Anatomy. — "*Thymus, spiracular sense organ and fenestra vestibuli (ovalis) in a* 63 *m.m. long embryo of Heptanchus cinereus*". By Prof. J. W. VAN WIJHE.

(Communicated at the meeting of September 29, 1923).

Many years ago I received this embryo from the Zoological Station at Naples. It was fixed in sublimate and preserved in alcohol. Just as another specimen it was treated with methylene blue, in order to make a skelet preparation of it.

This having proved quite successful with the one embryo, I decided to preserve the other, so as to make a series of cross sections later, in order also to be able to examine the remaining organs. I intended to wait with this until I had more of this rare material in different stages. I however received only one more embryo, 255 m.m. long, which was simply treated with alcohol and was much too large for making a series of sections. Here one would have to restrict oneself to only a few parts. For this specimen I am again indebted to the direction of the Station at Naples.

When in the autumn of 1922 I had finished with the development of the skeleton of Acanthias vulgaris [1]) I decided not to wait any longer, and a series of cross sections of the 63 mm. long embryo was made. The preservation proved to be excellent, notwithstanding the previous long treatment of HCl alcohol necessary for the elimination of the methylene blue from the remaining tissues, in order to restrict the colour to the cartilage. The staining of the sections with ammonia-carmine was also successful; but the light blue tint of the cartilage could not be intensified by the after-treatment with methylene blue or victoria blue. The reason for this remained unknown to me.

In the 255 m.m. long embryo, which had been in alcohol for many years, the cartilage suffered itself to be stained deep blue.

---

[1]) VAN WIJHE, J. W. Frühe Entwicklungsstadien des Kopf- und Rumpfskeletts von Acanthias vulgaris. Bijdragen tot de Dierkunde, publ. by the Kon. Zool. Genootsch. Natura Artis Magistra at Amsterdam. Afl. 22, Feestnummer voor MAX WEBER, 1922.

1. *Thymus.*

The development of the thymus in the Selachians was first described by DOHRN (1884). The facts then found by him were principally confirmed by later investigators. HAMMAR, who had given many years to the study of the structure, development and function of this organ in nearly all the principal groups of vertebrates, described the development in the Selachians in 1912, and gave a detailed account of the results of his predecessors.

He found, that in all vertebrates from fish up to man, the thymus continues to grow till the time of puberty. Then an involution period begins, wherein it as a rule atrophies, without totally disappearing.

The thymus, in all vertebrates, begins to form as a local proliferation of the epithelium of the gill clefts.

In man it appears principally, if not exclusively, on the third gill cleft, but in the Selachians, which generally have six gill clefts, a beginning of the thymus is described on each gill cleft. These however speedily disappear on the first and last, sometimes even on the last two gill clefts.

Not all investigators are of opinion that the thickening of epithelium cells of the first gill cleft (spiracle) may be considered as a thymus, and it is possible that here an interchange may have taken place with the place of origin of the spiracular sense organ.

Soon after its appearance, one can distinguish in the thymus two different kinds of cells, viz. a network of flat epithelial cells, which encloses groups of round cells in its meshes.

These round cells multiply themselves so quickly, that the network can no longer be discerned unless in very thin sections.

The whole organ, which formerly was pear-shaped and afterwards has the shape of a grape bunch, appears to be wholly constituted of round cells, which form a solid mass without lumen. These cells hardly have any protoplasm, and therefore give the appearance as if one only has to do with an accumulation of nuclei.

There are two opinions concerning the derivation of these round cells, which strongly resemble the lymphocytes of the blood. Many hold them for epithelium cells, which have rounded themselves off; others again take them to be true lymphocytes, which have penetrated the organ from the bloodvessels and the neighbouring mesenchym. The latter opinion is emphatically upheld by HAMMAR for all classes of vertebrates.

The question as to which of the two opinions is correct, cannot

be settled by the study of the 63 m.m. long embryo of Heptanchus, but a further question can be explained thereby, viz. whether the thymus has to be considered as a gland which has lost its original excretory duct and thus only has internal secretion left. It would then find itself in a similar condition as the anterior lobe of the hypophysis and the thyroid gland, which, however, in the embryo of vertebrates, always have an excretory duct which is only lost during the further course of development.

The thymus does not sever itself from the epithelium of the branchial gut in Cyclostomes and most of the bony fishes. This is however the case with the remaining vertebrates. But a true excretory duct, as a rule, does not appear. This would be expected in sharks, but FRITSCHE (1910) says: "Ein Lumen und einen Ausführungsgang habe ich bei Spinax ebensowenig auffinden können wie DOHRN bei seinen Haifischen."

In a very early stage of rays (Torpedo), they however noticed something which resembled an excretory duct.

In some of the sharks examined up to now, the body of the thymus separates itself directly, without a pedicle, from the epithelium of the branchial gut; while in others it still remains connected for some time by a stalk to the epithelium.

This stalk lacks the characteristics of an excretory duct, because it not only has no lumen, but also shows the same structure as the body of the thymus and consists almost exclusively of the rounded cells, which resemble lymphocytes.

In our embryo of Heptanchus we on the contrary find an excretory duct *in optima forma* for each of the thymus divisions (thymomeres) which are found on both sides of the body, one for each side from the second to the seventh branchial cleft. There are 8 gill clefts, but in the first (spiracle) and last the thymus is absent.

It is the largest in the second and third cleft and has the form of a bunch of grapes. The bunch is smaller in the 4th cleft, in the 5th still smaller, and in the 6th the thymus no longer has the bunch form, but is composed of a single acinus, into which the excretory duct opens.

In the 7th cleft every acinus is found missing from the short excretory duct.

In the figure of the section we see the large thymus of the 2nd branchial cleft. It runs over the top of the 1st epibranchial and then continues as the fairly long excretory duct. This has an obvious lumen, which with its one end opens at the top of the branchial

cleft, with the other reaches to the body of the thymus without entering it.

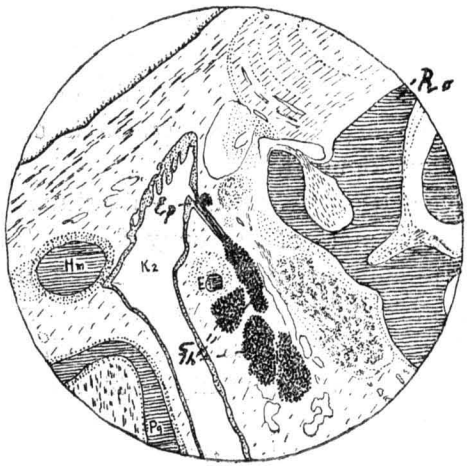

Fig. 1. Cross section through 2nd branchial cleft of a 63 m.m. long embryo of Heptanchus cinereus. In this and in the following figs. the cartilage (stained blue in section) is striated horizontally.

The wall of the duct is 2 cells thick, and is constituted of a double layer of fairly flat epithelium cells, amongst which not a single round cell is to be found.

The excretory duct of each of the remaining thymomeres shows a similar structure, viz. a double layered epithelial wall, encircling a lumen, which opens into its respective gill cleft.

These ducts from the 2nd caudally, gradually shorten; the last (6th) forming a rather unimportant, yet distinct attachment to the 7th branchial cleft.

The excretory ducts are not permanent. They later on lose their epithelial structure and lumen. This e. g. happened in the 225 m.m. long embryo. Here, in the place of the excretory duct of the first (anterior) thymomere, one finds a long pedicle, which appears as an outgrowth of the thymus. The pedicle runs over the top of the 1st epibranchial and reaches the wall of the branchial cleft.

It shows itself as a chord, which appears entirely to consist of lymphocyt-like round cells. No traces are left of the original epithelial structure and lumen. I however do not wish to deny the presence of a reticulum. It would also be possible to make it clear in the pedicle by appropriate methods.

For completeness the so called epithelial bodies and the supra-pericardial organ should also be mentioned. In the 63 m. m. embryo an epithelial body is found, immediately above the opening of the 1st and 2nd thymomere. Each little body is a round isolated cellmass, which resembles an acinus of the thymus in form and

size, but is more compact; owing to the fact that it has finer lymph-spaces than the thymus. No trace of such a body was to be found at the 3rd, 4th, 5th and 6th thymomere.

The suprapericardial body was discovered by VAN BEMMELEN [1]) (1885) at the end of the branchial gut. Later it was found in all classes of vertebrates. It is generally taken as the last indication of an abortive branchial pouch, and mostly appears on only one side of the body.

In 1906 BRAUS found it in the 67 m.m. long embryo of Heptanchus, which very likely originates from the same mother-animal as mine, and I can corroborate his statement. It is only well developed in the left half of the body, and shows itself as a little bladder, the lumen of which is encircled by a single layer of fairly columnar epithelium cells. It is to be seen on 35 sections, and is situated as BRAUS stated, behind the last visceral arch, in the angle which this makes with the ceratobranchial. Just as BRAUS, I found it near its posterior margin connected with the epithelium at the base of the branchial gut by a short pedicle.

On the right side the organ is rudimentary.

I found it represented by a flattened little group of epithelial cells without a lumen, and totally severed from the gut epithelium. This is visible in the sections passing through the posterior half of the vesicle on the left. BRAUS does not mention this little group.

His specimen was probably somewhat further developed than mine,

---

[1]) Owing to the presence of a suprapericardial body in the embryos of Heptanchus (VAN BEMMELEN in vain sought for it in the adult animal) one cannot assume that, in higher animals, this little body is the remains of a branchial cleft, which is present in the Notidanides as such. The morphological significance of this organ is a problem. One may of course believe that it is the remains of a branchial cleft, which still lies further caudally than the last (8th) of Heptanchus. BRAUS e. g. takes it to be the rest of a 10th branchial pouch.

He professes to find the remains of a (9th) branchial pouch in a slight protrusion of the intestinal wall behind the last branchial arch, in the angle between the last (7th) ceratobranchial, and a caudalwards directed protuberance on its ventral side.

Although this protuberance chondrifies continuous with the 7th ceratobranchial, he considers it to be the remains of an 8th branchial arch.

I cannot agree with these conceptions. In my specimen the rather long protuberance is still quite prochondral, and just like the prochondral cardiobranchial end, lies in the beginning of the oesophagus. In the protuberance I can only discern a processus muscularis of the 7th ceratobranchial, morphologically insignificant. An intestinal protrusion which could also be considered as a 9th branchial pouch, is not present, and I must consider it as an artificial product in the specimen of BRAUS.

and this little group more atrophied. He thought he saw an indication of an antimere of the left vesicle on the right side of the body, in the shape of a more caudally situated diverticulum of the branchial gut.

Let us however return to the thymus. The *genus* Heptanchus is indeed rightly regarded as the most primitive of the living Selachians. The number of visceral pouches (i. e. 8) surpasses that of all other fishes and higher animals. Only the anterior 5 are still formed in mammals.

Concerning the 63 m.m. long embryo of Heptanchus, we may now assume, that also its thymus appears in a more primitive form than in the development of higher animals.

The original function of the thymus could then not have been internal secretion only, but it must also have removed products through its excretory ducts.

Originally each thymomere was a true gland, according to the old notion, with an excretory duct even as was the case with the thyroid and the anterior lobe of the hypophysis.

The presence of excretory ducts is also of importance for the conception of the morphological significance of the gland. Since the researches of DOHRN, it is generally accepted that the thymus is a branchiomere organ, a division of which occurred on each branchial cleft.

Now Amphioxus has on each of its many branchial clefts a glandular body, which opens with its excretory duct into the top of the cleft. This branchionephros functions as an excretory organ, and for many years I have presumed, that it would prove homologous to the thymus of higher animals.

This presumption was strengthened, when in 1909 GOODRICH found that the branchionephros does not develop from the coelomic epithelium, as one would rather be inclined to assume for an excretory organ in chordates.

But he does not state that it develops from the branchial epithelium. His drawings however give this impression. Might this impression prove to be correct by later investigations, then the branchionephros develops from the same tissue as the thymus of higher animals. Cells resembling lymphocytes are never found in it. Lymphocytes do not occur in the blood of Amphioxus, the blood of which only consists of plasma, without any red or white blood corpuscles, just as the blood in its earliest stage in craniates.[1])

[1]) A few investigators profess to have found cells in the blood of Amphioxus. I have never observed any in my numerous sections of larvae and adult animals.

The presumed homology of the thymus and branchionephros has also been supported from the side of the craniates, now that, in the development of such a primitive form as Heptanchus, the presence in the thymus of excretory ducts, which in Amphioxus analogously open into the branchial clefts, has been shown.

If the branchionephros develops from the branchial epithelium, the chief difficulty to homologize it with the thymus, I think then lies in the period of development of this gland. One should expect the thymus to become perceptable in a very early period of its development, but this only happens very late.

The reason for this is because the original function no longer comes to development. It is taken over by the pronephros and the mesonephros. The other function of the thymus i.e. its internal secretion, caused by the lymphocytlike cells, must phylogenetically have originated much later.

2. *Spiracular sense organ.*

In no vertebrates does a division of the thymus come to development in the first branchial cleft (spiracle). It appears not even to be formed there at all. On the other hand, we find on the wall of the spiracle in the embryos or larvae of the more primitive fishes: Selachians, Ganoids and Dipnoi a sense organ, which is not met with on any of the remaining branchial clefts. These adult fishes also possess one.

We find it even in those forms (Dipnoi and Holostei) in which the spiracle, which is developed in the manner of an intestinal pouch, no longer breaks through outwardly.

It was discovered by Ramsay Wright in 1885, who found it as a protrusion of the medial wall of the spiracular visceral pouch of the Holostei (Lepidosteus and Amia). This protrusion (diverticulum) is directed upwards and surrounded by the cartilaginous auditory capsule; in other words, it lies in a canal of the lateral cartilaginous wall of the otic region of the skull, but otherwise has no relation to the auditory organ.

A similar canal in the cranial cartilage, into which a diverticulum of the spiracular wall penetrates, was discovered by Bridge in Polyodon. The same was also observed by Wright in the sturgeon. The presence of a sense organ in these Chondrostei is, however, not mentioned.

Wright found, that in the Holostei this sense organ is innervated by a branch of the ram. oticus of the facial nerve, which in the

Ganoids (Chondrostei and Holostei) is likewise overgrown by the cartilaginous auditory capsule, and of which (ram. oticus) it was known that it sends out branches in this region to the sense organs, belonging to the lateral line system.

These sense organs, called neuromasts (Nervenhügel) by WRIGHT, lie either free on the surface, or protected in little sacs, grooves or canals; all are of ectodermal derivation. Now it was noteworthy that the sense organ of the spiracular pouch also resembled the structure of a neuromast, although WRIGHT evidently thought it to be of entodermal origin. It seemed as if one here had the unexpected example of a sense organ of the Chordates, which did not originate from the ectoderm, although it was still supplied by a nerve, belonging to the lateral line system of the epidermal sense organs.

The study of the Dipnoi dispelled the singularity of this phenomenon. In this group PINKUS (1895) discovered in Protopterus annectens a little bladder with a sense organ on its wall, and imbedded in the cartilage of the otic region. The sense organ — evidently a neuromast according to the fig. — is supplied by a caudalwards running branch of the facial nerve, the branch belonging to the lateral line system.

PINKUS still describes two more caudalwards running branches from the lateral line system of the n. facialis. The one forms the well known anastomosis with the ramus lateralis vagi (and glossopharyngei) the other he calls ram. oticus. He, however, draws the origin (l.c. fig. 3) of these branches so close to each other that, according to my opinion, one has to consider them as the strongly developed homologue of the ram. oticus of the Ganoids.

Of this organ PINKUS says (l. c. p. 307) "Das Organ ist zweifellos ein Derivat des Seitenkanales. Ueber seine Bedeutung vermag ich übrigens nichts auszusagen, da vergleichend anatomische und entwicklungsgeschichtliche Thatsachen mir bisher fehlen".

For the knowledge of the development we are indebted to AGAR, (1906) who examined the first stages of the spiraculum in Lepidosiren and Protopterus.

He showed that this sense organ is of ectodermal origin. This seat of origin reaches the top of the solid gut protuberance, which represents the spiracle, and then severs itself from the ectoderm. The organ then naturally gives the impression of having been derived from the entoderm.

AGAR like PINKUS, was not aware of the work of RAMSAY WRIGHT, otherwise he would undoubtedly have mentioned, that the presence of a spiracular sense organ in Holostei was already known. He also

would not have neglected to point out, that, in the Holostei, we have no reason to believe in the entodermal origin of the sense organ, now that in the Dipnoi [1]) its formation from the ectoderm is manifest.

As opposed to PINKUS, AGAR says "This organ has no relation to the lateral line system of sense organs". To my opinion, however, it undoubtedly belongs to this system, because it possesses a neuromast, is supplied by a branch from the lateral line system of the facial nerve, and moreover is clearly of ectodermal origin in the Dipnoi.

The majority of epidermal sense organs, sinks under the epidermis during the ontogenetic period, and finds protection by the subcutaneous connective tissue. Only one organ having its seat of origin in the immediate vicinity of the spiracle, sinks therein, acquiring a considerable development.

In my opinion this not only happens when the spiracle no longer breaks through outwardly, retaining its opening into the gut, as in the Holostei, but also, when it moreover loses its connection with the gut, as in the Dipnoi.

Let us now proceed to the Selachians. In these WRIGHT examined the spiracle of a 60 m.m. long embryo of Mustelus. Here he found two diverticula, situated above each other, on the medial wall. The dorsal diverticulum reached till under the canalis semicircularis lateralis of the auditory organ, and was already discovered in a number of adult Selachians, by JOH. MÜLLER (1841).

The ventral diverticulum did not reach the cranial cartilage, and at one place contained columnar epithelium, which he took for sense organ epithelium, and which according to him, was supplied by the ram. praetrematicus of the facial nerve. This innervation would lead us to expect, that we have here to deal with a different sense organ to that in the Holostei. PHELPS ALLIS, however, in 1901, examined a 122 m.m. long embryo of Mustelus, and was able to trace the nerve from the organ till near the ram. oticus, the same branch which also supplies the sense organ in the Holostei.

Independent of WRIGHT's work, that of VAN BEMMELEN appeared in the same year (1885). The latter, besides in Mustelus, found both the diverticula in a great number of Selachians, in embryos as well

---

[1]) GREIL (1913) mentions the ectodermal origin of the sense organ ("Hyomandibular organ") in Ceratodus, and its innervation by a branch from the lateral line system ("ram. hypoticus") of the facial nerve. Whether the sense organ in Ceratodus is afterwards also surrounded by the cranial cartilage, I do not find mentioned.

as in the adult fishes. He found both (the dorsal and the ventral) simultaneously in the same animal, in the forms which now-a-days, after TATE REGAN, are called Galeoidei. In rays on the contrary, only the ventral diverticulum of the examined fishes: Raja, Torpedo, Trygon and Myliobatis was found to be present. The dorsal one was absent in concurrence with the results of JOH. MÜLLER, who found it in rays only in the family of the Rhinobatidae.

Vice versa the ventral diverticulum was found missing, while only the dorsal one was present in Acanthias and Heptanchus; each of which is a representative resp. of the groups Squaloidei and Notidanoidei.

On the ventral diverticulum a follicle, resembling an oval bladder, develops in all forms which possess it. It nearly touches the auditary labyrinth, is lined on the inside with columnar epithelium, and is connected to the wall of the spiracle by a pedicle, which may, or may not have a lumen. In an adult Torpedo the bladder was found to be very large.

As regards the morphological significance of the follicle, VAN BEMMELEN thought of the probability of a homologue with the suprapericardial body, which primarily is also a single little bladder. He says (l. c. p. 178) "[später] tritt aber der grosse Unterschied ein: die Suprapericardialkörper entwickeln sich zu drüsenartigen Gebilden [1]) die Spritzlochbläschen treiben nur eine oder zwei acinöse Ausstülpungen oder bleiben wohl ganz einfach."

VAN BEMMELEN further thought of the probability of considering the follicle, even as the suprapericardial body, as the remains of an original gill cleft.

My opinion is that this conception cannot be adhered to any longer, and that the follicle is a spiracular sense organ bladder.

VAN BEMMELEN did not consider this possibility, because he had evidently not observed a supplying nerve.

No mention is made of the appearance of a follicle from the dorsal protrusion of the spiracle in the Galeodei. We may thus accept that it is absent there.

Acanthias and Heptanchus only show the dorsal protrusion. Is the spiracular sense organ now also found missing in them or not?

VAN BEMMELEN speaks of a "dorsale Ausstülpung", but also calls it an "Anhang" of the spiracle. He says: (l. c. p. 176). "Bei erwachsenen Exemplaren von Acanthias endlich konnte ich den Anhang

---

[1]) Their structure in the Selachians, then has much in common with that of the thyroid gland, from which they, however, totally differ morphologically.

als ein sackförmiges, ungefähr 3 m.m. langes Gebilde aus dem Bindegewebe frei präpariren, seine Wände zeigten sich ausserordentlich dicht und inwendig glatt, das Epithelium hoch und drüsig. Ebenso zeigte sich der dorsale Anhang von Heptanchus, aber relativ noch kürzer". As it will presently be seen, he undoubtedly dissected out the sense organ bladder.

HOFFMANN (1899) inter alia also investigated the development of the diverticulum of the spiracle in Acanthias. He found it to make its appearance first in 28 m.m. long embryos and innervated by a branch from the lateral line system of the facial nerve.

He considers this branch, which also supplies epidermal sense organs, most likely homologous to the ram. oticus of the Ganoids. The diverticulum is soon directed forwards with its blind end, and unites itself there with the nerve. I can confirm this from my material of Acanthias.

HOFFMANN discovered the innervation, well knowing of the work of WRIGHT, from which he quotes in detail. He, however, missed the conclusion that a sense organ had to be present. He was too much under the impression of having here to do with the vestigial part of a branchial pouch, which had disappeared.

Besides the two embryos of Heptanchus, my own investigation also includes a series of sections (15μ thick) through embryos of Acanthias varying in length from 23 to 98 m.m.

In the 23 m.m. long embryo, the anterior wall of the spiracle forms a rostrally directed diverticulum, next to the auditory organ, from which it is separated by the jugular vein (the nervus facialis running under the vein). The diverticulum is to be seen on 7 sections anterior to the external opening of the spiracle, and has the shape of a cone flattened on one side, the axis of which runs parallel to the longitudinal axis, passing through the notochord. The three anterior ones of the seven sections pass through the top of the cone, which is distinguished by its columnar epithelium, so that the lumen appears for the first time on the third section. One also sees the termination of the branch of the ram. oticus connected here to the group of the columnar cells. HOFFMANN already pointed out, that one could stipulate, through this connection the situation of the organ before it is more clearly defined.

A cross section through the anterior margin of the external opening of the spiracle on the skin at the same time passed through the internal opening towards the intestine in an embryo of 39½ m.m. of which I in 1922 described the skull. The diverticulum is to be seen on 21 sections rostralwards. Just as in the embryo of 23 m.m.

it runs forwards along the auditory capsule and is separated from it by the jugular vein and the facial nerve [1]).

If we trace the diverticulum from the base rostrally, we see it after 8 sections already changed into a flat and narrow duct with a lateral and medial wall. The duct is prolonged over 4 sections, and then with nearly no change of lumen, passes over into the top part of the diverticulum, which is perceptable on 9 sections. The medial wall of this part has over its whole length a neuromast, whose posterior end is clearly defined. Near the rostral end (the blind top) of the diverticulum the branch of the ram. oticus unites with the neuromast.

We may now, proceeding from the anterior margin of the spiracle, distinguish three parts, seen resp. on 8, 4 and 9 sections which we shall call vestibulum of the spiracle, excretory duct and corpus of the sense organ bladder.

Excretory duct and corpus are partners, but the vestibulum is nothing more than an ordinary diverticulum of the anterior wall of a visceral pouch, and disappears later, in consequence of the enlargement of the external opening of the spiracle.

The vestibulum is still present in an embryo 69 m.m. long, but in embryos of 78 m.m. or more, it has disappeared. We then only see on a section, passing posterior to the anterior margin of the spiracle, the opening, which meanwhile has become very minute, of the excretory duct. Then the condition of the sense organ bladder principally corresponds to that of the organ which occurs in the adult animal. It then forms an appendix of the spiracle. The description by VAN BEMMELEN of the Galeoidei and rays also applies to the sense organ of Acanthias.

Probably these bladders are homologous in all the Selachians and of ectodermal origin. They have in some forms sunk somewhat deeper into the spiracle, than in others. We shall still examine the little bladder sowewhat closer in a series of cross sections of the Acanthias embryo 98 m.m. long.

The very minute opening in the anterior wall of the spiracle is only to be seen in one section. From here the organ passes rostralwards over 50 sections. It runs along the auditory organ from

[1]) During the translation of this paper I prepared a series of sagittal sections, stained with haematoxylin and eosin, of a 22 m.m. long embryo of Torpedo marmorata. I found the deep neuromast at the inner wall of the spiracle innervated by a branch of the ram. oticus, crossing the outer side of the vena jugularis, just as in Acanthias.

which, — as previously — it is seperated by the jugular vein and the facial nerve.

The corpus of the bladder, with its long neuromast, is visible on the anterior 21 sections. The excretory duct falls in the following 29 sections. The neuromast thus nearly constitutes half the length of the organ, and is much larger than in the lateral line system organs of the skin. Round the corpus one sees the mesenchym in more compact formation, the first stage of a connective tissue capsule. The excretory duct, immediately posterior to the corpus, shows a different construction than further caudalwards.

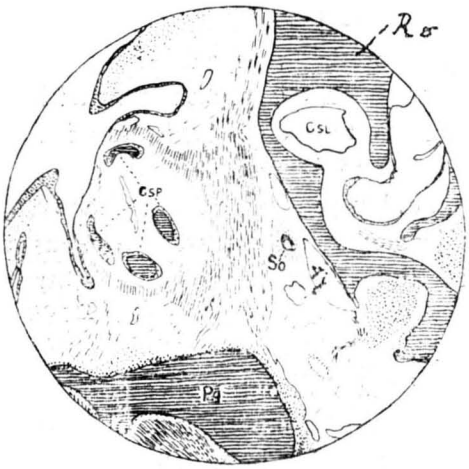

Fig. 2*a*. Cross section through the otic region of the skull and the anterior wall of the spiracle, from a 98 m.m. long embryo of Acanthias vulgaris.

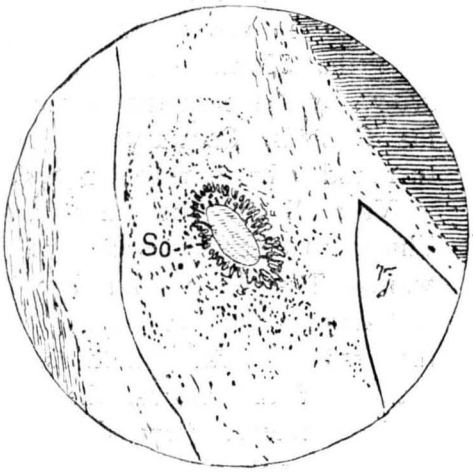

Fig. 2*b* shows the spiracular organ under high power. Its contents, mucus (stained blue in section) are seen as thin striations.

On the first 5 sections behind the corpus, the medial wall of the duct is thickened, as the result of the proliferation of the outer layer of epithelium cells. Here the oval lumen is wider than in other places. The longitudinal axis of the oval is more or less twice as long as in the corpus. On the following 24 sections this lumen continually decreases, the wall consisting of two layers of cells. Those of the inner layer are very flat, those of the outer layer may be called cubic.

It is of importance that the corpus of the sense organ bladder and the proximal part (5 sections) of the duct, should be filled with mucus, which in this stage (and later) allows itself to be stained blue, just as in the ampullary and canal organs of the lateral line system. In the distal part of the duct (24 sections) the mucus is present in lesser quantity.

From this we may see, that the spiracular sense organ shows itself to belong to the lateral line system of epidermal sense organs, which is generally also understood by the term mucus-organs. The direct proof has not yet been given, but may perhaps be found in stages earlier than those which I have studied.

The ram. oticus, in all the studied embryos, arises with a ganglion like thickening from the buccal ganglion of the facial nerve.

In the 39½ m.m. long embryo, it runs along the cartilage of the ear capsule — but not yet surrounded by the cartilage — dorsally and caudalwards. It sends off a few thin branches to the organs in the lateral line canal of the regio otica, and a thick branch, which goes to the spiracular sense organ across the jugular vein.

In the 98 m.m. long embryo, a part of the ram. oticus is overgrown by the cartilage of the ear capsule. This is also the case with the Ganoids. Contrary to the Selachians the sense organ itself is surrounded by cartilage in both Ganoids and Dipnoi.

We shall now pass on to the 63 m.m. long embryo of Heptanchus. The small external opening of the spiracle is here situated far backwards. The fissure like opening in the gut reaches still further rostralwards. If we accept that the beginning — the base — of the vestibulum falls on the section which passes through the anterior margin of this fissure, then the top of the vestibulum lies still 28 sections further forwards. In this top the sense organ bladder opens without an excretory duct. It can be traced in 12 sections rostralwards, along the auditory organ, from which it is separ-

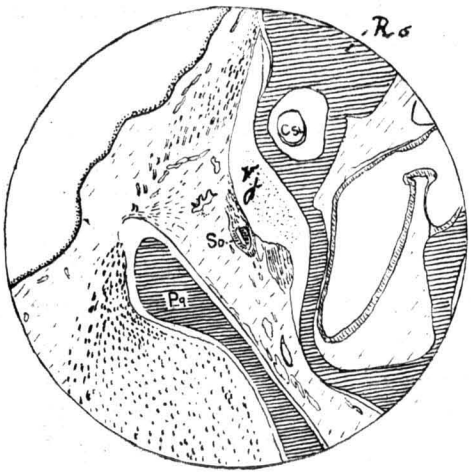

Fig. 3*a*. Cross section through the otic region of the skull of a 63 m.m. long embryo of Heptanchus cinereus.

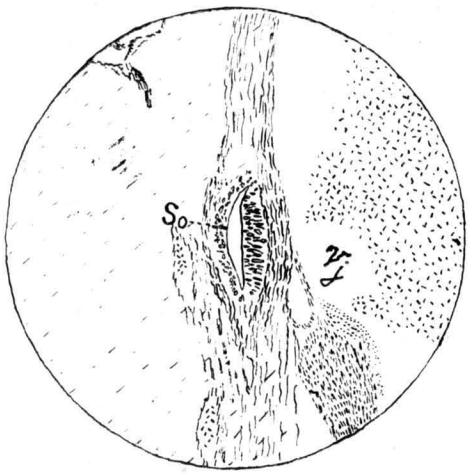

Fig. 3*b* shows the spiracular organ under higher magnification.

ated by the jugular vein. The neuromast on the medial wall just projects with its posterior margin from the vestibulum.

I was not successful in finding the supplying nerve. Perhaps it is owing to the intensely stained connective tissue capsule, which is more developed than in the largest of the examined embryos of Acanthias. In the 225 m.m. long embryo the organ was so badly preserved, that nothing of importance can be mentioned [1]).

3. *Fenestra vestibuli.*

In the 63 m.m. long embryo of Heptanchus, the attachment of the hyomandibular to the auditory capsule is brought about by a thin layer of connective tissue, wherein I can find no cavity of

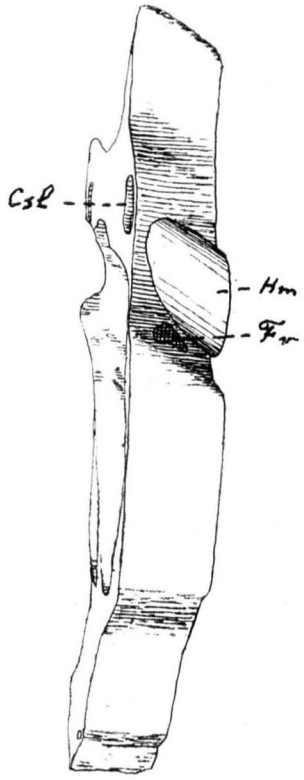

Fig. 4. Lateral surface of the model of a disk from the cartilage of the regio otica of an embryo of Heptanchus cinereus. The disk is placed in such a position that a part of the anterior surface with the canalis semicircularis lateralis, is just visible, and the fenestra vestibuli is not covered by the upper part of the hyomandibular.

[1]) Before the translation of this paper, the work of VITALI (Anat. Anzeig. 1911 and 1912) had escaped my notice, and I am indebted to Dr. BENJAMINS of Utrecht for having called my attention to it. As he remarks, this paratympanic organ in birds must be the homologue of the spiracular sense organ. An interesting referate of the works of VITALI on this organ by RUFFINI "Sull organo nervoso paratimpanico di G. VITALI od organo del volo degli uccelli" is to be found in "Archivio Italiano di Otologia Rinologia e Laringologia" publ. by GRADENIGO. Vol. 31, 1920.

articulation. It is prolonged over 49 sections, 15 μ thick. Immediately ventral to the anterior portion of this place of attachment one sees in the sections 5, 6 and 7 (in antero-posterior sequence) a connection through a small opening in the wall of the auditory capsule, between the mesenchym which in this stage fills the perilymphatic space, and the mesenchym outside the capsule. The posterior margin of the opening is not clearly defined, so that it remains dubious whether the hole is present in the next three sections or not. On the contrary the margins of the opening in the 255 m.m. long embryo, are clearly defined. The attachment of the hyomandibular to the capsule takes place here on about 59 sections 30 μ thick (in all the other embryos the sections are 15 μ thick).

The opening reaches from the 8th to the 25th section (counted antero-posteriorally). It is closed by a deeply red stained connective tissue, which also helps to connect the hyomandibular to the skull, and which is rather conspicuously surrounded by the blue colour of the cranial cartilage. The opening lies in the under part of the fossa for the hyomandibular, which partly covers it.

From the wax model of Mr. P. J. DE VRIES, made according to the method of BORN, one can see that the opening is not truly oval, but rather kidney-shaped, because the under margin forms a re-entering concavity. The mesenchym which formerly filled the perilymphatic spaces, has to a large extent disappeared and been replaced by a liquid, which is prevented from flowing out, by the connective tissue closing the opening.

The opening, owing to its position, has to be considered as the homologue of the fenestra vestibuli, which in Amphibians and Amniotes is closed by the stapes, and which according to general opinion would be absent in fishes.

Owing to the great length of the embryo, it must have been more or less fully developed, and it is improbable that the fenestra would not persist after birth.

I, however, had no opportunity of examining adult material. Irrespective of the autostylic Dipnoi and Holocephali, fishes are as a rule hyostylic. Their powerful hyomandibular functions in the first instance as a suspensorium. This fact evidently has to do with the absence of a fenestra vestibuli. Only two primitive forms viz. Heptanchus and Hexanchus are amphistylic. Their hyomandibular, owing to the firm attachment of the palatoquadrate to the skull, can only feebly function as a suspensorium. It is therefore conceivable, that the hyomandibular, at least in Heptanchus, may still have the function of transferring vibrations to the auditory organ.

The presence of the fenestra in the embryo is in any case a support to the old theory, which in later years has frequently been attacked, the theory namely: that the stapes in higher animals is homologous to the hyomandibular in fishes.

INDEX LETTERS.

Csl. Canalis semicircularis lateralis.
Csp. Cart. spiracularis. Each of the two spiracular cartilages (fig. 2*a*) is sectioned twice.
E. Top of the epibranchial of the first branchial arch.
Ep. Epithelial body.
Fv. Fenestra vestibuli (ovalis).
Hm. Hyomandibular.
K_2. Second branchial cleft.
Pq. Palatoquadrate.
Ro. Regio otica of the skull.
So. Spiracular sense organ.
Th. Thymus.
Vj. Vena jugularis.

LITERATURE.

AGAR, W. E. The Spiracular Gill Cleft in Lepidosiren and Protopterus. Anat. Anzeiger, Bd. 28, 1906.

ALLIS Jr. E. PHELPS. The Lateral Sensory Canals, the Eye Muscles and the Peripheral Distribution of certain of the Cranial Nerves of Mustelus laevis. Quart. Journ. of micr. Science, Vol. 45, 1901.

BEMMELEN, J. F. VAN, Ueber vermuthliche rudimentäre Kiemenspalten bei Elasmobranchiern. Mitth. a. d. zoologischen Station zu Neapel, Bd. 6, 1885.

BRAUS, H. Ueber den embryonalen Kiemenapparat von Heptanchus. Anatomischer Anzeiger, Bd. 29, 1906.

BRIDGE, F. W. On the Osteology of Polyodon folium. Phil. Trans. Roy. Soc. Vol. 169. 1879.

DOHRN, A. Die Entwicklung und Differenzirung der Kiemenbogen der Selachier. Mitth. a. d zoologischen Station zu Neapel, Bd. 5, 1884.

FRITSCHE, F. Die Entwickelung der Thymus bei Selachiern. Jenaische Zeitschr. f. Naturwiss. Bd. 46, 1910.

GOODRICH, E. S. On the Structure of the Excretory Organs of Amphioxus. Quart. Journ. of micr. Sc. Vol. 54, 1909.

GREIL, A. Entwickelungsgeschichte des Kopfes und des Blutgefässystemes von Ceratodus forsteri. Denkschr. d. Medicinisch Naturwiss. Ges. zu Jena, Bd. 4, 1913.

HAMMAR, J. AUG. Zur Kenntnis der Elasmobranchier-Thymus. Zool. Jahrbücher, Abt. f. Anat. und Ontog. Bd. 32, 1912.

HOFFMANN, C. K. Beiträge zur Entwicklungsgeschichte der Selachii. Morph. Jahrbuch, Bd. 27, 1899.

MÜLLER, JOH. Vergleichende Anatomie der Myxinoiden. Dritte Fortsetzung. Abh. der Kön. Akad. d. Wissensch. zu Berlin, 1841.

PINKUS, F. Die Hirnnerven des Protopterus annectens. Morph. Arbeiten, herausgeg. von Schwalbe. Bd. 4, 1895.

WRIGHT, R. RAMSAY. On the hyomandibular Clefts and Pseudobranchs of Lepidosteus and Amia. Journ. of Anat. and Phys. Vol. 19, 1885.