On the LAMINAR TISSUE *of Amphioxus.* By Professor POUCHET. (With Plate XXIX.)

THE chief results in this paper were communicated to the Société de Biologie at their meeting on May 1st, 1880.[1]

I. I was lately fortunate in obtaining large numbers of Amphioxus at Concarneau, and I used them in endeavouring to clear up a special point in their histology, which seems to have been only very imperfectly touched upon by the numerous authors who have studied this animal. I intend to speak of the system of canals and cavities, which from a point of view, which is perfectly legitimate, are comparable to fin rays. Both of these systems are differentiations of what may be termed "laminar tissue."

In 1849 M. de Quatrefages applied the term "singular" to the laminar tissue of Amphioxus. Since that time the system of spaces and canals which have been observed have not been the object of special study. The fact has been recognised that they do not communicate with the blood capillaries, but their histological structure has remained obscure. M. Reichert is the only author who has touched upon it, and he seems to have guessed at, rather than proved, their analogy with the constant elements of the laminar tissue. He expresses himself thus, "Da das bindegewebige Stroma uns als pellucide Grundsubstanz angesehen werden Kann und die dazu gehörigen Bindesubstanzkörper fehlen, so wäre es möglich dass das in Rede stehende netzförmige Gebilde den zellenhalftigen Theil des bindegewebigen Stromas darstellt, unter dessen Vermittelung die in grosser Menge verbreitete, ganz hyaline Grundsubstanz gebildet werde."[2]

In 1873 Stieda[3] contents himself with quoting these words of Reichert, which in fact contain the true state of the case.

Before Reichert's paper was published, Owsjannikow[4] described, in 1868, in the region of the tactile corpuscles, that is to say, round the mouth, connective-tissue cells, "gross, länglich, zuweilen sternförmig," and compared them to the cells of the cornea, an error of observation as will be seen further on.

[1] 'See 'Gazette Médicale' for May 22, 1880.

[2] Reichert, 'Arch. f. Anat.,' 1870.

[3] "Studien über den Amphioxus lanceolatus," 'Mém. de l'Académie de St. Petersberg,' viie Série, Tome xix, No. 7, 1873.

[4] "Ueber das centrale Nervensystem des Amphioxus lanceolatus," in Bullet. de l'Acad. de St. Petersbourg,' T. xii, 1868, p. 299.

P. Langerhans, in 1873, mentions[1] the same cells; he describes them as very much branched (reich verästelt), and compares them, as does Owsjannikow, to the cells of the cornea, and corroborates the description of Stieda, although this is limited to the quotation from Reichert, which rather goes against the existence of such cells.

Quite recently Ant. Schneider[1] admits the truth of Reichert's ideas, and at the same time describes (pp. 1 and 28) and figures in the laminar tissue of Amphioxus stellate fibro-plastic cells, inserted by their prolongations on the walls of the true capillaries around the mouth.

These quotations suffice to show the confusion and incompleteness of our knowledge of the laminar tissue of Amphioxus, and of the formed elements which it presents. As we shall presently show, even if it is possible to find isolated cells having the ordinary appearance of stellate fibro-plastic cells, it is quite exceptional at least in the adult. In our preparations we have found one or two in the thinnest region of the caudal lophioderm, quite at the extremity; with this exception we have not come across them. It is in fact a very decided character of the laminar tissue of Amphioxus that the formed elements analogous to the fibro-plastic cells never appear in an isolated state, and that they are always united again into scattered groups or in longer or shorter rows which are more or less regular, and anastomose to a greater or less extent, forming cords which are sometimes solid and sometimes hollow for a certain distance, or even form large spaces.

It is these cords, which are partly solid, partly hollowed, and generally slightly elongated, which have been described as "systems of canals." The supposed branched cells of Owsjannikow are only the enlargements of this very irregular network in the neighbourhood of the mouth.

II. As these cordons and spaces formed by the coalescence of the cells of the connective tissue are situated to a large extent in the layer of laminar tissue which surrounds the body of the animal, we shall first of all describe this,[3] adding, however, but little to the descriptions of Stieda.

Below the epidermis you get, passing from without inwards—

[1] "Zur Anatomie des Amphioxus lanceolatus," in 'Arch. f. Mik. Anat.,' 1873, p. 301.

[2] 'Beiträge zur vergleichenden Anatomie,' 4to, Berlin, 1879.

[3] Compare with this the structure of the skin in fishes. See Pouchet, "Du développement des poissons osseux," 'Journal de l'Anatomie,' May-June, 1879, pp. 288-289.

1. The dermis. 2. The subdermic layer. 3. The subcutaneous aponeurosis.

1. *The dermis*[1] is formed, as in fishes and Amphibia, of a thin layer which appears in section to be homogeneous, and which in Amphioxus presents upon its external surface a double rectangular striation, with the *crosses* corresponding to the nerve terminations which Langerhans[2] has so well figured.

2. *Subdermic layer.*—The subdermic layer[3] is characterised in Amphioxus by the presence of a large amount of structureless substance. This latter is as usual absolutely hyaline, and keeps this character even after the action of osmic acid in saturated solution.[4] It is traversed by fibres having the character of laminar fibres, stretching from the deep face of the dermis to the subcutaneous (cf. fig. 3A). These fibres are absolutely devoid of any nucleus and of any division into cells, a fact not without interest as bearing upon the much debated history of the genesis of the laminar fibres. We have pointed out in the following terms[5] the existence of similar fibres in the lophioderm of larval Axolotls measuring 60 to 80 mm. in length: "In sections cut 'à la planchette,' after treatment with osmic acid, the amorphous matter is seen to be traversed by vessels and nerves, and to enclose some fibro-plastic cells with regular spaces between them, invisible; but extremely fine laminar fibres are also seen. These run from the skin of one side to that of the other, in the same manner as the strings in an instrument in a very beautiful manner, all parallel to one another. These fibres seem, therefore, to have no relation to the fibro-plastic cells."

It was always difficult in Axolotl to pronounce an opinion on the independence of the fibres and cells on account of the presence of the latter. Whilst in Amphioxus there are no fibro-plastic cells furnished with any prolongations from which laminar fibres might be derived. These are also larger than in Axolotl, and are more deeply stained by carmine. No migratory corpuscles are found in the hyaline structureless portion of the subdermic tissue of Amphioxus.

3. *Subcutaneous aponeurosis.*—This membrane appears in

[1] "Cutis oder Lederhaut," Stieda.

[2] Loc. cit., fig. 13.

[3] "Das Unterhautgewebe" of Stieda.

[4] For the action of this reagent see Pouchet, "De l'emploi des solutions concentrées d'acide osmique," 'Journal de l'Anat.,' Sept.-Oct., 1876.

[5] G. Pouchet et Tourneux, 'Précis d'histologie humaine et d'histogenie,' 1878, p. 102.

section to be finely striated as in fishes. A lamellar structure must be attributed to it, and elongated nuclei appear in certain places. It belongs to a system of fibrous tissue widely distributed in the body of the animal; this is quite distinct from the laminar system properly so called, while in its physico-chemical characters it approaches the dermis. To this system belong the aponeuroses which separate the muscles and envelope the medulla, &c. The skin, the subdermic aponeurosis, and the fibrous partitions of which we have been speaking, when treated with osmic acid and picrocarmine, take a splendid red staining, while the structureless substance of the subdermic layer and other varieties of structureless substance which are sometimes found in the body of Amphioxus, remain uncoloured or become slightly yellow, but do not take the carmine.

III. We have said that the special character of the formed elements of the laminar tissue of Amphioxus was that they are never present in an isolated state nor as cells simply united, like the cells in the cornea of Amphibia, by fine prolongations. We shall first describe these elements and the network which they form in the "lophioderm"[1] of the caudal fin of Amphioxus, and afterwards their modifications in other parts of the body of the animal.

The caudal "lophioderm" presents a network with regular elongated meshes, which had already attracted the attention of M. de Quatrefages, and which at once calls to mind the figure which he gave at a time when the technical methods necessary for such a study were not yet in vogue.

This network is arranged in one plane in the centre of the lophioderme, which is very thin in this region. The meshes of the network are elongated in the neighbourhood of the anus, and measure 60 μ to 80 μ in length, by 10 μ in breadth (see fig. 1). The substance of the network is formed of rods, which measure generally 8 to 10 μ; they may, however, be narrower or broader than this. They are, in the region of which we are speaking, flattened out; they are solid. At the intersections of the network there are generally 6 to 10 larger or smaller nuclei. These are oval or almost spherical, irregularly placed, and separated by a distance which is generally less than their diameter. Sometimes several are placed in a row in one of the rods. They are then generally more or less obliquely placed. The rods after the action of osmic acid stain slightly with picrocar-

[1] *Lophioderm* is the termed used by M. Pouchet for the dermal fold which forms the substance of the median fin in Amphioxus and in Amphibia.—Ed.

mine. They represent the bodies of the cells united one with another, or rather, all fused together.[1]

The only tissue which approaches in histological structure that which we are about to describe, is the tissue of the cornea in the eyes of bony fish. We have elsewhere described this tissue thus:—"All the corneal cells appear fused together, so that it is impossible to distinguish their outline, the protoplasmic bodies of the cells forming a sort of membrane, with nuclei scattered here and there. The meshes of this network are small and separated by extremely broad rods, which forms a true lamellar system mixed up with the fundamental lamellar system of the organ, &c."[2]

In the lophioderm of Amphioxus the meshes of the network are broader than the rods, and these latter are disposed in the same plane; but the two tissues, nevertheless, present an analogy which enables one to place them side by side among the large number of varieties which the laminar tissue of vertebrates presents for our study.

Fig. 6 shows that in posterior region of the notochord the nuclei are very abundant; they are very small, and answer very well to the description and figure given by Stieda. On the other hand, they are scattered throughout the substance of this organ, which does not agree with the opinion of Kossman (see 'Verhandlungen der Phys. Med. Ges.,' Wurzburg, 1874).

In the deep layer under the dermis in the neighbourhood of the mouth, the network of cells of the laminar tissue has not the same appearance. It presents large irregular meshes, with a slightly sinuous contour. The nuclei are no longer accumulated at the junctions of the network. The rods are almost cylindrical, and are swollen here and there.[3] These swellings present a larger or smaller hollow in the centre, bounded on all sides by the protoplasm and the nuclei, which are arranged in a single row. In optical section these hollows appear to be simply fissures between the layers of cellular substance.

Sometimes these enlarge and spread to a greater or less extent, but they always remain very irregular. This is the

[1] It sometimes happens in certain preparations that at the intersections of the network there is a sort of cleavage, so that the nuclei appear to be each surrounded by a protoplasmic body with a polygonal outline.

[2] G. Pouchet et Tourneux, loc. cit., p. 611, et fig. 168.

[3] These swellings, scattered on the thinnest rods of the network, have no doubt given rise to the error of Owsjannikow, who thought he had seen the cellular bodies anastomising by their prolongations.

"system of canals"; it is very different from a capillary system properly so called, and its true nature has been very variously interpreted by different authors. In the neighbourhood of the notochord these cavities appear to enlarge and spread out. Fig. 3 B shows one of these cavities, pyriform in shape, the extremity being narrowed and running into a rod, which binds it to the network. Thus, this sort of hollow swellings forms a transition to other cavities which are quite separate from the network of cells, and which we are about to describe.

IV. The upper surface of the anterior extremity of the notochord presents isolated groups of connective-tissue cells, which completely envelope the cavities which they bound, being arranged in a single row, as in the swellings of the network in the neighbourhood of the mouth.

These groups of cells, always clearly bounded on the outside, are generally oval in form, or rather, polyhedral, where they are crowded together. The nuclei presents slight prominences in their interior, and, in certain individuals the cells contain various pigment granules of a reddish colour.[1]

The interior of the cavity is filled with a liquid, which is unacted on by osmic acid, and does not stain with most reagents. It appears to be watery. The further we recede from the extremity of the cord the larger do these laminar cavities become (see fig. 2). They become also slightly modified, and finally constitute the organs described under the very bad name "fin rays" (see figs. 4 and 5). The structure of these organs is extremely simple. They are cellular spaces, larger than the others, almost cubical in form, one wall of which, that which looks towards the notochord, is invaginated into the interior of the cavity itself, so as to fill up two thirds or three fourths of it. We call the invaginated portion the *papilla*. It is made of a structureless substance, presenting special characters which clearly distinguish it from that of the sub-dermic layer.

Stieda describes these parts very incompletely. He states, it is true, the analogy between these cavities and the system of canals, and describes their cellular covering, but the invaginated portion which Müller had already mentioned he has overlooked. Müller states that in the interior of these cavities there exists "eine durchsichtige Flüssigkeit und eine consistentere, aber weiche Masse." The cavities are bounded at the sides and below by the subcutaneous aponeurosis,

[1] Some specimens of Amphioxus present a reddish tint, others a greenish.

and are separated from one another by transverse partitions (see fig. 5), which are very thin.[1] The whole cavity, the papilla as well as the walls, is lined with a layer of cells, which closely resemble those lining the other spaces; there is no appreciable difference between the cells on the papilla and those on the walls. The contained liquid seems to be the same as that in the other spaces.

In the living animal the substance of the papilla can only be distinguished from the ambient liquid by the optical effects due to their differing density. In a specimen which has been preserved in alcohol the papilla had almost disappeared. Certain reagents have the same action, whilst others appear to swell them up. If the specimens are put into alcohol, even after the action of osmic acid, the papilla are altered in shape and drawn in. Their surface presents depressions, which gives them a jagged appearance in section.[2]

The use of reagents brings to view minute cavities inside the papilla, more or less irregular, and about the size of one of the nuclei. Suitable methods show that the amorphic substance of the papillæ contains nothing but some very fine and scattered fibres. Transverse sections (fig. 4) show very well the relations of the papillæ. There is a fibrous lamina occupying the middle line of the body, which, bifurcating at the level of the upper edge of the two lateral muscular masses, joins on each side the subcutaneous aponeurosis. The base of the papilla rests in the groove formed by this bifurcation, and is clearly marked out by its physico-chemical characters. The cavity which it contains, is itself bounded laterally and below by the subcutaneous aponeurosis.

The papilla is traversed towards its base by a layer of very fine fibres (fig. 4, *c*.) stretched between the two fibrous laminæ. In front and behind (fig. 5, *c*.) this sheet of fibres becomes raised up before being inserted into the partitions which separate the cavities. On the upper face of the layer a very loose and irregular network of extremely fine laminar fibres rise up and spread out in the centre of the papilla. None of these are directed downwards. When treated with osmic acid and then saturated with picro-carmine, the substance of the papilla assumes a slightly greenish tint, and moreover becomes slightly granular, and,

[1] Ant. Schneider, who believes that these cavities communicate with the system of canals, describes the latter as bounded by a membrane, with nuclei and finely striated fibres, and calls these fibres "muscular (?)," loc. cit., pp. 8, 9.

[2] This it is which Ant. Schneider (loc. cit., p. 8) has described as stalked, and supposes them to be contractile!

therefore, to a certain extent opaque. This character belongs to it alone; under the influence of the same reagents, the amorphous matter in other parts of the body of Amphioxus remains hyaline, and the layers of fibres become stained bright red.[1]

In certain cases (fig. 5) a very clear and definite stratification of the amorphous matter may be seen in the papilla. The peripheral portion and the base are clearer and less opaque than the central portion, and the boundary between these different portions is clearly marked out. The central portion, which is also the darkest, rests on the layer of transverse fibres, and is raised up against the partitions of the cavities to where the endothelium is reflected.

In the centre of the papilla this dark zone presents a large projection, which itself may be surmounted by an intermediate zone.

V.—What histological and morphological signification can be attributed to the papilla and to the containing cavity?

The papilla evidently belongs to the category of connective or laminar organs.

The amorphous substances are everywhere very abundant in Amphioxus. In transverse sections (fig. 4) two thick layers (*d*, *e*) of greenish amorphous substance, hyaline and devoid of laminar fibres, may be distinguished on each side of the median aponeurosis. Elsewhere the amorphous substance is very dark (*f*, *g*), and contains thick fibres,[2] and although no part is so opaque as the papilla, it is evident that it is a simple variety of connective-tissue, characterised by the absence of cellular elements, the great scarcity of fibres, and lastly, by the probable presence of a certain quantity of fatty matter in a finely divided state.

The meaning of the cavity which surrounds the papilla is also evident. It is a space which is more developed than the rest, and which tends to take on the character of a serous sac. Silver nitrate gives the same result as on serous membranes; in the only preparation we have made, the network of silver staining is very clear upon the lateral walls of the cavities, but does not show upon the papilla, but no doubt could be obtained there also by employing suitable methods.

[1] After the more prolonged action of osmic acid and alcohol the papilla becomes darkly coloured, and certain coloured oily globules appeared to be emitted by it. This seems to indicate the presence of fatty bodies in its composition.

[2] Comp. 'Aut. Schneider, loc. cit., p. 7.

When one observes the gradations of structure between these cavities, the small separate cellular cavities placed at the extremity of the cord, the terminal cavities of certain rods, and, lastly, the rudimentary cavities in the swellings of the network in the neighbourhood of the mouth, one is led to regard these various structures as the first indications of a system of serous cavities and lymphatics. Besides this, the cellular lining of these cavities has quite the characters of the lining of the embryonic serous sacs in the higher Vertebrata. We confine ourselves to merely suggesting this as a probable analogy, as we have no exact account of the histogenic development of these parts.

Lastly, what morphological signification shall we give to the organ represented by the papilla and its cavity?[1]

Here all terms of comparison leave us completely at fault; no homology whatever among vertebrates is to be thought of.[2] Their number corresponds neither with that of the spinal nerves, nor with that of the myotomes; they are about three or four times as numerous.

The connection which certain authors put forward[1] between the papilli and the fin-rays ought rather to be considered as an *analogy* than a true *homology*. Amphioxus is not altogether devoid of either cartilage or bone. It is cartilage which forms the skeleton of the oral tentacles; it is bony substance which forms the framework of the branchial skeleton. The papillæ, looked at from the point of view of general anatomy, would form a third kind of skeleton element. They would represent a first stage in the differentiation of amorphous matter which is about to form a special organ.

It should not be forgotten that amongst Teleostean fishes a certain number of bony organs of the dermal skeleton result simply from a similar differentiation of amorphous intercellular substance, such as we have shown in the bony plates of Syngnathians. In the latter case, the amorphous matter becomes individualised as calcareous salts are deposited in it; in the papilli of Amphioxus fatty matters are no doubt deposited in it. In any case, both belong directly to a numerous and varied group of amorphous intercellular substances.

[1] We may here note a peculiarity which presents itself in the sheath of the notochord of Amphioxus. In the most successful transverse sections this presents above a slight median prominence (cf. Ant. Schneider, loc. cit., pl. xiv, fig. 1), and below two slight prominences on each side of the median line, which no doubt might be considered as the first rudiments of the vertebral skeleton.

[2] Anton Schneider, loc. cit., p. 8.

We point out this analogy here without giving to it more weight than seems justifiable, in the absence of special researches. We may add that, from whatever point of view we look at the subject, either that of comparative anatomy or that of general anatomy, a considerable difference still remains between the papilla of Amphioxus enveloped in its serous cavity and the dermal bony productions of fishes. Up to the present day, at any rate, intermediate conditions are wanting which would show how such a morphological evolution could have taken place as is taken for granted in bringing these organs together, from a more or less pronounced phylogenetic point of view, under a common denomination. All just ground for establishing a real homology between them is wanting. Although the papillæ partake, along with true fin-rays, of the character of skeletal organs, they clearly possess a special nature, without homologue, in other vertebrates.

Muséum de Paris, 1 June, 1880.