Note on the Early Development of *Lacerta Muralis*.

By

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With Plates IV, V, VI.

The following paper contains an account of some observations on the early stages in the development of *Lacerta muralis*, begun during the summer of this year at the zoological station at Naples and completed in the morphological laboratory at Cambridge. It relates chiefly to the mode of formation of the germinal layers and to the early development of the kidney.

On my return from Naples I found that in June last Professor C. K. Hoffmann had published an account of the mode of formation of the germinal layers, and the results obtained by him agree generally with my own. As, however, Professor Hoffmann has published very few figures of the stages observed by him, and as my observations lead me to differ from him in one or two points of detail, it has seemed to me that it would not be useless to give a short account of my own results.

The segmentation, which conforms to the ordinary meroblastic type, has already been fully described and figured by

Kupffer and Benecke\textsuperscript{1} and by Balfour.\textsuperscript{2} Neither of these observers describes a segmentation cavity; but Hoffmann\textsuperscript{3} states that during the later stages of segmentation a cavity is present, the floor of which is formed by the yolk, the roof by the lower layer cells. Towards the close of segmentation it disappears.

This cavity Hoffmann considers equivalent to the segmentation cavity of the Icthyopsida.

I have observed cavities similar to that described by Hoffmann, but I have been unable to satisfy myself that they were not due to the action of the hardening reagents employed. The cavity described by Professor Hoffmann differs strikingly, as he himself points out, from the segmentation cavity of other vertebrates, in the fact that its floor is never formed of lower layer cells.

At the close of segmentation the blastoderm consists of a superficial layer of epiblast cells, which is generally stated to be a single cell thick; in my sections, however, the arrangement is very irregular, the epiblast being in some places two cells deep, in others more.

Beneath the epiblast is an irregular sheet of lower layer cells; this layer is in many places two or three cells deep, and the cells of which it is composed are large, irregular, loaded with yolk-granules, many having two or even more deeply-staining nuclei.

In the centre of the blastoderm the epiblast cells become more columnar than in the peripheral parts, and the lower layer cells become slightly more regular in their arrangement. An oval area pellucida is thus formed.

Hoffmann finds at this stage a marked thickening of the lower layer cells at the posterior extremity of the blastoderm.

The posterior region of the area pellucida now becomes dis-
tinsquished from the anterior by the presence of the primitive streak.

A median longitudinal section through an embryo with a commencing primitive streak is shown in fig. 1. Anteriorly the area pellucida is seen to be formed by an epiblastic layer of irregular columnar cells and a sheet of lower layer cells, the two layers being quite distinct. At a point (bp), however, the position of the future blastopore, these layers are replaced by a mass of closely-packed cells (pr), exhibiting no division into layers, and forming the primitive streak, which may in some cases at least extend backwards as far as the commencement of the area opaca.

The blastopore commences at the anterior end of this streak as a pit, open above, and closed below. This is shown in fig. 2.

The floor of this pit presently breaks up, and the blastopore assumes its normal condition, forming a communication between the archenteron and the exterior, its anterior wall forming a communication between the epiblast and the lower layer cells (see fig. 3).

From this time a change in the character of the lower layer cells takes place, beginning from the anterior wall of the blastopore, where they pass into the epiblast, and proceeding forwards. Instead of being large, irregular, full of yolk, as in the previous stages, they become columnar, lose their yolk, arrange themselves in a definite layer several cells deep, and take on the characters of normal hypoblast. A median longitudinal section through an embryo, in which about half the lower layer cells are thus converted, is seen in fig. 4.

This process is evidently an invagination comparable to that which takes place in an Elasmobranch. It especially resembles the process described by Scott and Osborne in the newt.

The first traces of mesoblast appear at a stage slightly earlier than that represented in fig. 4. Fig. 5, which shows a portion of a lateral section from the same series as that to which

fig. 4 belongs, shows the condition of the mesoblast shortly after its origin.

The blastopore being funnel shaped, with its narrow opening directed downwards, it appears in a lateral longitudinal section as a pit, closed below, and from its closed extremity the mesoblast grows forwards as a solid cap, separate from epiblast and hypoblast.

Transverse sections show that the mesoblast is in connection not only with the walls of the blastopore, but also with the axial strip of invaginated hypoblast. Figs. 6—13 are selected from a series of transverse sections of an embryo slightly older than that represented in fig. 4, and show the relations of the mesoblast. The figures are arranged in order from behind forwards, fig. 6 being posterior. Figs. 6—9 pass through the blastopore, and a sheet of mesoblast, continuous with its walls, is seen growing out on each side. In figs. 10 and 11, which pass through the posterior embryonic region in front of the blastopore, each sheet of mesoblast is seen to be free laterally, but to be continuous near the middle line with the axial strip of hypoblast, the cells of which will give rise to the notochord, and are easily distinguishable from the more peripheral hypoblast cells by their more elongated forms and by being more than one layer deep.

This mode of origin of the mesoblast, however, only holds good for the posterior part of the embryo. Anteriorly (fig. 11) the mesoblastic sheet loses its connection with the axial hypoblast and finally disappears (fig. 12), being replaced by branched cells, which are budded off, partly from the axial, partly from the lateral hypoblast. This mode of origin of the anterior mesoblast has been overlooked by Hoffmann.

The account above given is obviously in complete accord with the observations of Balfour, who described a stage a little later than that represented in figs. 6—13, with a widely-open, neuro-enteric canal, and a sheet of mesoblast on each

side, which had separated from the axial hypoblast—all the layers being, however, still fused in front of the blastopore.

The statement of Kupffer,¹ that the blastoporic invagination gives rise to a closed sac, the walls of which become the allantois, is of course inconsistent with the truth of the above observations; but it has been already so abundantly disproved, first by Balfour and afterwards by Stahl and Hoffmann, that it is not necessary here to do more than refer to it in passing.

The actual mode of development of the allantois was first figured by Balfour,² a copy of whose drawing is reproduced in the woodcut. The details of the process were worked out by Strahl.³

I have nothing to add to the account given by these authors, but I would call attention to a consequence of it which neither observer has, to my knowledge, remarked.

It is obvious from the woodcut that, as has been shown in detail by Strahl,⁴ the allantois arises as a process of the primitive streak, which projects at first backwards into the body cavity.

Now, if this be the case, when the primitive streak is bent ventrally during the establishment of the tail fold, the primitive streak must extend in the middle line from the posterior extremity of the medullary canal, round the end of the embryo, as far forwards as the point of connection of the allantoid stalk, with head cut; and therefore the proctodœum, when it arises, must not pass through the primitive streak.

Therefore, if we adopt the view of Balfour, that the primitive streak represents the position of the blastopore of other gastrulae, we shall be forced to conclude that, at any rate in this group of Craniata, the anus is in the position of a part of

² "On the Early Development of the Lacertilia," &c., this Journal xix.
⁴ Loc. cit.
the blastopore—a supposition which simplifies our ideas as to the origin of the vertebrate anus in general.

**Four Transverse Sections through the Hinder End of a Young Embryo of Lacerta Muralis (Balfour).**


The development of the kidney has been described by Braun. My observations lead me, however, to believe that his account of the mode of origin of the segmental tubules and of the Wolffian duct is erroneous.

According to him, the first part of the urino-genital system which appears is the Wolffian duct. He says: "In an embryo of Lacerta agilis, barely 5 mm. long, in sections just below the heart, I find the Wolffian duct lying close to the lateral mesoblast plates, in a region belonging neither to these nor to the protovertebræ, but lying between the two as a semicircular mass of cells, sharply defined towards the ectoderm, but passing gradually into the lateral mesoblast; in the middle of this cell mass is a lumen . . . ." which he considers to be the lumen of the Wolffian duct.

In the next stage described by Braun, a number of segmentally arranged vesicles are present, which are for a short time attached to the peritoneal epithelium, their cavities also opening for a short time into the body cavity, but which afterwards break away, form the well-known S-shaped tubes, and communicate with the Wolffian duct.

From this account it is evident that Braun has not investigated embryos less than 5 mm. long. I have been fortunate enough to obtain younger embryos, and have been led to somewhat different conclusions.

On the formation of the protovertebræ, each protovertebra does not at once become completely separated from the lateral mesoblast, but remains connected at a certain point with a continuous solid ridge of tissue, generally in early stages about two cells thick, which projects inwards from the peritoneal epithelium, thus forming an "intermediate cell mass" comparable with the structure so called in birds.

Figs. 15 and 16 show the characters of this ridge in an embryo of about seven protovertebræ; fig. 15 is taken from a vertebral region, and shows the ridge (i. c. m.) connecting the protovertebra with the peritoneal epithelium; fig. 16 is from the next intervertebral region, showing the ridge projecting freely inwards from the peritoneum. In fig. 16 traces of a prolongation of the body cavity into the intermediate cell mass may be observed. In an embryo with ten protovertebræ this cell mass, without losing its connection with the protovertebræ, swells up and becomes semicircular in
section, the convexity being directed outwards; this condition is shown for vertebral regions in fig. 17, for intervertebral in fig. 18.

At a stage with eleven protovertebrae, the vertebral portions of the intermediate cell mass, behind the fourth protovertebra, acquire a circular lumen, which is bounded by a single layer of columnar cells; this condition is seen in fig. 19. In fig. 20, which represents a section passing through the end of the same protovertebra as that from which fig. 19 is taken, the lumen is smaller; in the intervertebral region behind the lumen altogether vanishes, and the solid, swollen cell mass presents an appearance exactly like that seen in the preceding stage (fig. 18).

There is thus formed a series of cavities in the continuous intermediate cell mass, each situated opposite a protovertebra, and having its walls continuous both with the protovertebra and with the peritoneal epithelium. These cavities are separated from one another by the solid intervertebral parts of the intermediate cell mass.

In embryos with eleven protovertebrae there are five of these vesicles, opposite the fifth to the tenth protovertebra, the last two somites being as yet without them. In these last somites the intermediate cell mass is swollen and solid, as in the anterior region of an earlier embryo.

These cavities are, as will be seen from their subsequent history, the segmental vesicles described by Rathke and subsequent writers.

They have hitherto been described entirely separate from one another, and have been supposed (Braun., loc. cit) to arise as invaginations of the peritoneal epithelium.

When twelve protovertebrae are present the Wolffian duct begins to appear as a solid cord of cells, splitting off in the intervertebral region only from the intermediate cell mass, and passing, in the region of each protovertebra, into the wall of a segmental vesicle.

Figs. 21—23 represent three sections through about the sixth and seventh somites of an embryo with twelve proto-
vertebrae. Fig. 21, the most anterior, passes through a vertebral region, and shows the segmental vesicle, with its lumen; the section passes through the attachment to the peritoneum (which in the vertebral regions is becoming smaller), but not through the connection with the protovertebra. The next section (fig. 22), through the commencement of the intervertebral region, shows the solid cell mass, with a few cells (w. d.) split off from its outer portion. These cells are the rudiment of the Wolffian duct. In the next protovertebral region this cord ceases to be visible. Fig. 23 shows a section through the commencement of the next protovertebra, passing through the solid wall of the corresponding vesicle, which has no trace of the duct.

These cords of cells are present at this stage in four intervertebral areas, behind protovertebrae five to eight inclusive.

With the formation of the thirteenth protovertebra the solid rudiment of the Wolffian duct becomes more distinctly split off in the intervertebral regions, while opposite the protovertebrae it appears as a solid appendage of the wall of the segmental vesicles, with which it is perfectly continuous.

At the same time it extends backwards into the ninth intervertebral region.

In an embryo with fourteen protovertebrae there are eight segmental vesicles with a lumen opposite the protovertebrae five to twelve inclusive. All these have the Wolffian duct as a solid knob on their outer wall, while in the corresponding intervertebral regions there appears a distinct lumen in the duct, which is more or less completely split off from the rest of the intermediate cell mass.

The relations of the duct and vesicle in an embryo with fourteen somites are shown in fig. 24, from the second segmental vesicle of such an embryo. In this figure the segmental vesicle (s. v.) is seen to have a large lumen, and the solid Wolffian duct (w. d.) appears attached to its outer wall.

In fig. 25, from the next intervertebral region behind fig. 24, the Wolffian duct has a large lumen, and is attached to the solid intervertebral cell mass.
A section through the next protovertebra would repeat the features shown in fig. 24.

On the appearance of the fifteenth protovertebra the lumen of the Wolffian duct becomes continuous throughout the region of the first eight segments, and at the same time it acquires a communication with the cavity of each segmental vesicle in its course.

The first eight segmental tubules are therefore differentiated, continuously with the Wolffian duct, from a ridge of cells, continuous at first along its entire length with the peritoneal epithelium, and at certain points with the adjacent protovertebrae.

With regard to the tubules behind the eighth, they are developed from the intermediate cell mass in exactly the same way as those in front; but the Wolffian duct, instead of arising continuously with them, grows backwards as a free projection of the above-described portion, without coming into relation with adjacent structures. It is at first solid, but afterwards acquires a lumen, and becomes connected with the segmental vesicles in order from before backwards.

On the subsequent behaviour of the tubules and on the development of the metanephros I have no observations.

The most interesting feature in the preceding account of the early development of the lacertilian kidney is the close resemblance which it shows to exist between the process of development in that group and the process which has been shown by Sedgwick to exist in birds and Elasmobranchs. In both these groups Sedgwick has shown that the segmental tubules arise from a continuous cell mass connected with the peritoneal epithelium and with the mesoblastic somites, which cell mass is present from the very beginning of the process of mesoblastic segmentation.

In the anterior part of the Wolffian body of the chick

Sedgwick has shown that the Wolffian duct and segmental tubules arise continuously by differentiation of the cell mass. In the chick, as in the lizard, the independent origin of duct and tubules in the posterior region is probably a secondary character.

In conclusion, I wish to express my gratitude to the authorities of the Zoological Station at Naples for their kindness to me during my visit, and to Mr. Sedgwick for the advice and assistance which he has given me since my return to Cambridge.