, and at the same time the splitting into somatopleure and splanchnopleure commences (Pl. XIV, fig. 10, *s o* and *s p*).

The separation between the two sheets is first visible in its uppermost part, and thence extends outwards. By this means each of the protovertebræ becomes divided into two sheets, which are only connected at their upper ends and outside the region of the body. I speak of the whole lateral sheet as being composed of protovertebræ, because at this time no separation into vertebral and lateral plates can be seen; but I may anticipate matters by saying that only the upper portion of the sheet from the level of the top of the digestive canal, becomes subsequently the true protovertebræ; so that it is clear that the pleuro-peritoneal cavity extends primitively quite up to the top of the protovertebræ; and that thus a portion of a sheet of mesoblast, at first perfectly continuous with the splanchnic sheet from which is derived the muscular wall of the alimentary canal, is converted into a part of the voluntary muscular system of the body, having no connection whatever with the involuntary muscular system of the digestive tract.

The pleuro-peritoneal cavity is first distinctly formed at a time when only two visceral clefts are present. Before the appearance of a third visceral cleft in a part of the innermost layer of each protovertebræ (which may be called the splanchnic layer, from its being continuous with the mesoblast of the splanchnopleure), opposite the bottom of the neural tube, some of the cells commence to become distinguishable from the rest, and to form a separate mass. This mass becomes much more distinct a little later, its cells being characterised by being spindle-shaped, and having an elongated nucleus which becomes deeply stained by reagents (Pl. XV, fig. 11, *m p'*). Coincidently with its appearance the young Dog-fish commences spontaneously to move rapidly from side to side with a kind of serpentine motion, so that, even if I had not traced the development of this differentiated mass of cells till it becomes a band of muscles close to the notochord, I should have had little doubt of its muscular nature. It is indicated in figs. 11, 12, 13, by the letters *m p'*. Its early appearance is most probably to be looked upon as an adaptation consequent upon the respiratory requirements of the young Dog-fish necessitating movements within the egg.

Shortly after this date, at a period when three visceral clefts are present, I have detected the first traces of the spinal nerves.

At this time they appear in sections as small elliptical masses of cells, entirely independent of the protovertebræ, and closely applied to the upper and outer corners of the involuted epiblast of the neural canal (Pl. XV, fig. 11, *sp n*). These bodies are far removed from any mesoblastic structures, and at the same time the cells composing them are *not* similar to the cells composing the walls of the neural canal, and are not attached to these, though lying in contact with them. I have not, therefore, sufficient evidence at present to enable me to say with any certainty where the spinal nerves are derived from in the Dog-fish. They may be derived from the involuted epiblast of the neural canal, and, indeed, this is the most natural interpretation of their position.

On the other hand, it is possible that they are formed from wandering cells of the mesoblast—a possibility which, with our present knowledge of wandering cells, must not be thrown aside as altogether improbable.

In any case, it is clear that the condition in the Bird, where the spinal nerves are derived from tissue of the protovertebræ, is not the primitive one. Of this, however, I will speak again when I have concluded my account of the development of the protovertebræ.

About the same time that I have found the rudiments of the nerves the division of the mesoblast of the sides of the body into a vertebral and a lateral portion occurs. This division first appears in the region where the oviduct (Müller's duct) is formed (Pl. XV, fig. 11, *ov*).

At this part opposite the level of the dorsal aorta the two sheets, viz. the splanchnic and the somatic, unite together, and thus each lateral sheet of mesoblast becomes divided into an upper portion (fig. 11, *m p*), split up by transverse partitions into protovertebræ, and a lower portion not so split, but consisting of an outer layer, the true somatopleure, and an inner layer, the true splanchnopleure. These two divisions of the primitive plate are thus separated by the line at which a fusion between the mesoblast of the somatopleure and splanchnopleure takes place. The mass of cells resulting from the fusion at this point corresponds with the intermediate cell-mass of Birds (*vide* Waldeyer, 'Eierstock und Ei').

At the same time, in the upper of these two sheets, the splanchnic layer sends a growth of cells inwards towards the notochord and the neural canal. This growth is the commencement of the large quantity of mesoblastic tissue around the notochord, which is in part converted into the axial skeleton, and in part into the connective tissue adjoining this.

This mass of cells is at first quite continuous with the splanchnic layer of the protovertebræ, and I see no reason for supposing that it is not derived from the growth of the cells of this layer. The ingrowth to form it first appears a little after the formation of the dorsal aorta; but, as far as I have been able to see, its cells have no connection with the walls of the aorta.

What I have said as to the development of the skeleton-forming layer will be quite clear from figs. 11 and 12 *a*; and from these it will also be clear, especially from fig. 11 *a*, that the outermost layer of this mass of cells, which was the primitive splanchnic layer of the protovertebræ, still retains its epithelial character, and so can easily be distinguished from those cells which will form the skeleton. In the next stage which I have figured (fig. 12 *a*), this outer portion of the splanchnic layer is completely separated from the skeleton-forming cells, and at the same time. having united below as well as above with the outer (somatic) layer of the two layers of which the protovertebræ are formed, the two together form an independent mass (fig. 12, *m p*), similar in appearance and in every way homologous with the muscle-plate of Birds.

On the inner side of this, which we may now call the muscle-plate, is seen the bundle of earlier-developed muscles (fig. 12, *mp'*) which I spoke of before.

The section represented in fig. 12 is from a very considerably later embryo than that represented in fig. 11, so that the skeleton-forming cells, few in number in the earlier section, have become very numerous in the later one, and have grown up above the neural canal, and also below the notochord, between the digestive canal and the aorta. They have, moreover, changed their character; they were round before, now they have become stellate. As to their further history, I will only say that the layer of them immediately around the notochord and neural canal forms the cartilaginous centra and arches of the vertebræ, and that the remaining portion of them, which becomes much more insignificant in size as compared with the muscles, forms the connective tissue of the skeleton and of the parts around and between the muscles.

A muscle-plate itself is at this stage (shown in fig. 12) composed of an inner and an outer layer of epithelium (splanchnic and somatic) united at the upper and lower ends of the plate, and on the inner of the two lies the more developed mass of muscles before spoken of (*m p'*).

Each of these plates now grows both upwards and downwards; and at the same time connective-tissue cells appear between the plates and epidermis; but from where they come I do not know for certain; very probably they are derived from the somatic layer of the muscle-plate.

While the muscle-plates continue to grow both upwards and downwards, the cells of which they are composed commence to become elongated and soon acquire an unmistakably muscular character (Pl. XV, fig. 13, *m p*).

Before this has occurred the inner mass of muscles has also undergone further development and become a large and conspicuous band of muscles close to the notochord (fig. 13, *m p'*).

At the same time that the muscle-plates acquire the true histological character of muscle, septa of connective tissue grow in and divide them into a number of distinct segments, which subsequently form separate bands of muscle. I will not say more in reference to the development of the muscular system than that the whole of the muscles of the body (apart from the limbs, the origin of whose muscular system I have not yet investigated) are derived from the muscle-plates which grow upwards above the neural canal and downwards to the ventral surface of the body.

During the time the muscle-plates have been undergoing these changes the nerve masses have also undergone developmental changes.

They become more elongated and fibrous, their main attachment to the neural tube being still at its posterior (dorsal) surface, near which they first appeared. Later they become applied closely to the sides of the neural tube and send fibres to it below as well as above. Below (ventral to) the neural tube a ganglion appears, forming only a slight swelling, but containing a number of characteristic nerve-cells. The ganglion is apparently formed just below the junction of the anterior and posterior roots, though probably the fibres of the two roots do not mix till below it.

The main points which deserve notice in the development of the protovertebræ are—

(1) That at the time when the mesoblast becomes split herizontally into somatopleure and splanchnopleure the vertebral and lateral plates are one, and the splitting extends to the very top of the vertebral plate, so that the future muscle-plates are divided into a splanchnic and somatic layer, the space between which is at first continuous with the pleuro-peritoneal cavity.

(2) That the following parts are respectively formed by the vertebral and lateral plates:

(*a*) Vertebral plate. From the splanchnic layer of this, or from cells which appear close to and continuous with it, the skeleton, and connective tissue of the upper part of the body, are derived.

The remainder of the plate, consisting of a splanchnic and somatic layer, is entirely converted into the muscles of the trunk, all of which are derived from it.

(*b*) Between the vertebral plate and the lateral plate is a mass of cells where, as I mentioned above, the mesoblast of the somatopleure and splanchnopleure fuse together. This mass of cells is the equivalent of the *intermediate cell* mass of Birds (*vide* Waldeyer, 'Eierstock und Ei').

From it are derived the Wolffian bodies and duct, the oviduct, the ovaries and the testis, and the connective tissue of the parts adjoining these.

(*c*) The lateral plate. From the somatic layer of this is derived the connective tissue of the ventral half of the body; the mesoblast of the limbs, including probably the muscles, and certainly the skeleton. From its splanchnic layer are derived the muscles and connective tissue of the alimentary canal.

(3) The spinal nerves are developed independently of the protovertebræ, so that the protovertebræ of the Elasmobranchii do not appear to be of such a complicated structure as the protovertebræ of Birds.

The Digestive Canal.

I do not intend to enter into the whole history of the digestive canal, but to confine myself to one or two points of interest connected with it. These fall under two heads:

(1) The history of the portion of the digestive canal between the anus and the end of the tail where the digestive canal opens into the neural canal.

(2) Certain less well-known organs derived from the digestive canal.

The anus is a rather late formation, but its position becomes very early marked out by the hypoblast of the digestive canal approaching at that point close to the surface, whilst receding to some little distance from it on either side. The portion of the digestive tract I propose at present dealing with is that between this point, which I will call, for the sake of brevity, the anus, and the hind end of the body. This portion of the canal is at first very short; it is elliptical in section, and of rather a larger bore than the remainder of the canal. Its diameter becomes, however, slightly less as it approaches the tail, dilating again somewhat at its extreme end. It is lined by a markedly columnar epithelium. Though at first very short, its length increases with the growth of the tail, but at the same time its calibre continually becomes smaller as compared with the remainder of the alimentary canal.

It commences to become smaller, first of all, near, though not quite, at its extreme hind end, and thus becomes of a conical shape; the base of the cone being just behind the anus, while the apex of the cone is situated a short distance from the hind end of the embryo. The extreme hind end, however, at the same time does not diminish in size, and becomes relatively (if not also absolutely) much larger in diameter than it was at first, as compared with the remainder of the digestive canal. It becomes, in fact, a vesicle or vesicular dilatation at the end of a conical canal.

Just before the appearance of the external gills this part of the digestive canal commences to atrophy. It begins to do so close to the terminal vesicle, which, however, still remains as or more conspicuous than it was before. The lumen of the canal becomes smaller and smaller, and finally it becomes a solid string of cells, and these also soon disappear and not a trace of the canal is left.

Almost the whole of it has disappeared before the vesicle begins to atrophy, but very shortly after all trace of the rest of the canal has vanished the terminal vesicle also vanishes. This occurs just about the time or shortly after the appearance of the external gills—there being slight differences probably in this respect in the different species.

In this history there are two points of especial interest:

(1) The terminal vesicle.

(2) The disappearance of a large and well-developed portion of the alimentary canal.

The interest in the terminal vesicle lies in the possibility of its being some rudimentary structure.

In Osseous fishes Kuppfer has described the very early appearance of a vesicle near the tail end, which he doubtfully speaks of as the "allantois." The figure he gives of it in his earlier paper ('Archiv. für Micro. Anat.' vol. ii, pl. xxiv, fig. 2) bears a very strong resemblance to my figures of this vesicle at the time when the hind end of the alimentary canal is commencing to disappear; and I feel fairly confident that it is the same structure as I have found in the Dog-fish: but until the relations of the Kuppfer's vesicle to the alimentary canal are known, any comparison between it and the terminal vesicle in the Dog-fish must be to a certain extent guess-work.

I have, however, been quite unsuccessful in finding any other vesicular structure which can possibly correspond to the so-called allantoic vesicle of Osseous fish.

The disappearance of a large portion of the alimentary canal behind the anus is very peculiar. In order, however, to understand the whole difficulties of the case I shall be obliged to speak of the relations of the anus of the Dog-fish to the anus of Rusconi in the Lamprey, &c.

In those vertebrates whose alimentary canal is formed by an involution, the anus of Rusconi represents the opening of this involution, and therefore the point where the alimentary canal primitively communicates with the exterior. When, however, the "anus of Rusconi" becomes *closed*, the wall of the alimentary canal still remains at that point in close juxtaposition to the surface, and the new and final anus is formed at or close to that point. In the Dog-fish, although the anus of Rusconi is not present, still, during the closing of the alimentary canal, the point which would correspond with this becomes marked out by the alimentary canal there approaching the surface, and it is at this point that the involution to form the true anus subsequently appears.

The anus in the Dog-fish has thus, more than a mere secondary significance. It corresponds with the point of closing of the primitive involution. If it was not for this peculiarity of the vertebrate anus we would naturally suppose, from the disappearance of a considerable portion of the alimentary canal lying behind its present termination, that in the adult the alimentary canal once extended much farther back than at present, and that the anus we now find was only a secondary anus, and not the primitive one. It is perhaps possible that this hinder portion of the alimentary canal is a result of the combined growth of the tail and the persisting continuity (at the end of the body) of the epiblast with the hypoblast.

Whichever view is correct, it may be well to mention, in order to show that the difficulty about the anus of Rusconi is no mere visionary one, that Götte ("Untersuchung über die Entwickelung der Bombinator igneus," 'Archiv. für Micro. Anat.,' vol. v, 1869) has also described the disappearance of the hind portion of the alimentary canal in Batrachians, a rudiment (according to him) remaining in the shape of a lymphatic trunk.

It is, perhaps, possible that we have a further remnant of this "hind portion" of the alimentary canal amongst the higher vertebrates in the "allantois."

Organs developed from the Digestive Canal.

In reference to the development of the liver, pancreas, &c., as far as my observations have at present gone, the Dog-fish presents no features of peculiar interest. The liver is developed as in the Bird, and independently of the yolk.

There are, however, two organs derived from the hypoblast which deserve more attention. Immediately under the notochord, and in contact with it (*vide* Pl. XIV, fig. 10; XV, 11 and 12, *x*), a small roundish (in section) mass of cells is to be seen in most of the sections.

Its mode of development is shown in fig. 10, *x*. That section shows a mass of cells becoming pinched off from the top of the alimentary canal. By this process of pinching off from the alimentary canal a small rod-like body close under the notochord is formed. It persists till after the appearance of the external gills, but later than that I have not hitherto succeeded in finding any trace of it.

It was first seen by Götte (loc. cit.) in the Batrachians, and he gave a correct account of its development, and added that it became the thoracic duct.

I have not myself worked out the later stages in the development of this body with sufficient care to be in a position to judge of the correctness of Götte's statements as to its final fate. If it is true that it becomes the thoracic duct it is very remarkable, and ought to throw some light upon the homologies of the lymphatic system.

Some time before the appearance of the external gills another mass of cells becomes, I believe, constricted off from the part of the alimentary canal in the neighbourhood of the anus, and forms a solid rod composed at first of dark granular cells lying between the Wolffian ducts. I have not followed out its development quite completely, but I have very little doubt that it is really constricted off from a portion of the alimentary canal chiefly in front of the point where the anus appears, but also, I believe, from a small portion behind this.

Though the cells of which it is composed are at first columnar and granular (fig. 12, *s u, r*), they soon begin to become altered, and in the latter stage of its development the body forms a conspicuous rounded mass of cells with clear protoplasm, and each provided with a large nucleus. Later still it becomes divided into a number of separate areas of cells by septa of connective tissue, in which (the septa) capillaries are also present. Since I have not followed it to its condition in the adult, I cannot make any definite statements as to the fate of this body; but I think that it possibly becomes the so-called supra-renal organ, which in the Dog-fish forms a yellowish elongated body lying between the two kidneys.

The development of the Wolffian Duct and Body and of the Oviduct.

The development of the Wolffian duct and the Oviduct in the various classes of vertebrates is at present involved in some obscurity, owing to the very different accounts given by different observers.

The manner of development of these parts in the Dog-fish is different from anything that previous investigators have met with in other classes, but I believe that it gives a clearer insight into the true constitution of these parts than vertebrate embryology has hitherto supplied, and at the same time renders easier the task of understanding the differences in the modes of development in the different classes.

I shall commence with a simple description of the observed facts, and then give my view as to their meaning. At about the time of the appearance of the third visceral cleft, and a short way behind the point up to which the alimentary canal is closed in front, the splanchnopleure and somatopleure fuse together opposite the level of the dorsal aorta.

From the mass of cells formed by this fusion a solid knob rises up towards the epiblast (Pl. XV, fig. 11 *b*, *o v*), and from this knob a solid rod of cells grows backwards towards the tail (fig. 11 *c*, *o v*) very closely applied to the epiblast. This description will be rendered clear by referring to figs. 11 *b* and *c*. Fig. 11 *b* is a section at the level of the knob, and fig. 11 *c* is a section of the same embryo a short way behind this point. So closely does the rod of cells apply itself to the epiblast that it might very easily be supposed to be derived from it. Such, indeed, was at first my view till I cut a section passing through the knob. In order, however, to avoid all possibility of mistake I made sections of a large number of embryos of about the age at which this appears, and *invariably found* the large knob in front, and from it the solid string growing backwards.

This string is the commencement of the *Oviduct* or *Müller's duct*, which in the Dog-fish as in the Batrachians is the first portion of the genito-urinary system to appear, and is in the Dog-fish undoubtedly at first solid. All my specimens have been hardened with osmic acid, and with specimens hardened with this reagent it is quite easy to detect even the very smallest hole in a mass of cells.

As a solid string or rod of cells the Oviduct remains for some time; it grows, indeed, rapidly in length, the extreme hind length of the rod being very small and the front end continuing to remain attached to the knob. The knob, however, travels inwards and approaches nearer and nearer to the true pleuro-peritoneal cavity, always remaining attached to the intermediate cell mass.

At about the time when five visceral clefts are present the Oviduct first begins to get a lumen and to open at its front end into the pleuro-peritoneal cavity. The cells of the rod are first of all arranged in an irregular manner, but gradually become columnar and acquire a radiating arrangement around a central point. At this point, where the ends of all the cells meet, a very small hole appears, which gradually grows larger and becomes the cavity of the duct (fig. 12, *o v*). The hole first makes its appearance at the anterior end of the duct, and then gradually extends backwards, so that the hind end is still without a lumen, when the lumen of the front end is of a considerable size.

At the front knob the same alteration in the cells takes place as in the rest of the duct, but the cells become deficient on the side adjoining the pleuro-peritoneal cavity, so that an opening is formed into the pleuro-peritoneal cavity, which soon becomes of a considerable size. Soon after its first formation, indeed, the opening becomes so large that it may be met in from two to three consecutive sections if these are very thin.

Thus is formed the lumen of the Oviduct. The duct still, at this age, ends behind without having become attached to the cloaca, so that at this time the Oviduct is a canal closed behind, but communicating in front by a large opening with the pleuro-peritoneal cavity.

It has during this time been travelling downwards, and is now much nearer the pleuro-peritoneal cavity than the epiblast.

It may be well to point out that the mode of development which I have described is really not very different from an involution, and must, in fact, be only looked upon as a modification of an involution. Many examples from all classes in the animal kingdom could be selected to exemplify how an involution may become simply a solid thickening. In the Osseous fish nearly all the organs which are usually formed by an involution have undergone this change in their mode of development. I shall attempt to give reasons later on for the solid form having been acquired in this particular case of the Oviduct.

At about the time when a lumen appears in the Oviduct the first traces of the Wolffian duct become visible.

At intervals along the whole length, between the front and hind ends of the Oviduct, involutions arise from the pleuro-peritoneal cavity (fig. 12, *a, p, w d*) on the inside (nearer the middle line) of the Oviduct. The upper ends of these numerous involutions unite together and form a string of cells, at first solid, but very soon acquiring a lumen, and becoming a duct which communicates (as it clearly must from its mode of formation), at numerous points with the pleuro-peritoneal cavity. It is very probable that there is one involution to each segment of the body between the front and hind ends of the Oviduct. This duct is the Wolffian duct, which thus, together with the Oviduct, is formed before the appearance of the external gills.

For a considerable period the front end of the Oviduct does not undergo important changes; the hind end, however, comes into connection with the extreme end of the alimentary canal. The two Oviducts do not open together into the cloaca, though, as my sections prove, their openings are very close together. The whole Oviduct, as might be expected, shares in the general growth, and its lumen becomes in both sexes very considerably greater than it was before.

It is difficult to define the period at which I find these changes accomplished without giving drawings of the whole embryo. The stage is one considerably after the external gills have appeared, but before the period at which the growth of the olfactory bulbs renders the head of an elongated shape.

During the same period the Wolffian duct has undergone most important changes. It has commenced to bud off diverticula, which subsequently become the tubules of the Wolffian body (*vide* fig. 13, *w d*). I am fairly satisfied that the tubules are really budded off, and are not formed independently in the mesoblast. The Dog-fish agrees so far with Birds, where I have also no doubt the tubules of the Wolffian body are formed as diverticula from the Wolffian duct.

The Wolffian ducts have also become much longer than the Oviduct, and are now found behind the anus, though they do not extend as far forward as does the Oviduct.

They have further acquired a communication with the Oviduct, in the form of a narrow duct passing from each of them into an Oviduct a short way before the latter opens into the cloacal dilatation of the alimentary canal.

The canals formed by the primitive involution leading from the pleuro-peritoneal cavity into the Wolffian duct have become much more elongated, and at the same time narrower. One of these is shown in fig. 13, *p, w d*.

Any doubt which could possibly be entertained as to the true character of the ducts whose development I have described is entirely removed by the development of the tubules of the Wolffian body. In the still later stage than this further proofs are furnished involving the function of the Oviduct. At the period when the olfactory lobes have become so developed as to render the head of the typical elongated shape of the adult, I find that the males and females can be distinguished by the presence in the former of the clasping appendages.[1] I find at this stage that in the female the front ends of the Oviducts have approached the

[1] For the specimens of this age I am indebted to Professor Huxley.

middle line, dilated considerably, and commenced to exhibit at their front ends the peculiarities of the adult. In the male they are much less conspicuous, though still present.

At the same time the tubules of the Wolffian body become much more numerous, the Malpighian tufts appear, and the ducts cease almost, if not entirely, to communicate with the pleuro-peritoneal cavity. I have not made out anything very definitely as to the development of the Malpighian tufts, but I am inclined to believe that they arise independently in the mesoblast of the intermediate cell mass.

The facts which I have made out in reference to the development of the Wolffian duct, especially of its arising as a *series of involutions* from the pleuro-peritoneal cavity, will be found, I believe, of the greatest importance in understanding the true constitution of the Wolffian body. To this I will return directly, but I first wish to clear the ground by insisting upon one preliminary point.

From their development the Oviduct and Wolffian body appear to stand to each other in the relation of the Wolffian duct being the equivalent to a series, so to speak, of Oviducts.

I pointed out before that the mode of development of the Oviduct could only be considered as a modification of a simple involution from the pleuro-peritoneal cavity. Its development, both in the Birds and in the Batrachians as an involution, still more conclusively proves the truth of this view.

The explanation of its first appearing as a solid rod of cells which keeps close to the epiblast is, I am inclined to think, the following. Since the Oviduct had to grow a long way backwards from its primitive point of involution, it was clearly advantageous for it not to bore its way through the mesoblast of the intermediate cell mass, but to pass between this and the epiblast. This modification having been adopted, was followed by the knob forming the origin of the duct coming to be placed at the outside of the intermediate cell mass rather than close to the pleuro-peritoneal cavity, a change which necessitated the mode of development by an involution being dropped and the solid mode of development substituted for it, a lumen being only subsequently acquired.

In support of the modification in the development being due to this cause is the fact that in Birds the modes of development of the Wolffian duct and the Oviduct are inverted. The Wolffian duct there arises differently from its mode of development in all the lower vertebrates as a solid rod close to the epiblast.[1]

If the above explanation about the Oviduct be correct,

[1] If Romiti's observations ('Archives für Mikr. Anatom.,' vol. ix, p.

then it is clear that similar causes have produced a similar modification in development (only with a different organ) in Birds; while, at the same time, the primitive mode of origin of the Oviduct (Müller's duct) has been retained by them.

The Oviduct, then, may be considered as arising by an involution from the pleuro-peritoneal cavity.

The Wolffian duct arises by a series of such involutions, all of which are behind (nearer the tail) the involution to form the Oviduct.

The natural interpretation of these facts is that in the place of the Oviduct and Wolffian body there were primitively a series of similar bodies (probably corresponding in number with the vertebral segments), each arising by an involution from the pleuro-peritoneal cavity; and that the first of these subsequently became modified to carry eggs, while the rest coalesced to form the Wolffian duct.

If we admit that the Wolffian duct is formed by the coalescence of a series of similar organs, we shall only have to extend the suggestion of Gegenbaur as to the homology of the Wolffian body in order to see its true nature. Gegenbaur looks upon the whole urino-genital system as homologous with a pair of segmental organs. Accepting its homology with the segmental organs, its development in Elasmobranchii proves that it is not one pair, but a series of pairs of segmental organs with which the urino-genital system is homologous. The first of these have become modified so as to form the Oviducts, and the remainder have coalesced to form the Wolffian ducts.

The part of a segmental organ which opens to the exterior appears to be lost in the case of all but the last one, where this part is still retained, and serves as the external opening for all.

Whether the external opening of the first segmental organ (Oviduct) is retained or not is doubtful. Supposing it has been lost, we must look upon the external opening for the Wolffian body as serving also for the Oviduct. In the case of all other vertebrates whose development has been investigated (but the Elasmobranchii), the Wolffian duct arises by a single involution, or, what is equivalent to it, the other involutions having disappeared. This even appears to be the case in the Marsipobranchii. In the adult Lamprey the Wolffian duct terminates at its anterior end by a large

200) are correct, then the ordinary view of the Wolffian duct arising in Birds as a solid rod at the outer corner of the protovertebræ will have to be abandoned.

ciliated opening into the pleuro-peritoneal cavity. It will, perhaps, be found, when the development of the Marsipobranchii is more carefully studied, that there are *primitively* a number of such openings.[1] The Oviduct, when present, arises in other vertebrates as a single involution, strongly supporting the view that its mode of formation in the Dog-fish is fundamentally merely an involution.

The duct of the testes is, I have little doubt, derived from the anterior part of the Wolffian body; if so, it must be looked upon as not precisely equivalent to the Oviduct, but rather to a series of coalesced organs, each equivalent to the Oviduct. The Oviduct is in the Elasmobranchii, as in other vertebrates, primitively developed in both sexes. In the male, however, it atrophies. I found it still visible in the male Torpedos, though much smaller than in the females near the close of intra-uterine life.

Whether or not these theoretical considerations as to the nature of the Wolffian body and oviduct are correct, I believe that the facts I have brought to light in reference to the development of these parts in the Dog-fish will be found of service to every one who is anxious to discover the true relations of these parts.

Before leaving the subject I will say one or two words about the development of the Ovary. In both sexes the germinal epithelium (fig. 13) becomes thickened below the Oviduct, and in both sexes a knob (in section but really a ridge) comes to project into the pleuro-peritoneal cavity on each side of the mesentery (fig. 13, *p, o v*). In both sexes, but especially the females, the epithelium on the upper surface of this ridge becomes very much thickened, whilst subsequently it elsewhere atrophies. In the females, however, the thickened epithelium on the knob grows more and more conspicuous, and develops a number of especially large cells with large nuclei, precisely similar to Waldeyer's (loc. cit.) "primitive ova" of the Bird. In the male the epithelium on the ridge, though containing primitive Ova, is not as conspicuous as in the female. Though I have not worked out the matter further than this at present, I still have no doubt that these projecting ridges become the Ovaries.

[1] While correcting the proofs of this paper I have come across a memoir of W. Müller ('Über die Persistenz der Urniere bei Myxine Glutinosa,' 'Jenaische Zeitschrift,' vol. vii, 1873), in which he mentions that in Myxine the upper end of the Walffian duct communicates by numerous openings with the pleuro-peritoneal cavity; this gives to the suggestion in the text a foundation of fact.

The Head.

The study of the development of the parts of the head, on account of the crowding of organs which occurs there, always presents greater difficulties to the investigator than that of the remainder of the body. My observations upon it are correspondingly incomplete. I have, however, made out a few points connected with it in reference to some less well-known organs, which I have thought it worth while calling attention to in this preliminary account.

The continuation of the Pleuro-peritoneal Cavity into the Head.

In the earlier part of this paper (p. 346) I called attention to the extension of the separation between somatopleure and splanchnopleure into the head, forming a space continuous with the pleuro-peritoneal cavity (Pl. XIV, fig. 8 *a*, *p p′*); this becomes more marked in the next stage, and, indeed, the pleuro-peritoneal cavity is present for a considerable time in the head before it becomes visible elsewhere. At the time of the appearance of the second visceral cleft it has become for the most part atrophied, but there persist two separated portions of it in front of the first cleft, and also remnants of it less well marked between and behind the two clefts. The visceral clefts necessarily divide it into separate parts.

The two portions in front of the first visceral cleft remain very conspicuous till the appearance of the external gills, and above the hinder one of the two the fifth nerve bifurcates.

These two are shown as they appear in a surface view in fig. 14, *p p′*. They are in reality somewhat flattened spaces, lined by a mesoblastic epithelium, the epithelium on the inner surface of the space corresponding to the splanchnopleure, and that on the outer to the somatopleure.

I have not followed the history of these later than the time of the appearance of the external gills.

The presence of the pleuro-peritoneal cavity in the head is interesting, as showing the fundamental similarity between the head and the remainder of the body.

The Pituitary Body.

All my sections seem to prove that it is a portion of the epiblastic involution to form the mouth which is pinched off to form the pituitary body, and not a portion of the hypoblast of the throat. Since Gotte ('Archiv. für Micr. Anat.,' Bd. ix) has also found that the same is the case with the Batrachians and Mammalia, I have little doubt it will be found to be universally the case amongst vertebrates.

Probably the observations which lead to the supposition that it was the throat which was pinched off to form the pituitary body were made after the opening between the mouth and throat was completed, when it would naturally be impossible to tell whether the pinching off was from the epiblast of the mouth involution or the hypoblast of the throat.

The Cranial Nerves.

The cranial nerves in their early condition are so clearly visible that I have thought it worth while giving a figure of them, and calling attention to some points about their embryonic peculiarities.

From my figure (14) it will be seen that there is behind the auditory vesicle a nervous tract, from which four nerves descend, and that each of these nerves is distributed to the front portion of a visceral arch. When the next and last arch (in this species) is developed, a branch from this nervous mass will also pass down to it. That each of these is of an equal morphological value can hardly be doubted.

The nerve to the third arch becomes the glosso-pharyngeal (fig. 14, *g l*), the nerves to the other arches become the branchial branches of the vagus nerve (fig. 14, *v g*). Thus the study of their development strongly supports Gegenbaur's view of the nature of the vagus and glosso-pharyngeal, viz. that the vagus is a compound nerve, each component part of it which goes to an arch being equivalent to one nerve, such as the glosso-pharyngeal.

Of the nerves in front of the auditory sac the posterior is the seventh nerve (fig. 14, VII). Its mode of distribution to the second arch leaves hardly a doubt that it is equivalent to one such nerve as those distributed to the posterior arches. Subsequently it acquires another branch, passing forwards towards the arch in front.

The most anterior nerve is the fifth (fig. 14, V), of which two branches are at this stage developed. The natural interpretation of its present condition is, that it is equivalent to two nerves, but the absence of relation in its branches to any visceral clefts renders it more difficult to determine the morphology of the fifth nerve than of the other nerves. The front branch of the two is the ophthalmic branch of the adult, and the hind branch the inferior maxillary branch. The latter branch subsequently gives off low down, *i.e.*

near its distal extremity, another branch, the superior maxillary branch.

In its embryonic condition this latter branch does not appear like a third branch of the fifth, equivalent to the seventh or the glosso-pharyngeal nerves, but rather resembles the branch of the seventh nerve which passes to the arch in front, which also is present in all the other cranial nerves.

Modes of Preparation.

Before concluding I will say one or two words as to my modes of preparation.

I have used picric and chromic acids, both applied in the usual way; but for the early stages I have found osmic acid by far the most useful reagent. I placed the object to be hardened, in osmic acid (half per cent.) for two hours and a half, and then for twenty-four hours in absolute alcohol.

I then embedded and cut sections of it in the usual way, without staining further.

I found it advantageous to cut sections of these embryos immediately after hardening, since if kept for long in the absolute alcohol the osmic acid specimens are apt to become brittle.

List of Works referred to.

Gegenbaur.—'Anat. der Wirbelthiere,' III Heft, Leipzig, 1873.
A. Götte.—'Archiv. für Micr. Anat.,' vol. x, 1873.
" "Der Keim der forelleneies," 'Archiv. für Micr. Anat.,' vol. ix, 1873.
" "Untersuchung über die Entwickelung der Bombinator igneus," 'Archiv. für Micr. Anat.,' vol. v, 1869.
" "Kurze Mittheilungen aus der Entwicklungsgeschichte der Unke," 'Archiv. für Micr. Anat.,' vol. ix, 1873.
Kupffer.—'Archiv. für Micr. Anat.,' vol. ii, 1866, p. 473.
" Ibid., vol. iv, 1868, p. 209.
Kowalevsky.—"Entwickelungsgeschichte der Holothurien," 'Mémoirs de l'Acad. Impér. des Sciences de St. Petersbourg,' vii ser., vol. xi, 1867.
Kowalevsky, Owsjannikow, und Wagner.—"Entwickelung der Störe," 'Bulletin der K. Acad. St. Petersbourg,' vol. xiv, 1873.
Kowalevsky.—"Embryologische Studien au Wurmern und Arthropoden," 'Mémoirs de l'Acad. Impér. des Sciences de St. Petersbourg,' vol. xvi, 1871.
E. Ray Lankester, 'Annals aud Mag. of Nat. History,' vol. xi, 1873, p. 81.
W. Müller.—"Über die Persistenz der Urniere bei Myxine Glutinosa,' 'Jenaische Zeitschrift,' vol. vii, 1873.
Oellacher.—'Zeitschrift für Wiss. Zoologie,' vol. xxiii, 1873.
Owsjannikow.—"Entwickelung der Coregonus," 'Bul. der K. Akad. St. Petersbourg,' vol. xix.
Romiti.—'Archiv. für Micr. Anat.,' vol. ix, 1873.
Waldeyer.—'Eierstock u. Eie.'

Observations *on the* Development *of the* Pond-snail (*Lymnæus stagnalis*), *and on the* Early Stages *of other* Mollusca.

By E. Ray Lankester, M.A., Fellow and Lecturer of Exeter College, Oxford. (With Plates XVI and XVII.)

§ 1.—Some of the Developmental Phenomena of Mollusca.

Four years since, I determined to make a study of the developmental phenomena of a series of Mollusca, with the view of ascertaining from the minute comparison of a number of cases what phenomena might be common to the group, or be considered as indicating ancestral conditions inherited from common ancestors.

The success which had attended Fritz Müller's investigation of the Crustacea, and his celebrated "recapitulation hypothesis," according to which we have, in the development of every individual organism, a more or less complete epitome of the development of the species, so that the series of changing forms passed through between ovum and adult form are but a series of dissolving views or portraits (often very much marred) of its line of ancestors—this, I say, led me to hope that materials might be found in the developmental history of the Mollusca for constructing their genealogical tree. During the past fifteen years but little has been done in the study of the embryology of the Mollusca, and it was therefore to be expected that the application of improved methods of investigation and new hypotheses would yield valuable results. The result of my study of the development of the Lamellibranch *Pisidium* and of the Gasteropods *Aplysia, Neritina, Tergipes* and *Polycera,* are now in course of publication elsewhere.

I have also, during this spring, completed the examination of the development of the Cephalopod *Loligo* from an early stage of the ovarian egg up to the escape of the embryo from the egg-jelly, which, together with less complete accounts of the development of *Octopus* and *Sepia,* I hope soon to see published. Before proceeding to give here an account of observations on *Lymnæus* which I carried out during July in the laboratory of Exeter College, Oxford, I may briefly summarise the chief results of my previous observations, which are remarkably confirmed by the facts to be subsequently related in regard to *Lymnæus.*

Kowalevsky, in his account of the development of *Amphi-*