Anatomy. — *"The myelencephalic gland of Polyodon, Acipenser and Amia".* By Dr. C. J. VAN DER HORST. (Central Institute of Brain Research, Amsterdam). (Communicated by Dr. C. U. ARIËNS KAPPERS.)

(Communicated at the meeting of April 25, 1925).

When dissecting the brain of Polyodon I found a darkly pigmented, lozengeshaped mass surrounding the foremost part of the spinal cord closely behind the calamus scriptorius. This structure encircles the spinal cord very closely. At the dorsal side it reaches somewhat further caudad than at the ventral side. I prepared this mass with the brain out of the skull and have cut it in serial sections which are stained in different ways. While examining this mass, it appeared to be a bloodgland, the histological structure of which will be discussed closer in this paper.

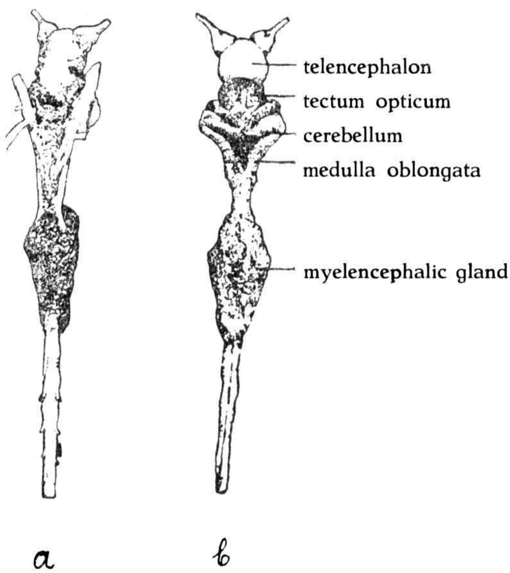

Fig. 1. Brain and frontal part of the spinal cord of Polyodon. *a* ventral, *b* dorsal.

In literature a similar gland is only known in Lepidosteus, where it has been accurately described and figured by CHANDLER. In this fish the gland covers a part of the medulla oblongata, being situated a little more frontally than in Polyodon. CHANDLER calls it "myelencephalic gland", a name which I wish to obtain here, though the gland is situated quite behind the myelencephalon in Polyodon. As this gland immediately strikes us while dissecting the brain, this somewhat more caudal situation can not be the reason that CHANDLER did not find it in Polyodon, no more than in Amia, where it is also very large and even situated on the myelencephalon, and in Acipenser, where the gland is to be found partly in front of and partly behind the calamus scriptorius. CHANDLER himself admits the possibility that the gland was removed together with the perimeningial tissue in the brains of the fishes, examined by him. This must have been indeed the case. In Polypterus, however, I could not find the gland though it certainly should still be present in the specimen dissected by me, if it had been there.

Neither could I find it in Megalops or in other teleosts. CHANDLER suggests the possibility, but not the probability, that the saccus endolymphaticus which covers the fourth ventricle in Protopterus according to BURCKHARDT may be related in some way to the bloodgland of Lepidosteus. Besides that here this is a real saccus endolymphaticus, so a part of the internal ear, this organ of Protopterus has quite a different microscopical structure than the myelencephalic gland of Lepidosteus, so that in my opinion all relation must be excluded. So this gland is found as a well defined structure only in Lepidosteus and Amia, Polyodon and Acipenser.

MOODIE described fossil fish brains from the coal measures of Kansas. In all probability these are brains of representatives of the Palaeoniscidae, a family related to the recent sturgeons. In these brains closely behind the calamus scriptorius a large eminence is found. MOODIE supposes this eminence to be an unpaired vagal lobe. But as such large vagal lobes are found only in Cyprinidae and Siluridae among recent fishes and not at all in Chondrostei, I think it very probable that this structure is also a myelencephalic gland.

In Amia the myelencephalic gland has the shape of a triangular mass that covers the fourth ventricle and the foremost part of the spinal cord. The apex of this triangle is turned to the front and reaches the caudal side of the cerebellum. At the base of the triangle the gland surrounds the spinal cord in a wide curve. According to my opinion the wideness of this curve cannot only be ascribed to shrinkage. There must be a good distance between the glandular tissue and the spinal cord in Amia, which is not the case in Polyodon and Acipenser. Behind the vagal root the gland is connected with a mass in the shape of a long quadrangle that reaches at the ventral side as far frontal as is the case with the gland at the dorsal side. The earlike lobes, described by CHANDLER in Lepidosteus, are lacking in Amia as well as in Polyodon and Acipenser.

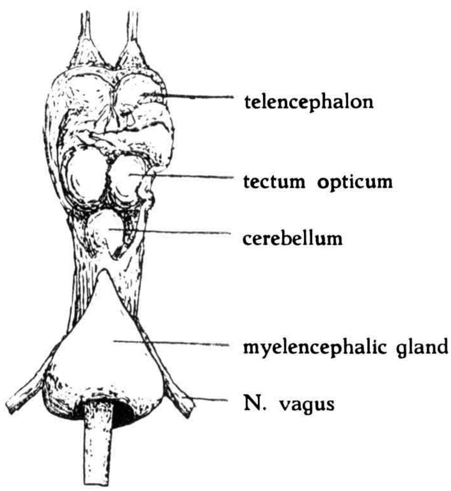

Fig. 2. Brain with myelencephalic gland of Amia.

In a young specimen of Acipenser ruthenus I found the myelencephalic gland near the calamus scriptorius. When seen from the dorsal side it has the same shape as the gland in Polyodon, but at the ventral side of the spinal cord the glandular tissue is not present, so that the gland is crescentic in cross section. In a very large specimen of Acipenser sturio a large mass of fat is found at the same place. So it is probable that the gland in Acipenser degenerates in older animals. This is certainly not the case in

Amia, because the specimens, examined by me, were adult ones. Of Polyodon I possessed only young specimens of about 20 to 25 cm. total length. So I can not find out, if the gland in Polyodon also becomes degenerated at higher age.

At the microscopical examination the gland appears to be situated in the perimeningial tissue wholly external to the meninx primitiva. Whereas in Amia there is a great space between the meninx primitiva and the glandular tissue, in Polyodon the gland immediately touches the meninx and in cross section it extends from the meninx to the perichondrium (fig. 6).

The microscopical structure of the myelencephalic gland is very much like that of the spleen. The glandular tissue itself consists of rather large polygonal cells. They have a large nucleus which may be simply round, but in general it is oval or of an irregular shape (fig. 3). Often it is curved, sometimes S-shaped, sometimes the nucleus may consist of some pieces, which, nearly without mutual connection, lie scattered in the protoplasm.

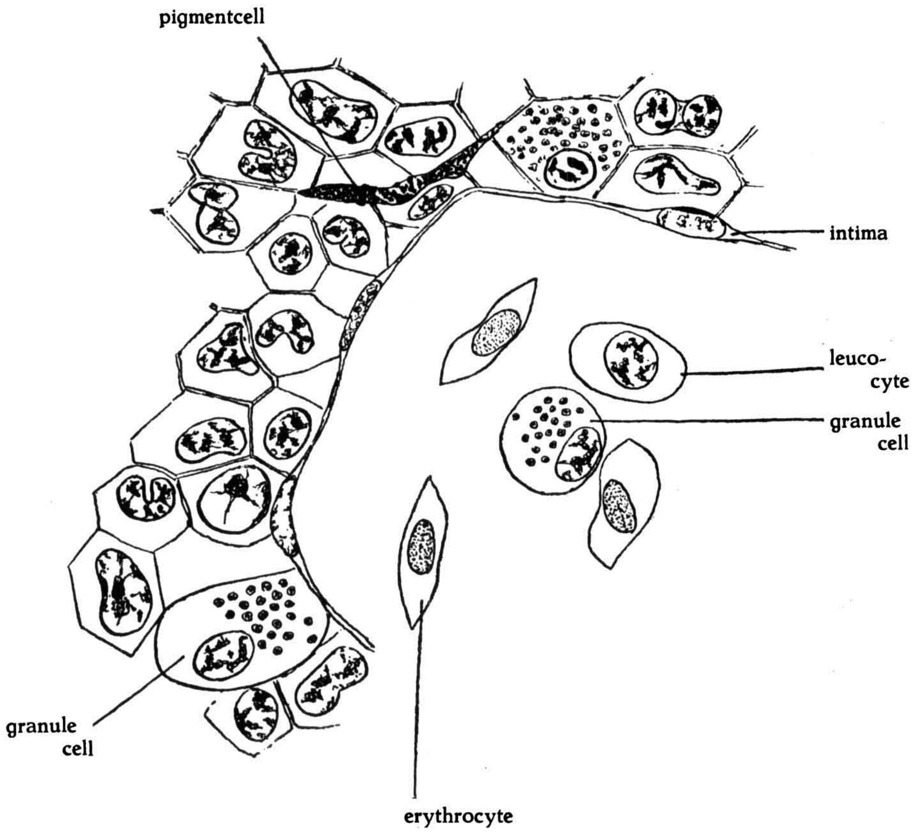

Fig. 3. Tissue and bloodsinus of the myelencephalic gland of Polyodon.

In this respect, as also in size, reaction to dyes and structure of the protoplasma and the nucleus these cells quite agree with the leucocytes, which are found numerously in the bloodvessels. I think I have the right to suppose that these cells are young leucocytes which find their origin here, though I did not find mitoses. But often these cells can be seen projecting partly in a vessel.

In this tissue, cells are scattered with a more regular nucleus, which strike us by a great number of granules in the protoplasma. Some are not or hardly larger than the leucocytes, but most of them are up to twice as large. These same cells are found in certainly the same if not in greater number in the bloodvessels, also I found some projecting partly in a vessel. The number of granules in these cells varies very much. In general the small ones have few granules, the larger ones possess more, some are wholly filled with granules. These granules are strongly stained with haematoxyline, so that contrastaining with eosine had no result. Especially in Weigert-preparations these cells are very obvious, the granules being stained intensely black by this method. My material does not allow me to examine, whether we have before us oxyphil or eosinophil leucocytes with coarse granules or basiphil mastcells. According to CHANDLER the granular cells are intensively stained with eosine, KAPPERS found them stained with picrid acid. On the other hand SUNDWALL mentions that the granular cells occurring in other animals in similar places which will be discussed later on show a decided affinity to basic deyes.

According to Miss SABIN, who described the development of the different bloodcells in the chicken, the granulocytes have no relation whatsoever to the endothelial cells. Also Miss DANTSCHAKOFF has demonstrated that the granulocytes are extravascular from origin. I found this confirmed in Polyodon. All endothelial cells have a much smaller nucleus than the leucocytes or the granulocytes. But these two latter ones are closely related to each other. As has already been said before, the granulocytes with few granules are not larger than the leucocytes and the presence or the lacking of these few granules in that case is the only difference between these cells.

In this tissue a venous network of capillaries and sinusses is found, almost quite filled with erythrocytes. These erythrocytes are oval in shape, like in all lower vertebrates, and seen from the side they are spindleshaped, showing often a slight depression in the centre (fig. 3). They have a rather small oval nucleus, in which the chromatine is regularly distributed. In this respect they can be distinguished at once from the leucocytes, in which the chromatine is irregularly distributed. According to CHANDLER the erythrocytes are scattered freely throughout the gland, entirely independent of vessels of any sort. I could not find this in my preparations, the erythrocytes being found only in the bloodvessels. Also I think it probable that the cells, called erythrocytes by CHANDLER, are not erythrocytes, as they quite deviate from the characteristic form of these cells in other animals.

The bloodsinusses are only bordered by a well defined intima, consisting of very flat endothelial cells with oval nuclei, so they have quite the character of perimeningial bloodsinusses. In the larger sinusses the intima is continuous (fig. 3). But in the smaller ones interruptions seem to occur in the intima. This is certainly the case, where leucocytes or granulocytes protrude in the vessels.

At the periphery the gland is more compact and the capillaries cross it in all directions, but they are few in number compared with the amount of glandular tissue. In the midst of the gland and at the dorsal side of the spinal cord, however, the number of capillaries is much more numerous. Also these capillaries run in fronto-caudal direction for the greater part. The glandular tissue between them is reduced to columns with a breadth of a few cells.

At the caudal side and dorsal to the spinal cord the glandular tissue passes in a tissue which seems to be adipose tissue. At least in the celloidine sections this tissue proves to consist of a great number of round cavities with protoplasma and some nuclei between them ; cellmembranes could not be discerned here (fig. 4). This same tissue was found also at the frontal side of the gland, but here ventrally to the spinal cord. In paraffine sections this tissue is greatly deformed and lookes like a reticular mass. CHANDLER

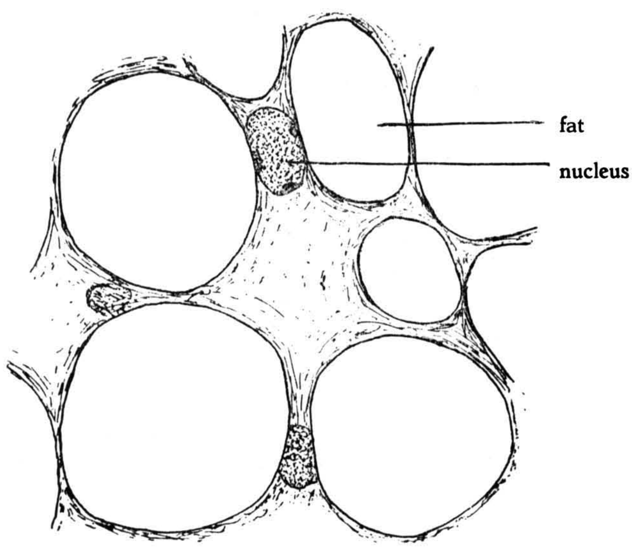

Fig. 4. Adipose tissue near the myelencephalic gland of Polyodon.

describes it as such in Lepidosteus. However, with this difference that the reticular network is found here scattered everywhere between the glandular tissue, so that CHANDLER supposes it to be a framework for the entire structure. In this tissue the bloodvessels of the gland collect in some sinusses, which are situated, for the greater part at least, frontally at the ventral and caudally at the dorsal side of the spinal cord.

In Amia the triangular structure covering the oblongata also consists of similar glandular tissue. As has been said before, in this animal the gland is connected behind the vagus root with a mass of tissue, situated at the ventral side of the oblongata. In the parts connecting the dorsal and ventral masses the glandular tissue is substituted gradually by the reticular connective tissue. Probably this is also of an adipose character, though I can not say this with certainty. This tissue is very obvious in Amia by the great number of veins, so that the whole mass ventral of the oblongata has a somewhat red color. The dorsal side of the gland itself in Amia is covered also by a thin layer of the reticular tissue, whereas in Polyodon the glandular tissue is in direct contact with the perichondrium of the vertebral canal.

A very striking element of the gland and of the surrounding tissue are large, profusely branched pigmentcells. In Polyodon they are especially striking, the gland being nearly wholly black. In Amia I found less of these cells and they are much smaller than the pigmentcells in the membranes that cover the midbrain and the cerebellum. According to CHANDLER these pigmentcells send their branches freely in all directions between the glandu-

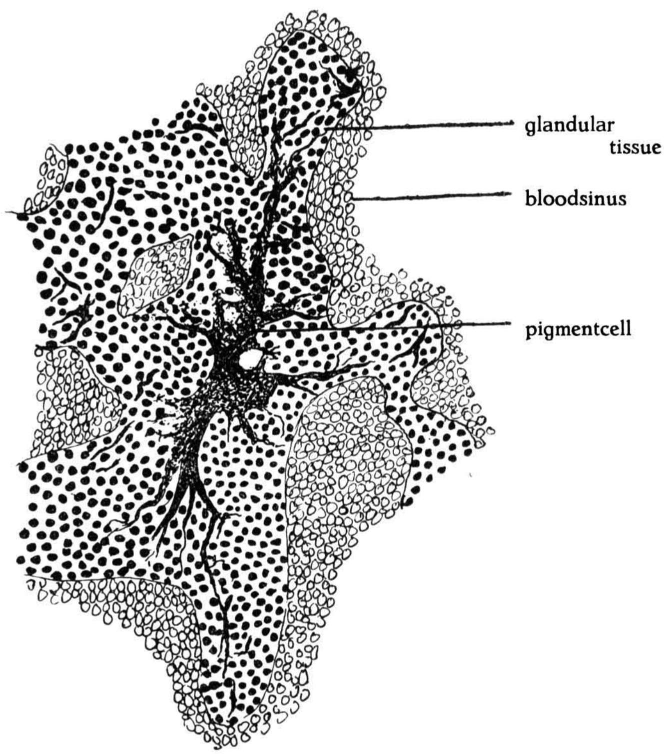

Fig. 5. Pigmentcell in the myelencephalic gland of Polyodon.

lar tissue in Lepidosteus. In Polyodon, however, I found these black pigmentcells in a dense layer immediately below the surface of the gland, where they arborize principally parallel to the surface, so that in cross sections they look like lines and are seen only in their real shape in the first sections that touch the gland, there where the surface of the gland is almost in the plane of the sections (fig. 5). Between the glandular tissue only a few of these cells are situated and these are scattered and send their branches in all directions. However, they increase in number again around the spinal cord, where they arborize again parallel the surface of the spinal cord. In the connective tissue frontal and caudal to the gland only few of these pigmentcells are situated except at the surface of the large bloodsinusses in this tissue, where there are many.

Concerning the bloodsupply of the gland I observed the following in Polyodon: Where the carotis interna reaches the brain near the infundibulum, it sends a large branch in caudal direction, the arteria encephalica posterior, described by STERZI in selachians. The right and the left artery run together in the perimeningeal tissue at the ventral side of the midbrain and the medulla oblongata in caudal direction, giving off again and again larger or smaller branches which penetrate into the brainsubstance. In the most caudal part of the oblongata I twice found an anastomosis of the right and the left artery; a total union to an arteria basilaris, however, as has been described by STERZI in selachians, does not exist here. But taking in account the more caudal relations, the right artery may be compared with the arteria basilaris. Later on the left artery splits up in two branches. One branch runs along the left to the dorsal side of the spinal cord and seems to serve principally for the bloodsupply of the spinal cord. The other branch curves in ventral direction and runs there caudad at a great distance from the spinal cord but still situated in the perimeningeal tissue. In the frontal part of the gland this artery approaches the spinal cord again, but here it is much smaller, so in this way it is not excluded that small sidebranches have been given off, though I could not find them. I will go on to call this artery the left one, just as the other the right one, though these names are suitable only in respect to the situation of the arteries in front of the gland. In the gland itself these names are not properly suitable, as we will see. In the mean time also the right artery has given off a sidebranch that runs along the right side of the spinal cord in dorsal direction, while the right artery itself goes on in caudal direction. Soon it splits in two equal parts, one of which runs approximately straightly caudad, while the other part curves in ventral direction but soon approaches again the spinal cord, where both parts of the right artery continue in caudal direction symmetrically situated, in the same way as happens with the right and the left artery below the medulla oblongata. In this respect the right artery corresponds to the arteria basilaris of selachians that splits also in two vessels running parallel to each other. In the foremost part of the gland these two arteries approach the spinal cord more and more, so that finally they are taken up in the meninx primitiva, which is much thickened here at the ventral side (fig. 6). In this thickened part of the meninx also the left artery penetrates running here in caudal direction between the two other arteries. The two parts of the right artery remain in the meninx primitiva and seem to serve mainly for the bloodsupply of that part of the spinal cord, that is surrounded by the gland. Gradually they diminish in size and at the caudal end of the gland they have almost disappeared. The left artery leaves the meninx primitiva again and arborizes in the gland in a rather regular way. The small arterial capillaries fall into the much wider sinusses. With regard to the veins in relation with this gland, none of my series is in such a condition that all details could be observed completely. At the caudal end of the gland a number of sinusses and vessels is situated in the reticular tissue, surrounding the spinal cord. More caudad these sinusses decrease in size and at the end of my series they have almost quite disappeared.

It was very evident that the blood is gathered from the gland in two veins situated at both sides of the gland immediately against its periphery. In the best series, in which the brain with the gland is prepared out of the cartilaginous skull and vertebrae this lateral vein is thickest in about the

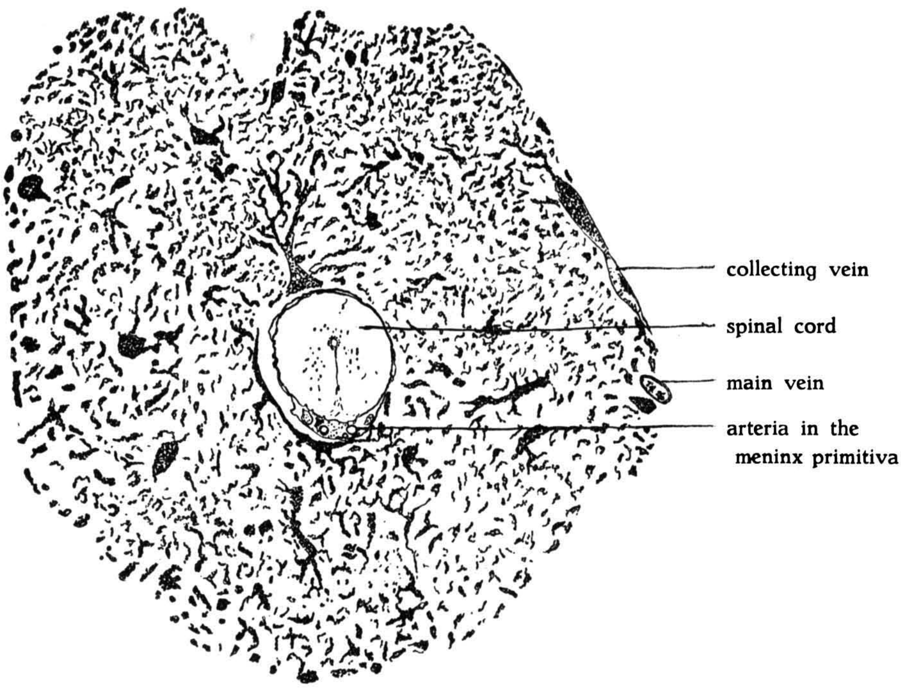

Fig. 6. Cross section showing the cervical cord surrounded by the myelencephalic gland of Polyodon. Only the bloodcavities of the gland are figured.

midst of the gland, also it has here a well developed wall. From this place the vein spreads as well in frontal as in caudal direction over the surface of the gland. The vein is cut off at place where it curves away from the gland in lateral direction, so I suppose that it leaves the vertebral canal here (fig. 6).

In another series a sheath of cartilage is left around the gland, but by the difference in contraction of the cartilage and the glandular tissue the sections are very much folded, some are even torn, so this series in general is less useful. However, I found here a vein leaving the vertebral canal together with a spinal nerve. At the frontal side a vessel strikes us, running at the dorsal side of the spinal cord from the gland to the choroid plexus of the fourth ventricle and splitting up in a number of sinusses lying on this menbrane (see also fig. 6). This vessel can be distinguished immediately from the other sinusses and veins by the great number of pigment cells lying along the thin wall in the same way as is the case in arteries.

Against the wall of other venous bloodvessels also pigment cells are found but in far smaller number.

Furthermore frontal of the gland and at the ventral side of the spinal cord a great number of venous cavities is found, which anastomose again and again. A little caudal of the calamus scriptorius a part of this cavities curves around the spinal cord in order to spread on the choroidal roof of the fourth ventricle.

Most of the blood, however, seems to flow away through a wide vein, that leaves the perimeningial tissue in the ventral midline.

It still remains an open question whether the blood runs from the gland to the choroidal roof through the dorso-median vein or in the opposite direction from the choroid to the gland. The latter seems more probable to me. In the first place because an artery comes from the midbrain in the dorsomedian line that arborises in the choroidal plexus and secundly both venae encephalicae posteriores, that run, according to STERZI, parallel the vagus and discharge the blood from the plexus in Selachians are lacking here.

As said before I found this myelencephalic gland only in Polyodon, Acipenser and Amia, while it has been described also by CHANDLER in Lepidosteus, which is confirmed by KAPPERS.

In other fishes, Cyclostomes, Selachians, Teleosts, Polypterus, which I have studied, I found no gland. However, this does not prove that there is really nothing at all. As one of the components of the gland I have described the very characteristic granular cells. I found — like KAPPERS — the same granular cells in great numbers in Ceratodus, scattered on the choroid plexus of the fourth ventricle between the numerous bloodvessels and in the meningial tissue. Indeed these granular cells seem to occur in vertebrates in general. So SUNDWALL has described them accurately in different mammals, they are mentioned also by DEWEY. GOLDMANN described them in rats as pyrrolcells. It is difficult to say in how far the mastcells, described by MCKIBBEN in Necturus, as also the "Wanderzellen" mentioned by KOLMER, correspond to the granulecells, as I found in Polyodon.

As said by MCKIBBEN, the mastcells in the meninges of Necturus, if studied superficially look like neurones on account of their long thin branches. Also the "Wanderzellen" figured by KOLMER in Triton have the same shape. It is remarkable that these cells occur also at the ventricular side of the choroid membrane. Although I do not doubt KOLMER's statement, I have never seen this in the preparations of the different amphibians, which I studied. According to KOLMER these cells occur in the choroid plexus of all vertebrates, so in the same place, where SUNDWALL and other authors found the more simply shaped granulecells. So it seems probable to me that we are dealing here with the same cells and that the difference in shape is only the result of the functional state and perhaps of the fixation. LEHNER also has shown that mastcells may make amoeboid movements and lately NEUMANN mentioned that also the eosinophil cells are able to do so.

I did not find the granule cells that occur in such a large number in the myelencephalic gland in the choroid of the fourth ventricle in Polyodon. This indicates that these cells, scattered in other vertebrates, are accumulated in a structure of definite form in these four fishes. This seems also to be the case in reptiles, but in a much less degree, at least KOLMER mentions accumulations of a typical shape on the choroid plexus near the calamus scriptorius.

Different authors have indicated (KAPPERS, KOLMER, a.o.) that the plexus can be vascularised to such an extent that it can be looked upon as a bloodgland. So KOLMER found all lymphatic elements in it in a great number especially in the dog and says that it gives the same impression as small pieces of bone marrow scattered on the choroid.

In the four mentioned ganoids we do not only find a localisation but also an enormous increase of the granule cells compared with other vertebrates. And furthermore the organ has the typical structure of a bloodgland.

There are different opinions about the function of the granule cells. Some authors suppose the granules to be nucleines, others derivatives of haemaglobine and the opinion exists also that the granules contain oxydases. I think that the granule cells are of phagocytic character. In the blood-cavities of the myelencephalic gland I found many more or less deformed erythrocytes and these were always in the neighbourhood of the granule cells. Also the granules are always stained in the same way as the nuclei of the erythrocytes. This was striking especially in the Weigert preparations, where only these granules and nuclei are intensively black, whereas the nuclei of the other cells are red by the contrastaining with paracarmin.

For an experimental research of the function of the granule cells, especially in relation to their situation on the choroid or in the neighbourhood of the brain, the fishes, mentioned in this article, are a very good material, because here the whole organ and together with this all granule cells can be removed easily, whereas in other vertebrates this is excluded on account of their widely spread occurrence.

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