A Renewed Study *of the* Germinal Layers *of the* Chick.

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The formation of the germinal layers in the chick has been so often and so fully dealt with in recent years, that we consider some explanation to be required of the reasons which have induced us to add to the long list of memoirs on this subject. Our reasons are twofold. In the first place the principal results we have to record have already been briefly put forward in a 'Treatise on Comparative Embryology' by one of us; and it seemed desirable that the data on which the conclusions there stated rest should be recorded with greater detail than was possible in such a treatise. In the second place, our observations differ from those of most other investigators, in that they were primarily made with the object of testing a theory as to the nature of the primitive streak. As such they form a contribution to comparative embryology; since our object has been to investigate how far the phenomena of the formation of the germinal layers in the chick admit of being compared with those of lower and less modified vertebrate types.

We do not propose to weary the reader by giving a new version of the often told history of the views of various writers on the germinal layers in the chick, but our references to other investigators will be in the main confined to a comparison of our results with those of two embryologists, who have published their memoirs since our observations were made. One of them is L. Gerlach, who published a short memoir[1] in April last, and the other is C. Koller, who has published his memoir[2] still more recently. Both of them cover part of the ground of our investigations, and their results are in many, though not in all points, in harmony with our own. Both of them, moreover, lay stress on certain features in the development which have escaped our attention. We desired to work over these points again, but various circumstances have prevented our doing so, and we have accordingly thought it best to publish our observations as they stand, in spite of their incompleteness, merely indicating where the most important gaps occur.

[1] "Ueb. d. entodermale Entstehungswiese d. Chorda dorsalis," 'Biol. Centralblatt,' vol. i, Nos. 1 and 2.

[2] "Untersuch. üb. d. Blätterbildung im Hühnerkeim," 'Archiv. f. mikr. Anat.,' vol. xx, 1881.

Our observations commence at a stage a few hours after hatching, but before the appearance of the primitive streak.

The area pellucida is at this stage nearly spherical. In it there is a large oval opaque patch, which is continued to the hinder border of the area. This opaque patch has received the name of the embryonic shield—a somewhat inappropriate name, since the structure in question has no very definite connection with the formation of the embryo.

Koller describes, at this stage, in addition to the so-called embryonic shield, a sickle-shaped opaque appearance at the hinder border of the area pellucida.

We have not made any fresh investigations for the purpose of testing Koller's statements on this subject.

Embryologists are in the main agreed as to the structure of the blastoderm at this stage. There is (Pl. XIII, Ser. A, 1 and 2) the epiblast above, forming a continuous layer, extending over the whole of the area opaca and area pellucida. In the former its cells are arranged as a single row, and are cubical or slightly flattened. In the latter the cells are more columnar, and form, in the centre especially, more or less clearly, a double row; many of them, however, extend through the whole thickness of the layer.

We have obtained evidence at this stage which tends to show that at its outer border the epiblast grows not merely by the division of its own cells, but also by the addition of cells derived from the yolk below. The epiblast has been observed to extend itself over the yolk by a similar process in many invertebrate forms.

Below the epiblast there is placed, in the peripheral part of the area opaca, simply white yolk; while in a ring immediately outside and concentric with the area pellucida, there is a closely-packed layer of cells, known as the *germinal wall*. The constituent cells of this wall are in part relatively small, of a spherical shape, with a distinct nucleus, and a granular and not very abundant protoplasm; and in part large and spherical, filled up with highly refracting yolk particles of variable size, which usually render the nucleus (which is probably present) invisible (A, 1 and 2). This mass of cells rests, on its outer side, on a layer of white yolk.

The sickle-shaped structure, visible in surface veins, is stated by Koller to be due to a special thickening of the germinal wall. We have not found this to be a very distinctly marked structure in our sections.

In the region of the area pellucida there is placed below the epiblast a more or less irregular layer of cells. This layer is continuous, peripherally, with the germinal wall; and is composed of cells, which are distinguished both by their flattened

or oval shape and more granular protoplasm from the epiblast-cells above, to which, moreover, they are by no means closely attached. Amongst these cells a few larger cells are usually present, similar to those we have already described as forming an important constituent of the germinal wall.

We have figured two sections of a blastoderm of this age (Ser. A, 1 and 2) mainly to show the arrangement of these cells. A large portion of them, considerably more flattened than the remainder, form a continuous membrane over the whole of the area pellucida, except usually for a small area in front, where the membrane is more or less interrupted. This layer is the hypoblast (*hy.*). The remaining cells are interposed between this layer and the epiblast. In front of the embryonic shield there are either comparatively few or none of these cells present (Ser. A, 1), but in the region of the embryonic shield they are very numerous (Ser. A, 2), and are, without doubt, the main cause of the opacity of this part of the area pellucida. These cells may be regarded as not yet completely differentiated segmentation spheres.

In many blastoderms, not easily distinguishable in surface views from those which have the characters just described, the hypoblastic sheet is often much less completely differentiated, and we have met with other blastoderms, again, in which the hypoblastic sheet was completely established, except at the hinder part of the embryonic shield; where, in place of it and of the cells between it and the epiblast, there was only to be found a thickish layer of rounded cells, continuous behind with the germinal wall.

In the next stage, of which we have examined surface views and sections, there is already a well-formed primitive streak.

The area pellucida is still nearly spherical, the embryonic shield has either disappeared or become much less obvious, but there is present a dark linear streak, extending from the posterior border of the area pellucida towards the centre, its total length being about one third, or even less, of the diameter of the area. This streak is the *primitive streak*. It enlarges considerably behind, where it joins the germinal wall. By Koller and Gerlach it is described as joining the sickle-shaped structure already spoken of. We have in some instances found the posterior end of the primitive streak extending laterally in the form of two wings (Pl. XV, fig. L). These extensions are, no doubt, the sickle; but the figures given by Koller appear to us somewhat diagrammatic. One or two of the figures of early primitive streaks in the sparrow, given by Kupffer and Benecke,[1]

[1] "Photogramme d. Ontogenie d. Vogel." Nova Acta. K. Leop. Carol, 'Deutschen Akad. d. Naturfor.,' Bd. x, 41, 1879.

correspond more closely with what we have found, except that in these figures the primitive streak does not reach the end of the area pellucida, which it certainly usually does at this early stage in the chick.

Sections through the area pellucida (Pl. XIII, ser. B and C) give the following results as to the structure of its constituent parts.

The epiblast cells have undergone division to a considerable extent, and in the middle part, especially, are decidedly more columnar than at an earlier stage, and distinctly divided into two rows, the nuclei of which form two more or less distinct layers.

In the region in front of the primitive streak the cells of the lower part of the blastoderm have arranged themselves as a definite layer, the cells of which are not so flat as is the case with the hypoblast cells of the posterior part of the blastoderm, and in the older specimens of this stage they are very decidedly more columnar than in the younger specimens.

The primitive streak is however the most interesting structure in the area pellucida at this stage.

The feature which most obviously strikes the observer in transverse sections through it is the fact, proved by Kölliker, that it is mainly due to a proliferation of the epiblast cells along an axial streak, which, roughly speaking, corresponds with the dark line visible in surface views. In the youngest specimens and at the front end of the primitive streak, the proliferated cells do not extend laterally beyond the region of their origin, but in the older specimens they have a considerable lateral extension.

The hypoblast can, in most instances, be traced as a distinct layer underneath the primitive streak, although it is usually less easy to follow it in that region than elsewhere, and in some cases it can hardly be distinctly separated from the superjacent cells.

The cells undoubtedly formed by a proliferation of the epiblast, form a compact mass extending downwards towards the hypoblast; but between this mass and the hypoblast there are almost always present along the whole length of the primitive streak a number of cells, more or less loosely arranged, and decidedly more granular than the proliferated cells. Amongst these loosely arranged cells there are to be found a certain number of large spherical cells filled with yolk granules. Sometimes these cells are entirely confined to the region of primitive streak, at other times they are continuous laterally with cells irregularly scattered between the hypoblast and epiblast (Ser. C, 2), which are clearly the remnants of the undifferentiated cells of the embryonic shield. The junction between these cells and the cells of the primi-

tive streak derived from the epiblast is often obscure, the two sets of cells becoming partially intermingled. The facility with which the cells we have just spoken of can be recognised varies moreover greatly in different instances. In some cases they are very obvious (Ser. C), while in other cases they can only be distinguished by a careful examination of good sections.

The cells of the primitive streak between the epiblast and the hypoblast are without doubt mesoblastic, and constitute the first portion of the mesoblast which is established. The section of these cells attached to the epiblast, in our opinion, clearly originates from the epiblast; while the looser cells adjoining the hypoblast must, it appears to us, be admitted to have their origin in the indifferent cells of the embryonic shield, placed between the epiblast and the hypoblast, and also very probably in a distinct proliferation from the hypoblast below the primitive streak.

Posteriorly the breadth of the streak of epiblast which buds off the cells of the primitive streak widens considerably, and in the case of the blastoderm with the earliest primitive streaks extends into the region of the area opaca. The widening of the primitive streak behind is shown in Ser. B, 3; Ser. C, 2; and Ser. E, 4. Where very marked it gives rise to the sickle-shaped appearance upon which so much stress has been laid by Koller and Gerlach. In the case of one of the youngest of our blastoderms of this stage in which we found in surface views (Pl. XV, fig. 1) a very well-marked sickle-shaped appearance at the hind end of the primitive streak, the appearance was caused, as is clearly brought out by our sections, by a thickening of the hypoblast of the germinal wall.

There is a short gap in our observations between the stage with a young primitive streak and the first described stage in which no such structure is present. This gap has been filled up both by Gerlach and Koller.

Gerlach states that during this period a small portion of the epiblast, within the region of the area opaca, but close to the posterior border of the area pellucida, becomes thickened by a proliferation of its cells. This portion gradually grows outwards laterally, forming in this way a sickle-shaped structure. From the middle of this sickle a process next grows forward into the area pellucida. This process is the primitive streak, and it is formed, like the sickle, of proliferating epiblast cells.

Koller [1] described the sickle and the growth forwards from it of the primitive streak in surface views somewhat before Gerlach; and in his later memoir has entered with considerable detail

[1] "Beitr. z. Kenntniss d. Hühnerkeims im Beginne d. Bebrütung," 'Sitz. d. k. Akad. Wiss.,' iv Abth., 1879.

into the part played by the various layers in the formation of this structure.

He believes, as already mentioned, that the sickle-shaped structure, which appears according to him at an earlier stage than is admitted by Gerlach, is in the first instance due to a thickening of the hypoblast. At a later stage he finds that the epiblast in the centre of the sickle becomes thickened, and that a groove makes its appearance in this thickening which he calls the "Sichel-rinne." This groove is identical with that first described by Kupffer and Benecke[1] in the sparrow and fowl. We have never, however, found very clear indications of it in our sections.

In the next stage, Koller states that, in the region immediately in front of the "Sichel-rinne," a prominence appears which he calls the Sichelknopf, and from this a process grows forwards which constitutes the primitive streak. This structure is in main derived from a proliferation of epiblast cells, but Koller admits that some of the cells just above the hypoblast in the region of the Sichelknopf are probably derived from the hypoblast. Since these cells form part of the mesoblast it is obvious that Koller's views on the origin of the mesoblast of the primitive streak closely approach those which we have put forward.

The primitive streak starting, as we have seen, at the hinder border of the area pellucida, soon elongates till it eventually occupies at least two thirds of the length of the area. As Koller (loc. cit.) has stated this can only be supposed to happen in one of two ways, viz. either by a progression forward of the region of epiblast budding off mesoblast, or by an interstitial growth of area of budding epiblast. Koller adopts the second of these alternatives, but we cannot follow him in doing so. The simplest method of testing the point is by measuring the distance between the front end of the primitive streak and the front border of the area pellucida at different stages of growth of the primitive streak. If this distance diminishes with the elongation of the primitive streak then clearly the second of the two alternatives is out of the question.

We have made measurements to test this point, and find that the diminution of the space between the front end of the primitive streak and the anterior border of the area pellucida is very marked up to the period in which the medullary plate first becomes established. We can further point in support of our view to the fact that the extent of the growth lateralwards of the mesoblast from the sides of the primitive streak is always less in front than behind; which would seem to indicate that the front part of the streak is the part formed latest. Our view as to the

[1] 'Die erste Entwick. an Eier d. Reptilien.' Königsberg, 1878.

elongation of the primitive streak appears to be that adopted by Gerlach.

Our next stage includes roughly the period commencing slightly before the first formation of a groove along the primitive streak, known as the primitive groove, and terminating immediately before the first trace of the notochord makes its appearance. After the close of the last stage the primitive streak gradually elongates, till it occupies fully two thirds of the diameter of the area pellucida. The latter structure also soon changes its form from a circular to an oval, and finally becomes pyriform with the narrow end behind, while the primitive streak occupying two thirds of its long axis becomes in most instances marked by a light linear band along the centre, which constitutes the primitive groove.

In surface views the primitive streak often appears to stop short of the hinder border of the area pellucida.

During the period in which the external changes, which we have thus briefly described, take place in the area pellucida, great modifications are effected in the characters of the germinal layers. The most important of these concern the region in front of the primitive streak; but they will be better understood if we commence our description with the changes in the primitive streak itself.

In the older embryos belonging to our last stage we pointed out that the mesoblast of the primitive streak was commencing to extend outwards from the median line in the form of two lateral sheets. This growth of the mesoblast is continued rapidly during the present stage, so that during the latter part of it any section through the primitive streak has approximately the characters of Ser. 1, 5.

The mesoblast is attached in the median line to the epiblast. Laterally it extends outwards to the edge of the area pellucida, and in older embryos may even form a thickening beyond the edge (fig. G). Beneath the denser part of the mesoblast, and attached to the epiblast, a portion composed of stellate cells may in the majority of instances be recognised, especially in the front part of the primitive streak. We believe these stellate cells to be in the main directly derived from the more granular cells of the previous stage. The hypoblast forms a sheet of flattened cells, which can be distinctly traced for the whole breadth of the area pellucida, though closely attached to the mesoblast above.

In sections we find that the primitive streak extends back to the border of the area pellucida, and even for some distance beyond. The attachment to the epiblast is wider behind; but the thickness of the mesoblast is not usually greater in the median line than it is laterally, and for this reason probably the

posterior part of the streak fails to show up in surface views. The thinning out of the median portion of the mesoblast of the primitive streak is shown in a longitudinal section of a duck's blastoderm of this stage (fig. D). The same figure also shows that the hypoblastic sheet becomes somewhat thicker behind, and more independent of the parts above.

A careful study of the peripheral part of the area pellucida, in the region of the primitive streak, in older embryos of this stage, shows that the hypoblast is here thickened, and that its upper part, *i.e.* that adjoining the mesoblast, is often formed of stellate cells, many of which give the impression of being in the act of passing into the mesoblast above. At a later stage the mesoblast of the vascular area undoubtedly receives accessions of cells from the yolk below; so that we see no grounds for mistrusting the appearances just spoken of, or for doubting that they are to be interpreted in the sense suggested.

We have already stated that during the greater part of the present stage a groove, known as the primitive groove, is to be found along the dorsal median line of the primitive streak.

The extent to which this groove is developed appears to be subject to very great variation. On the average it is, perhaps, slightly deeper than it is represented in Ser. I, 5. In some cases it is very much deeper. One of the latter is represented in fig. G. It has here the appearance of a narrow slit, and sections of it give the impression of the mesoblast originating from the lips of a fold; in fact, the whole structure appears like a linear blastopore, from the sides of which the mesoblast is growing out; and this as we conceive actually to be the true interpretation of the structure. Other cases occur in which the primitive groove is wholly deficient, or at the utmost represented by a shallow depression along the median axial line of a short posterior part of the primitive streak.

We may now pass to the consideration of the part of the area pellucida in front of the primitive streak.

We called attention to a change in the character of the hypoblast cells of this region as taking place at the end of the last stage. During the very early part of this stage the change in the character of these cells becomes very pronounced.

What we consider to be our earliest stage in this change we have only so far met with in the duck, and we have figured a longitudinal and median section to show it (Pl. XIII, fig. D). The hypoblast (*hy*) has become a thick layer of somewhat cubical cells several rows deep. These cells, especially in front, are characterised by their numerous yolk spherules, and give the impression that part of the area pellucida has been, so to speak, reclaimed from the tarea opaca. *Posteriorly, at the front end of the primitive streak,*

the thick layer of hypoblast, instead of being continuous with the flattened hypoblast under the primitive streak, falls, in the axial line, into the mesoblast of the primitive streak (Pl. XIII, fig. D).

In a slightly later stage, of which we have specimens both of the duck and chick, but have only figured selected sections of a chick series, still further changes have been effected in the constitution of the hypoblast (Pl. XIV, Ser. H, 1 and 2).

Near the front border of the area pellucida (1) it has the general characters of the hypoblast of the duck's blastoderm just described. Slightly further back the cells of the hypoblast have become differentiated into stellate cells several rows deep, *which can hardly be resolved in the axial line into hypoblast and mesoblast*, though one can fancy that in places, especially laterally, they are partially differentiated into two layers. The axial sheet of stellate cells is continuous laterally with cubical hypoblast cells.

As the primitive streak is approached an axial prolongation forwards of the rounded and closely-packed mesoblastic elements of the primitive streak is next met with, and at the front end of the primitive streak, where this prolongation unites with the epiblast, it also becomes continuous with the stellate cells just spoken of. In fact, close to the end of the primitive streak it becomes difficult to say which mesoblast cells are directly derived from the primitive layer of hypoblast in front of the primitive streak, and which from the forward growth of the mesoblast of the primitive streak. There is, in fact, as in the earlier stage, a fusion of the layers at this point.

Sections of a slightly older chick blastoderm are represented in Pl. XIV, Ser. I, 1, 2, 3, 4 and 5.

Nearly the whole of the hypoblast in front of the primitive streak has now undergone a differentiation into stellate cells. In the second section the products of the differentiation of this layer form a distinct mesoblast and hypoblast laterally, while in the median line they can hardly be divided into two distinct layers.

In a section slightly further back the same is true, except that we have here, in the axial line above the stellate cells, rounded elements derived from a forward prolongation of the cells of the primitive streak. In the next section figured, passing through the front end of the primitive streak, the axial cells have become continuous with the axial mesoblast of the primitive streak, while below there is an independent sheet of flattened hypoblast cells.

The general result of our observations on the part of the blastoderm in front of the primitive streak during this stage is to show that the primitive hypoblast of this region undergoes

considerable changes, including a multiplication of its cells; and that these changes result in its becoming differentiated on each side of the middle line, with more or less distinctness, into (1) a hypoblastic sheet below, formed of a single row of flattened cells, and (2) a mesoblast plate above formed of stellate cells, while in the middle line there is a strip of stellate cells in which there is no distinct differentiation into two layers.

Since the region in which these changes take place is that in which the medullary plate becomes subsequently formed, the lateral parts of the mesoblast plate are clearly the permanent lateral plates of the trunk, from which the mesoblastic somites, &c., become subsequently formed; *so that the main part of the mesoblast of the trunk is not directly derived from the primitive streak.*

Before leaving this stage we would call attention to the presence, in one of our blastoderms of this stage, of a deep pit at the junction of the primitive streak with the region in front of it (Pl. XIV, Ser. F, 1 and 2). Such a pit is unusual, but we think it may be regarded as an exceptionally early commencement of that most variable structure in the chick, the neurenteric canal.

The next and last stage we have to deal with is that during which the first trace of the notochord and of the medullary plate make their appearance.

In surface views this stage is marked by the appearance of a faint dark line, extending forwards, from the front end of the primitive streak, to a fold, which has in the mean time made its appearance near the front end of the area pellucida, and constitutes the head fold.

Pl. XV, Ser. K, represents a series of sections through a blastoderm of this stage, which have been selected to illustrate the mode of formation of the notochord.

In a section immediately behind the head fold the median part of the epiblast is thicker than the lateral parts, forming the first indication of a medullary plate (Ser. K, 1). Below the median line of the epiblast is a small cord of cells, not divided into two layers, but continuous laterally, both with the hypoblast and mesoblast, which are still more distinctly separated than in the previous stage.

A section or so further back (Ser. K, 2) the axial cord, which we need scarcely say is the rudiment of the notochord, is thicker, and causes a slight projection in the epiblast above. It is, as before, continuous laterally, both with the mesoblast and with the hypoblast. The medullary plate is more distinct, and a shallow but unmistakable medullary groove has made its appearance.

As we approach the front end of the primitive streak the notochord becomes (Ser. K, 3) very much more prominent,

though retaining the same relation to the germinal layers as in front.

In the section immediately behind (Ser. K, 4) the convex upper surface of the notochord has become continuous with the epiblast for a very small region. The section, in fact, traverses the front end of the primitive streak.

In the next section the attachment between the epiblast and the cells below becomes considerably wider. It will be noticed that this part of the primitive streak is placed on the floor of the wide medullary groove, and there forms a prominence known as the anterior swelling of the primitive streak.

It will further be noticed that in the two sections passing through the primitive streak, the hypoblast, instead of simply becoming continuous with the axial thickening of the cells, as in front, forms a more or less imperfect layer underneath it. This layer becomes in the sections following still more definite, and forms part of the continuous layer of hypoblast present in the region of the primitive streak.

A comparison of this stage with the previous one shows very clearly that the notochord is formed out of the median plate of cells of the earlier stage, which was not divided into mesoblast and hypoblast, together with the short column of cells which grew forwards from the primitive streak.

The notochord, from its mode of origin, is necessarily continuous behind with the axial cells of the primitive streak.

The sections immediately behind the last we have represented show a rudiment of the neurenteric canal of the same form as that first figured by Gasser, viz. a pit perforating the epiblast with a great mass of rounded cells projecting upwards through it.

The observations just recorded practically deal with two much disputed points in the ontogeny of birds, viz. the origin of the mesoblast and the origin of the notochord.

With reference to the first of these our results are briefly as follows:

The first part of the mesoblast to be formed is that which arises in connection with the primitive streak. This part is in the main formed by a proliferation from an axial strip of the epiblast along the line of the primitive streak, but in part also from a simultaneous differentiation of hypoblast cells also along the axial line of the primitive streak. The two parts of the mesoblast so formed become subsequently indistinguishable. The second part of the mesoblast to be formed is that which gives rise to the lateral plates of mesoblast of the head and trunk of the embryo. This part appears as two plates—one on each side of the middle line—which arise by direct differentiation

from the hypoblast in front of the primitive streak. They are continuous behind with the lateral wings of mesoblast which grow out from the primitive streak, and on their inner side are also at first continuous with the cells which form the notochord.

In addition to the parts of mesoblast, formed as just described, the mesoblast of the vascular area is in a large measure developed by a direct formation of cells round the nuclei of the germinal wall.

The mesoblast formed in connection with the primitive streak gives rise in part to the mesoblast of the allantois, and ventral part of the tail of the embryo (?), and in part to the vascular structures found in the area pellucida.

With reference to the formation of the mesoblast of the primitive streak, our conclusions are practically in harmony with those of Koller; except that Koller is inclined to minimise the share taken by the hypoblast in the formation of the mesoblast of the primitive streak.

Gerlach, with reference to the formation of this part of the mesoblast, adopts the now generally accepted view of Kölliker, according to which the whole of the mesoblast of the primitive streak is derived from the epiblast.

As to the derivation of the lateral plates of mesoblast of the trunk from the hypoblast of the anterior part of the primitive streak, our general result is in complete harmony with Gerlach's results, although in our accounts of the details of the process we differ in some not unimportant particulars.

As to the origin of the notochord, our main result is that this structure is formed as an actual thickening of the primitive hypoblast of the anterior part of the area pellucida. We find that it unites posteriorly with a forward growth of the axial tissue of the primitive streak, while it is laterally continuous, at first, both with the mesoblast of the lateral plates and with the hypoblast. At a later period its connection with the mesoblast is severed, while the hypoblast becomes differentiated as a continuous layer below it.

As to the hypoblastic origin of the notochord, we are again in complete accord with Gerlach; but we differ from him in admitting that the notochord is continuous posteriorly with the axial tissue of the primitive streak, and also at first continuous with the lateral plates of mesoblast.

The account we have given of the formation of the mesoblast may appear to the reader somewhat fantastic, and on that account not very credible. We believe, however, that if the view which has been elsewhere urged by one of us, that the primitive streak is the homologue of the blastopore of the lower vertebrates is accepted, the features we have described receive an adequate explanation.

The growth outwards of part of the mesoblast from the axial line of the primitive streak is a repetition of the well-known growth from the lips of the blastopore. It might have been anticipated that all the layers would fuse along the line of the primitive streak, and that the hypoblast as well as part of the mesoblast would grow out from it. There is, however, clearly a precocious formation of the hypoblast; but the formation of the mesoblast of the primitive streak, partly from the epiblast and partly from the hypoblast, is satisfactorily explained by regarding the whole structure as the blastopore. The two parts of the mesoblast subsequently become indistinguishable, and their difference in origin is, on the above view, to be regarded as simply due to a difference of position, and not as having a deeper significance.

The differentiation of the lateral plates of mesoblast of the trunk directly from the hypoblast is again a fundamental feature of vertebrate embryology, occurring in all types from Amphioxus upwards, the meaning of which has been fully dealt with in the 'Treatise on Comparative Embryology' by one of us. Lastly, the formation of the notochord from the hypoblast is the typical vertebrate mode of formation of this organ, while the fusion of the layers at the front end of the primitive streak is the universal fusion of the layers at the dorsal lip of the blastopore, which is so well known in the lower vertebrate types.