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On the Mesodermic Origin and the Fate of the So-called Mesectoderm in Petromyzon.

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Introductory.

About 20 years ago v. Kupffer (85) described in the embryos of *Petromyzon* an epithelial structure extending, between the ectoderm and the somatic plate of the mesoderm, from the head to the posterior boundary of the branchial region, and described it under the name of the neurodermis; subsequently, he bestowed on it the name branchiodermis. Seventeen years later the same structure was again discovered by Koltzoff (02), who identified it with the mesectoderm which was described by Miss Platt (94) in *Necturus* embryos. Subsequently, so far as *Petromyzon* is concerned, nothing was published until last year, when a paper by Schalk (13) appeared, although the corresponding layer of cells was described by A. Dohrn (02) in Selachii and by Brauer (04) in Gymnophiona.

For a long time the origin and fate of the layer in question engaged my attention. Last summer I was able to re-examine my sections and to confirm observations which I had previously published in a paper entitled "Die Bildungsweise und erste Differenzierung des Mesoderms beim Neunauge (*Lampetra mitsukurii*, Hatta)," in which the origin and differentiation of the so-called mesectoderm are described and illustrated by a series of microphotographs. To my regret the paper, which was ready for press when the great war broke out, could not be sent to the editor of a certain scientific journal in Belgium, who had promised to publish it in his journal. The present note is an attempt to communicate some of the principal points of that paper which relate to the mesectoderm. The other organs dealt with in the above-mentioned paper have already been described in preliminary notes or in my previous papers.

1. *Origin of the Mesectoderm.*

The previous authors who deal with mesectoderm invariably assume its ectodermic origin. The layer was so named by Platt, because, in spite of its supposed ectodermic derivation, it takes on in its further differentiation features like a mesodermic structure. About the mode in which the layer arises from its assumed mother-layer, positive evidence has not as yet been given by any of the authors, except Schalk, who endeavours to make intelligible, by means of figures and descriptions, the precise mode in which it originates.

According to v. Kupffer and Koltzoff, the mesectoderm is formed of cells liberated partly from the medullary cord, but in the main from the ectoderm forming the lateral walls of the head and of the branchial section of the body. The cells of the branchial region are regarded by them as being proliferated from the ganglionic placodes. These cells, whether medullary or ectodermic in origin, are classed together by the authors and designated as *ectodermal*, because the cells from the two sources become so much intermingled as to be indistinguishable, when once they have left their mother-layers.

But the cells of the mesectoderm become differentiated, as their later history shows, in two directions: viz. into the cephalic nerves with their internal ganglia on the one hand, and into the tissues which give rise to the cartilaginous branchial basket and the connective tissue on the other. For this reason probably the mesectoderm has been designated either as neurodermis or as branchiodermis, until Koltzoff (02) identified it with the corresponding structure found by Platt in *Necturus lateralis*. Neither v. Kupffer nor Koltzoff were able to distinguish in the mesectoderm the nerve cells from the other elements, but each states that both the nerves and connective tissue are derived from one and the same source.

Koltzoff distinguishes in the mesectoderm the dorsal division, which is found above and within the cephalic ganglia, and the ventral division, which extends below them towards the ventral surface. This attempt amounts to nothing and is founded only on histological distinction; the dorsal division is a network of the strings caused by the connection of the cells with one another by their protoplasmic processes, while the ventral division of the layer consists of a typical epithelium of columnar cells. But this histological difference is a temporary one, and does not indicate, as Koltzoff believes, a differentiation of the nerve cells from the other elements of the mesectoderm.

The principal point in which the results by Schalk differ from those of the authors above mentioned consists in the origin of the mesectodermal cells, which, according to him, is confined absolutely to the ectoderm; he denies positively that any of these cells have a medullary origin. He says, however, nothing definite about the nerve cells or the mesoderm somites, except that the sclerotomes give rise to the trabeculæ and parachordals.

If I understand Schalk correctly, there are two phases in the liberation of the mesectodermal cells from the ectoderm. In embryos about ten days old selected from his material, the heads of which have just begun to be raised above the yolk, more or less conspicuous groups of cells are proliferated from the ectoderm, at about the level of the chorda and uninterruptedly from the eye backwards along the whole extent of the branchial tract of the enteric canal, and these cells push their way ventrally between the ectoderm and mesoderm. The cells of this first phase are, the author believes, identical with those of the branchiodermis of Kupffer.

In quite young embryos the production of these cells goes on throughout one continuous streak, but in a little more advanced stage it is concentrated in certain centres, which Schalk believes to have been detected by him and which resemble the nerve placodes of Kupffer. This concentration indicates the beginning of the second phase of cell production.

In the second phase of the formation of mesectoderm which Schalk describes, each centre of cell production is found close behind each visceral pouch, except the hyomandibular, which is destitute of such a centre. The statements are illustrated by his text-figs. 16 and 17. The centres are produced by local thickenings of the ectoderm, which appear from before backwards one after another and proliferate the cells in a continuous layer, which becomes pushed backwards so as to be mixed with those of the branchiodermis, so that the cells of both lots can no longer be distinguished one from the other. But he says nothing definite as to whether the branchiodermis, or the cells directly descended from the placodes, represent the formative elements for the branchial cartilage bars. He says merely quite indefinitely: "Wenn nun auch jene kleinen Plakoden möglicherweise an der Bildung der Branchialnerven Anteil haben, so glaube ich doch behaupten zu können, dass ein Teil der aus jenen Epidermisplakoden auswandernden Zellen bei der Bildung der Kiemenknorpel verwandt werden" (14, p. 55).

Schalk is correct in so far as he excludes the medullary cells from the origin of the mesectoderm; in other respects the results given by him do not add anything to what everybody had assumed.

I have observed all the occurrences which Schalk gives in his figures and found that they have nothing to do with the mesectoderm.

How and when the cell proliferation of the placodes in the branchial region is closed, we cannot learn from the statements given by him at all. But his text-fig. 18 shows a great resemblance to a section from a series of frontal sections through an embryo, 12 to 13 days old, in my possession, which is just hatched or is about to break the chorion. At such a stage as this the mesectoderm is, of course, already fully established and has commenced even to be differentiated, as is clearly seen in the figure referred to; the continuous epithelium is divided by the outgrowth of the visceral pouches into branchiomeres, and the proximal part of each branchiomeric piece is swollen up (Schalk's text-fig. 18, p. 6), giving rise to the cartilaginous visceral bar.* The ectoderm lying in close contact with the thickened part of the mesectodermal epithelium, which is shown in the figure by Schalk as continuous with the ectoderm, is a thickening produced by active multiplication of the component cells of the ectoderm (text-fig. 1, A).

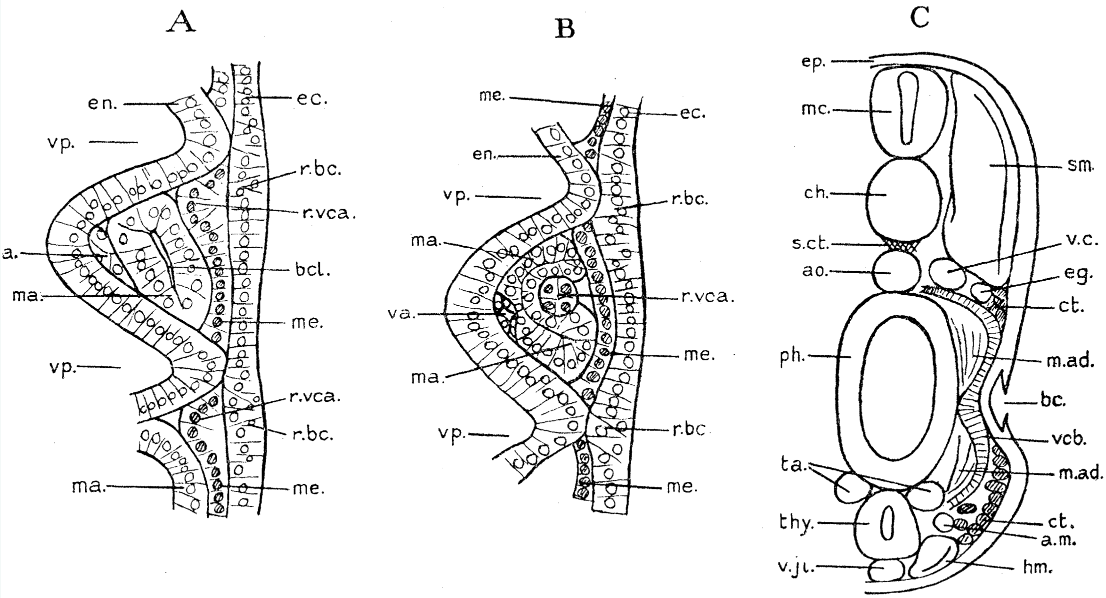
This ectodermal thickening assumes an oval outline with its long axis vertical and grows inwards (text-fig. 1, B), pressing against the rudiment of the cartilaginous visceral bar. But the conical bottom of the entodermal visceral pouch in front pushes its way laterally and backwards, and presses upon the invaginating ectodermic pouch, finally fusing with it. On the 14th day this spot becomes perforated and forms an oval slit with its long axis vertical, and the gill slit is thus established (text-fig. 1, C). The rudiment of the cartilaginous visceral bar is found close behind this orifice. Now, the thickening of the ectoderm which Schalk saw was the developing gill-cleft and had no genetical relation to the cartilaginous visceral bar.

A thick section might induce one to assume continuity of the thickened ectoderm and the likewise thickened mesectoderm; but, in reality, both the parts are separated by a sharply defined boundary line, as careful observations of thin sections prove without difficulty.

In spite of his efforts, Schalk could not detect, as he says, a corresponding placode in the hyoid segment. Here, in fact, no ectodermal thickening for the gill-cleft takes place at all, because the hyomandibular pouch in front of this visceral area does not break out to the exterior, but is transformed into the velar cavity; it has no direct respiratory function, but performs an auxiliary service to it.

I may be permitted to add a few words on the placodes of nervous nature in the branchial region, in order to avoid possible confusion of them with the ectodermal thickening for the gill-cleft above stated. V. Kupffer (94) was the first who described the ganglionic placodes, termed by him the epibranchial placodes. The placodes of the epibranchial ganglia are situated at the level of the dorsal edge of the lateral plates, consequently at a much higher level than that at which the ectoderm thickens for the gill-clefts, and the placode appears in each branchiomere from the vagus segment, which represents the fourth branchiomere, counting from the premandibular arch to the hindmost branchiomere behind the last or eighth visceral pouch. These placodes are, of course, purely nervous and have nothing to do with the mesectoderm; they represent the posterior section of what I have called

* The cartilaginous visceral bar in *Petromyzon* is not to be confounded with the true visceral arch formed in higher craniota.



xt-fig. 1.—A and B show frontal sections, of which B shows a little further advanced stage. C represents a transverse section through a postotic visceral arch of a much advanced larva. The nuclei of the mesectoderm cells and the structures derived from the mesectoderm are shaded. *a.m.*, muscular artery ; *ao.*, aorta ; *bc.*, branchial cleft ; *bcl.*, branchiocœle ; *ch.*, chorda ; *ct.*, connective tissue ; *ec.*, ectoderm ; *eg.*, epibranchial ganglion ; *en.*, entoderm ; *ep.*, epidermis ; *hm.*, hypoglossal muscle ; *ma.*, mesodermal visceral arch ; *m.ad.*, adductor muscle ; *mc.*, medullary canal ; *me.*, mesectoderm ; *ph.*, pharynx ; *r.bc.*, rudiment of branchial cleft ; *r.va.*, rudiment of vascular arch ; *r.vca.*, rudiment of visceral cartilaginous arch ; *s.ct.*, subchordal connective tissue ; *sm.*, scleromyotome ; *ta.*, truncus arteriosus ; *thy.*, thyroid gland ; *va.*, visceral vascular arch ; *v.c.*, cardinal vein ; *vcb.*, visceral cartilaginous bar ; *v.ji.*, vena jugularis impar ; *vp.*, visceral pouch.

in my paper above referred to (14*b*) the ventral series of cephalic ganglia. The epibranchial placodes are not only cut off from their mother-layer, but have been already transformed into the definitive nervous system of the branchial apparatus, when the ectoderm commences to be thickened for the gill-clefts.

About the fate of the foremost placode, which is situated, as seen in text-fig. 14 by Schalk, immediately behind the optic cup, the author gives no definite account. Judging from the position in which it is found, and from what he says about it, the placode must be taken to be the rudiment of the lens which belongs to the trigeminal region. Here the circumstances are not so simple as shown in the figure. The ophthalmic and trigeminal placodes and the placode for the lens have coalesced at their bases and are distinguishable from one another only by the divergence of their distal parts, and they embrace between them the second mesodermic somite and a part of its mandibular fold, being closely apposed to one another. The placodes are purely nervous in nature and have no genetic relation to the mesectoderm at all, although they are at certain stages in close contact with the latter. In the course of the seventh day, therefore, before the first appearance of the epibranchial placodes, the placode for the trigeminal group is constricted off from the ectoderm.

Finally, the origin of the branchiodermic cells in their first stage, of which Schalk speaks, seems to be unintelligible. Judged from his text-fig. 12 and the accompanying statements, the cells are brought into their position from the ectoderm not by cell-multiplication going on in this germinal layer but simply by liberation of some of those composing the layer. If such a case as given by Schalk really occurs, it might be looked upon as a case of delamination. But the occurrence of delamination, even for the formation of the ventral parts of the mesoderm, as W. Scott (82) and, later, Mollier (06) assume, or for the origin of the pericardium and endocardium, as asserted by Shipley (87), has been disproved, and, according to my experience, occurs in no case at least in the development of *Petromyzon*. In the series of sections in my possession I find no similar case to that of Schalk, except some sections frontally cut through the lower margin of the well-established mesectoderm.

According to the results obtained by myself the so-called mesectoderm is not so peculiar a structure as it appeared to the previous observers, but it is the mesoderm itself, a part of the somites or, as we may term them, the scleromyotomes. It is represented at its first appearance, as observed in the mandibular arch, by scattered free cells, which later coalesce for the most part, to form a typical epithelium. In the postotic region the mesectoderm is, on the contrary, from the first epithelial.

In early stages there are found only two kinds of free cells: the blood vascular cells and the mesectoderm cells. The cells of both kinds appear almost at the same stage; at about the fifth day from the fertilisation a few vascular cells are to be observed in the space below the chorda, and between the floor of the pharynx and the ectoderm which represents in these early stages the ventral wall of the body.*

On the contrary, the mesectodermic cells, the earlier traces of which are seen already to the close of the fourth day, appear as a rule between the ectoderm and the somatic plate of the mesoderm. When established the mesectoderm is confined to the head and the branchial extent of the body.

What interests us is that the mesectoderm is in the postotic region nothing else than the ventro-lateral edge of the scleromyotome which has

* As to the full account on the characteristics of the blood vascular cells and on the development of the vascular system I refer to my other papers (00, 07, 14*a*).

grown downwards by active cell-multiplication (text-fig. 2). The growth is produced not by the rearrangement of free cells cast off, but by the outgrowth of a continuous epithelium a single cell thick, which pushes its way between the somatic layer of the lateral plates and the ectoderm until the ventral edge of this epithelium reaches the mid-ventral line of the thyroid groove. The epithelium thus produced is what is called the mesectoderm.

The downward growth of the mesectoderm can readily be traced step by step. On the fifth day, where the growth of the layer begins, the ventro-lateral edge, for instance, of the fifth scleromyotome* is produced a short distance downwards and is wedge-shaped in cross-section. The cutis layer of the scleromyotome passes over into the muscle plate round the apex of the wedge. On the sixth day the mesectoderm in its anterior portion is so broad that its lower margin is found as low as the thyroid groove, while farther backwards the layer is narrowed so that it is represented in the seventh scleromyotome by a short wedge-shaped process of the latter. It is only in the course of the eighth day that the mesectoderm is fully established in the posterior branchial region.

The formation of the mesectoderm is, therefore, commenced in the anterior region and goes on backwards (text-fig. 3). In its early stages of formation the mesectoderm is an epithelium composed of flattened cells; but it thickens gradually as its component cells assume a tall columnar character which is, doubtless, brought about by their mutual pressure resulting from repeated cell-multiplication within the layer.

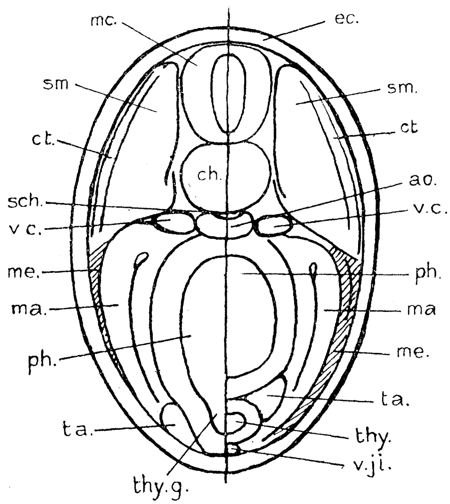
On a cross-section through a visceral arch six layers of epithelium are now seen: the innermost is the entodermal pharynx wall; the next outer feeble layer represents the first rudiments of the vascular arch; then follow the splanchnic and somatic layers of the lateral plates representing the mesodermic visceral arch; and between the latter layer and the outermost layer, the ectoderm, intervenes an intensely stained epithelium of tall columnar cells, which represent the mesectoderm. The mesectoderm is gradually diminished in thickness from the level of its middle height downwards into its sharp-edged lower margin.

Although the mesectoderm is only a single cell thick in most parts, at the dorsal edge, where it is in connection with the ventro-lateral edge of the scleromyotome, it is divided into two layers which pass over into the cutis layer and the muscle plate layer of the scleromyotome respectively. The mesectoderm can, therefore, be regarded as a fold arising from the above-

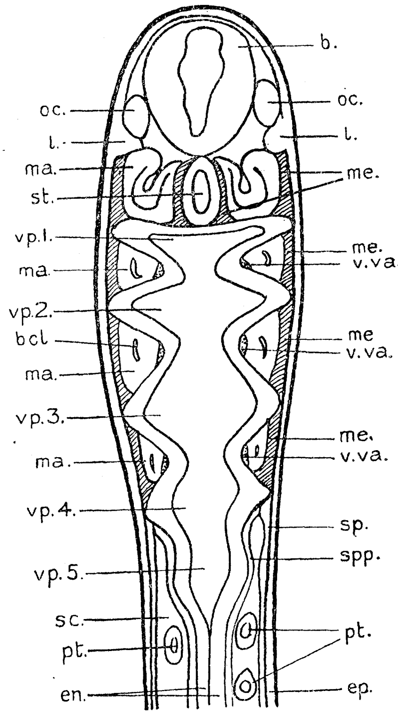
* The anterior three mesodermic somites found in front of the auditory vesicle are not transformed into typical scleromyotomes; nevertheless they are reckoned as such. The fifth scleromyotome is accordingly the second postotic.

mentioned edge of the scleromyotome, and wedging itself in between the meso- and ectoderm.

The manner in which the mesectoderm develops reminds us of the folding



TEXT-FIG. 2.



TEXT-FIG. 3.

TEXT-FIG. 2.—Diagrammatic representation of a transverse section through a postbranchial visceral arch, showing down-growth of the cutis-layer of scleromyotome to give rise to mesectoderm (left) and the established mesectoderm (right). The mesectoderm and its rudiments are shaded. *ct.*, cutis layer ; *sch.*, subchordal cells ; *thy.g.*, thyroid groove. For other letters, see the explanation of the previous diagram.

TEXT-FIG. 3.—Diagrammatic representation of a frontal section showing the position of the mesectoderm in relation to other layers. The posterior visceral pouches are in formation, and the lateral plates are being cut into the mesodermic visceral arches. The mesectoderm is shaded. *b.*, brain ; *bcl.*, branchiocœle ; *ep.*, epidermis ; *l.*, placode of lens ; *ma.*, mesodermal visceral arch ; *me.*, mesectoderm ; *oc.*, optic cup ; *pt.*, pronephric tubule ; *v.va.*, rudiment of vascular arch ; *sc.*, splanchnocœle ; *spp.*, splanchnopleure ; *sp.*, somatopleure ; *st.*, stomodæum ; *vp.*, visceral pouch.

of the corresponding edge of the scleromyotomes in the postbranchial region, by means of which the scleromyotomes grow downwards so as to provide the ventral somatic walls of the body with the muscular and the cutis layers. The downward growth of the scleromyotomes in both cases is, I venture to assume, analogous, and the two structures thus brought about are serially homologous. The difference consists in that the mesectoderm is destitute of the muscle plate layer. Though to a very small extent, the mesectoderm is divided into two layers, and there can be no objection to our regarding the inner of these as homologous with the muscle plate.

The causes of this modification in the branchial region are to be sought in the changes in that region produced by the respiratory mechanism and its skeletal framework. As will be stated later on, the mesectoderm is converted to a great extent into the cartilaginous branchial bar, which is a special skeletal arrangement for the respiratory mechanism. The remainder of the layer supplies the elements for the subcutaneous tissue, while, in the postbranchial region, the whole of the cutis layer is employed in the formation of this tissue. The demand for the formative elements of this tissue has caused, as I believe, the cutis layer of the scleromyotomes in the branchial region to be developed so vigorously as to call the mesectoderm into existence, although the muscle plate in the branchial region is almost entirely suppressed. The consequence of this suppression is that the somatic walls of the branchial chamber are destitute of the segmental muscles, and have to fill this deficiency by the so-called hypoglossal muscles which with their cutis layers undergo an exceedingly modified mode of development,* as was pointed out by Neal (97) and was confirmed by Koltzoff (02),† and by myself (14*a*, 14*b*).

The great modifications met with in the prootic section of the head cause the mesoderm to be modified to a still greater extent than in the postotic branchial region. Accordingly some peculiarities occur in the formation of the mesectoderm in this region.

In this section of the head there are formed three mesodermic somites, the third being situated just in front of the auditory vesicle. Of these three somites the hindermost shows a structure very similar to a postotic somite; the middle somite, under which the lateral plates‡ develop into the enormous mesodermic mandibular arch, is represented by a narrow epithelial fold of the archenteric roof, which ascends along the lateral wall of the medullary canal and strikes with its distal extremity against the trigeminal placode of the

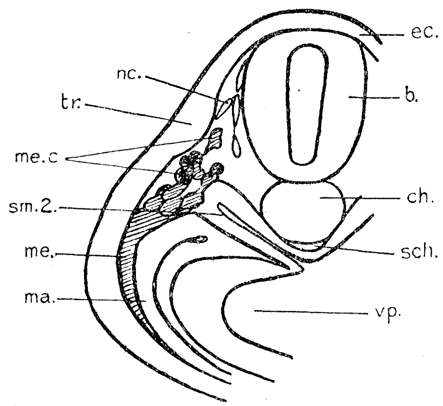
* The hypoglossal muscles are produced by the forward bending and shifting of the ventral part of some postbranchial scleromyotomes. They are the only segmental muscles in the somatic walls of the branchial chamber.

† As Koltzoff (02) remarks, Goette (90) gives incorrectly four somites in front of the auditory vesicle.

‡ Koltzoff is the only author who gives a detailed account of his second somite. But he failed to detect the free somite, which is very small, whilst he regards the dorsal part of the colossal lateral plates as their somitic part.

ectoderm; whilst the first somite is represented by the anterior blind end* of the epithelial archenteron, which is folded off from the rest as a median unpaired pocket. This somite is so small that it is practically destitute of the lateral plates.

In the two posterior of the three somites the outer wall, which corresponds to the cutis layer of a postotic somite, gives off free cells which push their way between the ectoderm and the somitic layer of the lateral plates (text-fig. 4). The free cells are quickly increased to some extent by the cells



TEXT-FIG. 4.—Diagrammatic representation of a transverse section through the second somite, showing the proliferation of the mesectoderm cells from the lateral layer of the somite. *me.c.*, mesectoderm cells (shaded); *nc.*, nerve cells; *sm.*2, second somite standing still in connection with the pharynx; *tr.*, placode for trigeminal ganglion. For other letters, see the explanation of the previous diagrams.

budded out from the lateral layer of the somitic fold. The somites are cut off from the archenteric roof only on the fifth day, while the cell-proliferation begins at the early part of the fourth day, therefore earlier than the stage at which the ventro-lateral edge of the postotic scleromyotomes begins to be produced into the mesectoderm. The free cells soon make up a thick columnar epithelium which represents the mesectoderm of this preotic region. In contrast to that in the postotic region, the mesectoderm is here not confined to the lateral part, but is spread into the ventral wall of the body, which gives rise to the stomodæum by invagination.

At the same time, but on a smaller scale, the cells wander out of the lateral layer of the somites into the space between the medullary canal and the ectoderm opposite and above the ganglion placodes. They do not assume

* The first somite may not be confounded with the anterior blind sac of the pharynx of Kupffer; for the explanation of both the structures I refer to my paper above given (14*b*).

an epithelial character, but make up a simple network of cells with the nervous cells coming forth from the nerve ridge, by which the epidermic ectoderm is connected with the walls of the medullary canal. This network corresponds to that part of the mesectoderm which Koltzoff distinguishes as the dorsal division from the epithelial ventral part of it. The dorsal division is accordingly confined to the preotic region, while the ventral division is to be traced uninterruptedly to the corresponding part in the postotic branchial region and constitutes one continuous structure from the mandibular arch to the hindmost visceral arch.

The network forming the dorsal division becomes gradually more complicated, owing to further growth of both the nervous and mesectodermic cell-strings; so that the elements of both kinds are not easily distinguished from each other, as Koltzoff complains. The cells of the dorsal division ought not, therefore, to be overlooked at the earlier stage of their appearance, a phase in which the nervous cells coming downwards from the medullary roof and the mesectoderm cells arising from the lateral layer of the somites do not as yet meet with each other. Then, in the following stages, both kinds of cells are not very difficult to trace into the points from which they start respectively. At the dorsal corner of the lateral layer of the somites active cell-divisions can be observed which are repeated during the course of development for not less than 12 hours, and the course taken by the resulting cells in passing into the network is not difficult to make out. The nerve-cell strings passing downwards almost vertically can also be traced with certainty. The nerve fibres developed from these strings associate with those from the ganglion of epidermic origin and make their way between the mesectoderm and somitic layer of the lateral plates, as was obvious already in an embryo of the eighth day.

The foremost somite gives off the mesectoderm cells not only from its outer wall, as in the following somites, but also from the anterior wall of the blind pocket by which the first somite on each side stands in connection with its fellow. The cells from both sources fill up the space between the ectoderm and the somite and the space below the anterior extremity of the brain. The free cells lying in close contact with the ectoderm are transformed into the epithelial mesectoderm, which can by no means be distinguished from the ventral division in the posterior region and is continuous to it; those inside are developed into the network of muscle fibres which occupies the interior of the upper lip in later stages.

While the outer walls of the first, second, and third somites and also the anterior wall of the first are broken up into the mesectoderm cells, the inner walls of these three somites, which correspond to the muscle plate of the postotic somites, give rise to the six muscles of the eye,* which are distinctly differentiated already at the close of the ninth day or at the commencement of the 10th day. The preotic section of the head is consequently totally destitute of the segmental muscles derived from the somites. This deficiency is, as pointed out by Kupffer, made good by a few myotomes behind the auditory vesicle, the dorsal parts of which are bent and shifted forwards into the head.

The mesectoderm is, therefore, the product of that part of the mesodermic somites which corresponds to the cutis layer. The dorsal division, which is distinguished by Koltzoff from the ventral division, is derived from the three preotic somites and confined to the preotic section of the head; it never assumes an epithelial structure, but makes a simple network with the nerve-cell strings.

On the contrary, the ventral division is a continuous layer of typical epithelium, extending from the anterior margin of the lateral plates to the posterior boundary of the branchial region and as high as the lateral plates. This division of the mesectoderm is cut into nine vertical epithelial bands, when the lateral plates are divided into nine visceral arches.

That part of the mesectoderm which assumes the epithelial character in the snout may be regarded as the anterior continuation of the ventral division. If this assumption is correct, all the three preotic somites, just like the following somite in the postotic branchial region, contribute elements to the ventral division of the mesectoderm.

2. *Fate of the Mesectoderm.*

While the nerve-cell strings in the pre- and postotic region are transformed into the nerves and ganglia, the mesectodermic elements of the dorsal division are converted mainly into the connective tissues standing in relation to the nerves and ganglia; a small portion of them, which lies in contact with the medullary walls, gives rise to the most anterior section of the cranial skeleton, *i.e.* the trabeculæ.

The nine mesectodermic bands, into which the continuous layer of the ventral division is divided by the visceral invagination of the pharynx-walls, undergo the following differentiation. On frontal sections through a just-hatched larva, the first stage of the differentiation is very obvious. At the level of the visceral pouch, the entodermal wall of the pharynx is in close contact with the ectoderm, while at the level of the visceral arch, between the two layers, are contained the vascular cells, the mesodermic arch and the

* Detailed accounts on the development of the ophthalmic muscles I have given in my above-mentioned paper (14*b*).

mesectoderm. The proximal half of the last-named epithelium is thickened so as to be raised inwards into a ridge, which shows on cross-sections a pyramidal outline and represents the first rudiment of the cartilaginous visceral arch. This stage of differentiation is represented in text-fig. 18 by Schalk, which is correct, except the connection of the mesectoderm with the ectoderm.

The cells forming the rudiment of the cartilage acquire a radial arrangement (text-fig. 1, A) and are soon constricted off from the remainder of the layer; this stage is followed by a stage in which they are wedged in between one another, so as to form one row (B). On cross-sections through a larva of the 14th day this rudiment of the cartilage looks like a bar consisting of piled-up discs, in which three sections are distinguishable (C): a dorsal and a ventral section curved outwards and the middle section bowed inwards. While the ventral section touches with its distal extremity the lateral division of the vena jugularis impar, the proximal end of the dorsal section lies in the corner between the dorsal aorta and the chorda and under the anterior cardinal vein. In the course of the 15th day, the aorta together with the roof of the pharynx is separated from the chorda by enormous development of the reticular subchordal connective tissue. Accordingly the dorsal end of the rudiment of cartilage is also brought downwards, so as to be forced into the corner between the aorta and the roof of the pharynx which has been pressed down. It is interesting that the band of connective tissue which before and after this change connects the rudiment of cartilage with the chorda is drawn out into a string stretched between both the structures.

In sharp contrast to other visceral arches, the hyoid arch does not undergo this dislocation of the cartilage bar, which is, on the contrary, shifted by stages a little upwards, and the cardinal vein passes into the mandibular vein *under* the cartilage bar. This peculiar feature is the first step towards the fulfilment of the function which the hyoid arch has secondarily acquired; it enters into the formation of the primordial skull, leaving the service of respiration.

The differentiation of the mesectoderm into the visceral arch takes place at first in the visceral arch behind the hyoid arch and proceeds backwards to the following arches, which undergo the same process one after another. For a long while the mesectoderm in the hyoid arch is not cut off from that in the mandibular arch. And it delays its differentiation into the rudiment of the cartilaginous hyoid arch, which is, however, obvious before the same process commences its work in the hindmost visceral arch.

The rudiment of the cartilaginous visceral arch shifts inwards, when it is detached from the remainder of the mesectoderm, and presses and separates at last the lateral plates, the mesodermic visceral arch, into the inner adductor and the outer constrictor muscles, with which the cartilaginous arch is invested.

The remainder of the mesectoderm constricted off from the rudiment of the cartilaginous visceral arch is stretched at the same time to the outside of the rudiment of arch so as to line the whole inner surface of the ectoderm ventral to the chorda level, and assumes the characteristic feature of the subcutaneous tissues which underlie the ectoderm, except the ventral part for the hypoglossal muscles. This differentiation of the mesectoderm is very obvious in larvæ of the 13th to the 14th day.

The mandibular arch, which we may now consider, is characterised particularly by the enormous mesodermic arch which it contains and which is folded upon itself, thrust inwards by the invaginating stomodæum, so that four layers of the folded lateral plates are obvious on a cross-section through this arch. While the ventral edge of the folded mesodermic arch, by which the somatic layer passes over into the splanchnic plate, is separated by the bottom of the stomodæum from its counterpart on the opposite side, the dorsal edge, by which the two layers of the lateral plates also run into one another, is divided only by the carotid artery from the chorda. The ventral division of the mesectoderm, following this folding of the mesoderm, is brought into the same topographical relations to the stomodæum and to the chorda.

The dorsal edge of this division of the mesectoderm is brought into contact with the lateral wall of the chorda, above the carotid artery, as is very clearly seen on the 9th to 10th day. On the 13th day the cells composing this edge are concentrated into a characteristic compact mass which is soon constricted off from the remainder of the layer. This compact cell mass constitutes the earliest traces of what are known since Sewertzoff (97) as the anterior parachordals. The rudiment of the anterior parachordal is on cross-sections wedge-shaped and looks as if produced from the lateral wall of the chorda.

On the 14th day the rudiment of the anterior parachordal can be traced as far as the branching of the facial artery from the carotid, which marks the boundary between the first and second somites, and it ends backwards rather suddenly in front of the roots of the vascular mandibular arches and of the carotids. The parachordal rudiment is most prominent at a little distance from the root of the vascular mandibular arch and grows gradually lower toward the snout, while it is decreased suddenly in height backwards.

The parachordal rudiment cannot, therefore, be distinguished genetically from the rudiment of a cartilaginous visceral arch; both the structures are, I believe, serially homologous with one another. The prominent point of the parachordal rudiment develops into a transverse bar of cartilage, which is, I assume, the rudimentary remnant of the equivalent of its corresponding visceral cartilage bar. This bar is, in the mandibular arch, reduced to its last remnant, because it has been shut off from the respiratory mechanism. The further fate of the cartilage bar interests us in developing into that important element of the primordial skull, which is known since Parker (83) as the palato-quadrate; the redevelopment of this rudimentary remnant into so conspicuous an element of the cranial skeleton is due to nothing but the law of "Funktionswechsel" first enunciated by Dohrn.

The remainder of the mesectoderm detached from the rudiment of the anterior parachordal is, in the mandibular arch as elsewhere, converted into the subcutaneous tissues, which develop in distinction to those in other arches not only beneath the ectoderm, the skin of the cheek, but also beneath the epidermis of the stomodæum, the cover of the mouth cavity.

As Gaup (06) remarks, the single origin of the parachordal, which Koltzoff (02) maintains and Schalk (13) confirms, is incorrect. On the contrary, the posterior parachordal of Sewertzoff (97) is represented in reality by the medial horizontal process of the cartilaginous hyoid arch itself, and the transverse process, which, according to Sewertzoff, is very similar to an ordinary visceral bar in the following arches, is nothing more than the visceral bar itself in the hyoid arch.

The anterior and posterior parachordals are separated for a long time by interposition of the large auditory capsule. Both the rudiments grow respectively backwards and forwards to meet and be fused together with each other only in a larva about thirty days old, in which the auditory capsule retreats and is detached from the chorda. But for a long time the transition is obvious, because both the rudiments are decreased in thickness towards their point of meeting.

The trabecula is formed in front of the root of the facial artery and the rudimentary vascular arch in the premandibular segment; its centre lies close to this vascular root. From this centre it grows anteriorly along the basal wall of the brain and over the posterior cerebral artery, which is the anterior prolongation of the carotid artery. The more it is prolonged, the more it diverges hand in hand with the artery from the median line, so that it lies opposite the optic cup rather on the lateral wall of the brain and embraces, with its counterpart on the opposite side from right and left, its infundibulum, and is finally lost on the lateral wall of the latter.

The mesectoderm cells giving rise to the centre of the trabecula are doubtless those derived from the first somite, and are marked off from those of the second somite by the facial artery, which appears much earlier than the mesectoderm. But the forward growth of the anterior parachordal seems to be carried on largely at the cost of the dorsal division of the mesectoderm from the second somite, viz., the cells which lie close to the chorda, and to be continued uninterruptedly to the formation of the trabecula in front; so that the trabecula is practically not formed separately from the anterior parachordal, as was believed by previous observers, but as the prolongation of the latter. The trabecula has, however, a special centre for itself, marked by a slight thickening in the rudiment.

It is, however, still an open question, whether the trabecula is to be put in the series of the visceral arches or not. But it is obvious that the rudiment of the trabecula is genetically identical with the latter, because it is formed of material identical with that giving rise to the visceral arches and because it comes forth in a metamere identical with that which has a cartilaginous visceral arch for itself.

To avoid misconception, a few words may be said about the relation of the mesectoderm to the metamery of the body. The epithelial bands into which the ventral division of the mesectoderm is divided are in numerical as well as topographical correspondence with the mesodermic visceral arches, that is to say, branchiomeric. The cartilaginous visceral arches are derived from these branchiomeric bands, also branchiomeric in arrangement.

The mesodermic visceral arches arise from two unsegmented continuous layers, the lateral plates, which are divided by nothing but evagination of the visceral pouches, which is independent of the process dividing the mesoderm into the somites. This metameric repetition in the entodermal pouches has, therefore, no direct relation to the mesodermic somites at all, which represent the primary metamery of the body. In spite of its being the product of the segmented mesoderm, the mesectoderm is also an unsegmented continuous layer, until it is divided by the visceral invagination into branchiomeric bands. The branchiomery in this part of the meso- and mesectoderm, which is brought about in a passive way, is, therefore, not of the same value as the body-metamery.

The branchiomery is furthermore in segmental accordance with the ventral series of the ectodermic placodes for the ganglia.* But this series of ganglia is never in direct segmental connection with the mesodermic somites.

Still another organic system, which is in segmental accordance with the

* This series of ganglia consists of the facial and glossopharyngeal ganglia and of the series of the epibranchial ganglia. The placode for the lens may be put in this series, for it can by no means be distinguished from the ganglionic placodes, so far as its origin and mode of development are concerned.

visceral arches, is the vascular. In the first formed vascular system, in which the arterial and venous systems are not yet differentiated, we have before us a vascular system of the Annelid type (Hatta (07), Keiser (14)), which consists of a dorsal and a ventral longitudinal vessel connected with each other by vascular rings which are repeated in each body somite (Hatta (07), (14*a*), Keiser (14)). The anterior division of this ring vessel system is represented by the vascular visceral arches (Goette (90)), and is followed by Mayer's Quergefässe in the pronephric region, while in the region still further posterior the segmental character of this series of vessels is obscure, because in this region there is no organ of segmental arrangement standing in direct relation with the vessels.

The origin of the ring vessels, whether entodermic (Goette (90)) or mesodermic (Hatta (14*a*), Keiser (14)), is by no means mesomeric, because the vascular cells are derived, even in the strictly segmented branchial region, from an organ which is not segmented in accordance with the mesodermic somites.*

The pronephros is the only organ in which the vasomery, *i.e.* branchiomery, is connected with the mesomery. But there is an incontestable fact which shows that the segmental repetition of Mayer's vessels in the pronephric region is not primary but secondary. The first pronephric artery, which is evident in a larva of the 10th day, corresponds with the 11th vasomere (counting the premandibular vascular arch as the first vasomere), and the first pronephric tubule with which the artery stands in relation, is the product of the 7th mesodermic somite† (Hatta (97), (00), (14*a*), (14*b*)), while this somite is no longer found over the first pronephric tubule, when the tubule is cut off from it.

There are two movements by which the segmental discordance between the nephromeres and the myomeres is brought about, viz., the pronephros is gradually pushed backwards by the outgrowth of the visceral pouches; the somites, *i.e.* myotomes, on the contrary, move forwards after their formation, so that a few anterior of them are pushed over the preotic section of the head to give rise to the capitis muscles. The ring vessels, *i.e.* Mayer's vessels, occur in the visceral arches as well as in the pronephros only when the visceral pouches are formed and the backward movement of the pronephros has already been carried out. The vasomeres are thus put secondarily in relation to the pronephros, although the latter

* According to Goette, the vascular cells (in branchial region) are derived from the enteric wall; according to my results, which are confirmed by Keiser, the cells are derived from the mesodermic visceral arches.

† In my papers formerly published (97, 00) the first pronephric tubule is regarded as the product of the fourth scleromyotome, which corresponds to the seventh, when the three preotic somites are counted in.

is the descendant of the somites. In short, the branchiomery and mesomery are independent of each other.

The cartilaginous visceral arches and their equivalents in the primordial skull are branchiomeric organs, and have no genetic relation to the mesomeres.

Therefore, it seems extremely probable that the participation of the sclerotomes in the branchial elements and in their equivalents, which is maintained by Koltzoff, Schalk, and others, is, if such actually occurs, nothing but accidental.

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LITERATURE.

80. Balfour, F., "A Treatise on Comparative Embryology," London, 1880–1881.
04. Brauer, A., "Beiträge zur Kenntnis der Entwicklung und Anatomie der Gymnophionen. IV.—Die Entwicklung der beiden Trigeminusganglien," 'Zool. Jahr., Suppl. Bd. 7.
02. Dohrn, A., "Studien zur Urgeschichte des Wirbeltierkörpers. XXII.—Weitere Beiträge zur Beurteilung der Occipitalregion und der Ganglienleiste der Selachier," 'Mitteil. Zool. Stat. Neapel.,' vol. 15.
06. Gaup, E., "Die Entwicklung des Kopfskelettes," 'Hertwig's Handb. vergl. exper. Entw.-lehre d. Wirbelt.,' vol. 3.
90. Goette, A., "Entwicklungsgeschichte des Flussneunauge (*Petromyzon fluviatilis*)," 'Abh. entw. gesch. d. Tiere,' Part 5.
91. Hatta, S., "On the Formation of Germinal Layers in Petromyzon," 'Journ. Coll. Sci. Imp. Univ. Tokyo,' vol. 5.
92. —— "Contributions to the Morphology of Cyclostomata. I.—On the Formation of the Heart in Petromyzon," *Ibid.*, vol. 8.
97. —— "Preliminary Note on the Development of the Pronephros in Petromyzon," 'Ann. Zool. Jap.,' vol. 1.
00. —— "Contributions to the Morphology of Cyclostomata. II. On the Development of the Pronephros and Segmental Duct in Petromyzon," 'Journ. Coll. Sci. Imp. Univ. Tokyo,' vol. 13.
01. —— "On the Relation of the Metameric Segmentation of Mesoblast in Petromyzon to that in Amphioxus and in the Higher Craniota," 'Ann. Zool. Jap.,' vol. 5.
07. —— "Bemerkungen über die früheren Entwicklungsstudien des Gefässystems bei Ammocoeten," 'Journ. Agric. Coll., Imp. Univ., Sapporo,' vol. 4.
14*a*. —— "Ueber die Entwicklung des Gefässystems beim Neunauge (*Lampetra mitsukurii*, Hatta)."
14*b*. —— "Die Bildungsweise und die erste Differenzierung des Mesoderms beim Neunauge (*Lampetra mitsukurii*, Hatta)."
14. Keiser, W., "Untersuchungen über die erste Anlage des Herzens, der beiden Längsstämme und des Blutes bei Embryonen von *Petromyzon planeri*," 'Jen. Zeitschr. Natur.,' vol. 51.

13. Schalk, Alban, "Die Entwicklung des Cranial- und Visceralskeletts von *Petromyzon fluviatilis*," 'Arch. Mikr. Anat.,' vol. 83.

99. Koltzoff, N. K., "Metamerie des Kopfes von *Petromyzon planeri*," 'Anat. Anz.,' vol. 16.

02. —— "Entwicklungsgeschichte des Kopfes von *Petromyzon planeri*," 'Bull. Soc. Imp. Natural.,' Moscow, vol. 15.

90. v. Kupffer, C., "Die Entwicklung von *Petromyzon planeri*," 'Arch. Mikros. Anat.,' vol. 35.

94. —— "Die Entwicklung des Kopfes von Ammocoetes," 'Stud. vergl. Entw. gesch. des Kopfes d. Cranioten,' Part 2.

95. —— "Die Entwicklung der Kopfnerven von Ammocoetes Planeri," *Ibid.*, Part 3.

85. —— "Ueber die Entwicklung des Kiemenskelettes von Ammocoetes und die organogene Bestimmung des Ektoderms," 'Verh. Anat. Ges., 9.Vers. in Basel.'

06. Mollier, S., "Die erste Entstehung der Gefässe und des Blutes bei Wirbeltieren," 'Hertwig's Handb. vergl. exp. Entw.-lehre d. Wirbelt.,' vol. 1.

97. Neal, H. V., "The Development of the Hypoglossus Musculature in Petromyzon and Squalus," 'Anat. Anz.,' vol. 13.

83. Parker, W. K., "On the Skeleton of the Marsipobranch Fishes," 'Phil. Trans., London.

94. Platt, Julia B., "Ontogenetische Differenzierung des Ektoderms in Necturus," 'Arch. mikr. Anat.,' vol. 43.

98. —— "The Development of the Cartilaginous Skull and of the Branchial and Hypoglossal Musculature in Necturus," 'Morphol. Jahrb.,' vol. 25.

93. —— "Ectodermic Origin of the Cartilages of the Head," 'Anat. Anz.,' vol. 8.

82. Scott, W. B., "Beiträge zur Entwicklungsgeschichte der Petromyzonten," 'Morphol. Jahrb.,' vol. 7.

95. Sewertzoff, A., "Die Entwicklung der Occipitalregion der niederen Vertebraten in Zusammenhang mit der Frage über die Metamerie des Kopfes," 'Bull. Soc. Imp. Natural.,' Moscow, vol. 2.

97. —— "Beitrag zur Entwicklungsgeschichte des Wirbeltierschädels. Vorläufige Mitteil.," 'Anat. Anz.,' vol. 13.

87. Shipley, A., "On some Points in the Development of *Petromyzon fluviatilis*," 'Quart. Journ. Micr. Sc.,' vol. 27.