UC-NRLF

B 3 670 170

GIFT OF
asa c. chandler

462

308 t
C455

UNIVERSITY OF CALIFORNIA PUBLICATIONS

IN

ZOOLOGY

Vol. 9, No. 2, pp. 85-104, pls. 10-12

December 28, 1911

ON A LYMPHOID STRUCTURE LYING
OVER THE MYELENCEPHALON
OF *LEPISOSTEUS*

BY
newford
ASA C. CHANDLER

CONTENTS

	PAGE
Introduction	85
Occurrence and homologies	87
In genus <i>Lepisosteus</i>	87
Absence in other Ganoids	87
Possible homology with " <i>Saccus endolymphaticus</i> " in <i>Protopterus</i>	88
General morphology	88
Histology	90
Development	93
18-22 mm. stages	93
55 mm. stage	94
Summary	95
Literature cited	97

INTRODUCTION

The present paper is a preliminary report on a peculiar gland-like, lymphoid structure discovered over the myelencephalon of *Lepisosteus* while dissecting out the brain in the laboratory of Professor B. G. Wilder, at Cornell University, during the fall of 1910. The work was begun in Cornell University during the college year 1910-11, and later carried on in the Zoological Laboratory at the University of California.

Grateful acknowledgments are here made to Professors C. A. Kofoid and J. Frank Daniel, of the University of California,

A Thesis submitted to the Department of Zoology in partial fulfillment of the requirements for the degree of Master of Science in the College of Natural Sciences, of the University of California, May, 1912.

and to Professors H. D. Reed, B. F. Kingsbury and W. A. Hilton, of Cornell University, for their supervision and assistance, as well as for the material used, and to Professor B. G. Wilder for his helpful advice and the free use which he allowed of the specimens in his neurological collection.

The structure here described, which I shall provisionally call the myelencephalic gland, was discovered while dissecting out the brain of a long-nosed gar, *Lepisosteus osseus*. While dissecting away the cartilage bit by bit in the region of the hind brain, there was noted a deeply pigmented mass of tissue lying over the myelencephalon, and directly behind the cerebellum. The first inclination was to tear this off with the cartilage and dura mater as merely a pigmented mass of connective tissue, such as often fills the subdural space in teleosts (see Wiedersheim, 1909, fig. 200, p. 294). Since, however, it was seen to have a rather definite form, and to be in closer relation to the brain than to any other part of the head, it was left in position, and carefully dissected out with the brain, to the pial covering of which it was firmly attached.

Though many eminent and competent scientists have worked over the brain of *Lepisosteus*, the myelencephalic gland seems almost universally to have been overlooked. The only reference to it which could be found in the literature on the brain of the ganoids is by Herrick (1891). In his plate 13, figure 9, he figures the dorsal aspect of the brain of *Lepisosteus osseus*, showing the structure in question lying over the myelencephalon. No reference is made to it in the text, and in the description of the figure he merely says: "The bilobed mass lying behind the cerebellum is not of a nervous character." But, as pointed out by Wilder (1891), the membranous parietics of the brain are an important morphological feature of the organ, and should be considered in any treatment of the brain as a whole. As will be shown later, the "bilobed mass" of Herrick is in direct connection with the parietics of the brain, and therefore should not be omitted from a morphological study of the brain because it is not of nervous tissue.

Parker and Balfour (1882) carefully worked out the brain of the adult *Lepisosteus*, and demonstrated the delicate thin-

walled "vesicle of the thalamencephalon," which had previously escaped notice and is readily destroyed in dissection, yet they make no reference to any gland-like structure lying over the myelencephalon, and the usual opening into the fourth ventricle is shown in their figure. They also made sections of the entire head of embryos up to the 26 mm. stage in working out the development of the brain, but, since the gland is not recognizable, as such, up to that stage, it escaped their notice again.

In tearing off the gland in a dissection of the brain, it is impossible not to tear off the posterior medullary velum with it, which accounts for the foramen of Magendie usually shown in figures of the brain of *Lepisosteus*, as seen in the figures of Busch (1848), Mayer (1864), Owen (1868, vol. 1, fig. 174), Huxley (1872, fig. 38), Wilder (1875, pl. 2, fig. 7), Parker and Balfour (1882, pl. 25, fig. 47B), and Allen (1907, pl. 6, figs. 11 and 12). Kingsbury (1897, pl. 6, fig. 5) has a diagrammatic sketch of a cross-section of the myelencephalon in which he shows the roof of the fourth ventricle undisturbed, but without the gland.

OCCURRENCE AND HOMOLOGY

The possibility that this gland might be an abnormal development presented itself, and to determine this point two other specimens of *Lepisosteus osseus* were dissected. The same structure was found in each of them, and was of the same size and shape. It was also found in *Lepisosteus platystomus*, only one specimen of which was available. No example of *L. tristaechnus*, or alligator gar, could be obtained for dissection, but on account of the similarity of this species to *L. platystomus* in other respects, and also because of the very close similarity of the gland in the two more divergent species examined, it is probable that this organ will be found in the third species.

It was thought that some such development would be found in other types of ganoid fishes, and in the hope of finding something at least suggestive of it, numerous specimens of ganoid brains in the collection of Dr. Wilder were examined, including examples of *Acipenser*, *Scaphirhynchus*, *Polyodon*, and *Amia*. In none of these, however, could any indication of it be found,

though the possibility exists that in some it may have been present, and have been torn off in dissection. *Polyodon* has a very deeply pigmented pial covering of the brain, but it remains thin and membranous over the fourth ventricle. I myself exposed several brains of *Amia* with the myelencephalic gland especially in mind and found no trace of such a gland there. This was unexpected, since *Amia* is without doubt the form nearest allied to *Lepisosteus*.

In an article on the central nervous system of *Protopterus annectens*, only the preliminary report of which was accessible to me, Burckhardt (1892) figures the dorsum of this dipnoan brain with a structure possessing numerous diverticula lying over the hind-brain, which he calls the "saccus endolymphaticus." This figure is reproduced by Wiedersheim (1909, fig. 201A, p. 296). In a longitudinal section of the same brain (fig. 202B in Wiedersheim) this "saccus" has seemingly been removed, as the myelencephalon is covered only by the much folded choroid plexus. Whether or not Burckhardt's "saccus endolymphaticus" of *Protopterus* is in any way related to the myelencephalic gland in *Lepisosteus*, I am unable to say at the present time, but judging from the name and its appearance in the figure, it is highly improbable. If this should be found to be homologous, it is all the more strange that it does not appear in *Amia*.

GENERAL MORPHOLOGY

The general form of the gland in *Lepisosteus osseus* is not bilobed as described by Herrick (1891), but it is trilobed. The main body of the gland is slightly wider than long, and is thickened just cephalad of the middle, sloping off towards the front and back, and with a pronounced median sulcus caudally, causing the posterior border to be emarginate. From the antero-lateral angles there projects on either side an ear-like lobe, very definite and constant in shape and size, connected with the main body by a rather slender neck (fig. A). These ear-like projections are entirely surrounded by cartilage, making their dissection rather difficult, although they readily hold their shape and position when freed from the cartilage.

Fig. A. Dorsal aspect of brain of *Lepisosteus osseus*, with myelencephalic gland in situ. $\times 10$. *ant. l. cb.*, anterior lobe of cerebrum; *post. l. cb.*, posterior lobe of cerebrum; *opt. l.*, optic lobe; *trilob. cbl.*, trilobed cerebellum; *myelen. gl.*, myelencephalic gland; *sp. cd.*, spinal cord.

In *Lepisosteus platystomus* the gland is almost identical in form to that in *L. osseus*, but the ear-like projections are relatively shorter and stouter than in any specimen of the latter species which I have examined.

The size of the gland as compared with the rest of the brain is considerable. The width of the main body in a 25 cm. specimen is slightly greater than the width of the optic lobes, which form the widest dimensions of the brain, while the width from tip to tip of the lateral lobes is more than twice that of the cerebrum at its greatest width. Its length is approximately that of the cerebellum, and its thickness such that it attains the level of the dorsum of the cerebellum. In adults the size of the gland as compared with the rest of the brain is even greater than this.

The entire brain of *Lepisosteus* is covered by a deeply pigmented membrane which has a peculiar metallic appearance, in which the enormous pigment cells appear under the binocular microscope as a sprinkling of pepper. This covering membrane seems to be directly continuous with the covering of the myelencephalic gland, or with the gland itself, since in microscopic section there is no distinctly differentiated outer covering. In other words, the structure seems to be a very highly developed and enlarged portion of the pigmented covering of the brain.

HISTOLOGY

In order to determine the histological structure of the myelencephalic gland, the best specimen available was fixed in formalin, imbedded in paraffin, and cut in sections 12 microns in thickness. The sections were then stained in Delafield's haematoxylin, and counter-stained with eosin or with picro-fuchsin.

When placed under the microscope, it became at once apparent that the structure under study was not of nervous tissue, but appeared as an enormous development of the pial covering of the brain. In some of the sections there appeared to be a cavity, but this proved to be nothing more than a very large central blood vessel or sinus. In the section drawn (pl. 10, fig. 1) the true pia mater is indistinctly differentiated from the gland across the dorsum of the fourth ventricle and is fused with it. Where the pia mater curves down over the side of the

medulla, however, the tissue of the gland is continued into it as shown in the region marked "A" in plate 10. As stated above, there is no apparent differentiated covering of the gland.

Of the various histological elements present, the most conspicuous are the extremely large and irregular, black pigment cells, scattered irregularly throughout the whole organ. These cells are similar to those in the pigmented covering of the brain, except that they are not so flattened, and send their branches freely in all directions. Under the high power of the microscope, they show the typical granular structure of melanin pigment cells, and the granules are often somewhat scattered at the periphery, where the cell has been cut. Some of these pigment cells measure fully eighty microns from tip to tip of their branches.

In the middle of the dorsal part of the gland there is an open reticulum of connective tissue which is gradually encroached upon by the more solid substance (pl. 10, fig. 1). From the microscopical appearance of other parts of the gland, there is much evidence that this reticulum forms the framework for the entire structure, the other elements being netted in it. The appearance of this network in a "solid" part of the gland may be seen in plate 11, figure 2, which represents such a portion highly magnified. Farther cephalad than the region shown in plate 10, figure 1, which represents a section slightly in front of the middle, the network becomes more and more open, until, on the sides under the cerebellum, nothing is left but the reticular connective tissue with a few pigment cells in it. Blood vessels are of frequent occurrence, running in all directions, and ranging from very large ones visible to the naked eye to very minute capillaries. Even in the open network of connective tissue surrounding the hinder part of the cerebellum, and constituting the cephalic portion of the structure in question, blood vessels ramify quite freely. The pia mater on the sides of the medulla, and surrounding the cerebellum, is almost a solid mass of blood vessels, which are densely crowded with corpuscles, so crowded, in fact, that they appear as solid masses of tissue, and their identity was for some time in doubt. Caudally, the open network of connective tissue is lost entirely, and the gland appears solid throughout, and denser than the portion drawn (pl. 11, fig. 2).

The histological elements found in the connective tissue network of the gland, in addition to pigment cells, are of three kinds: (1) large, clear cells with small, deeply staining nuclei (pl. 11, fig. 2, *eryth.*); (2) large cells more or less deeply clouded with blue in material stained in haematoxylin, and showing evidence of reticular chromatin network (pl. 11, fig. 2, *leuc.*); and (3) cells filled with masses of granules staining deep red with eosin, and yellow with piero-fuchsin (pl. 11, fig. 2, *gran. m.*).

The first cells above mentioned seem to be erythrocytes, as they have precisely the same appearance as those filling the vessels in the pia mater, where they are associated with fairly numerous leucocytes. They are scattered freely throughout the gland, entirely independent of vessels of any sort. This is very remarkable for an animal which has a closed blood system, and no explanation for such a phenomenon, if they really be erythrocytes, has yet been found. These cells range from eight to ten microns in diameter, are clear and transparent, with small, round, deeply-staining nuclei, and are irregular in outline, though this may be due to slight shrinkage or contact with other cells.

The second cells above described have much the appearance of large leucocytes. They are very uniformly round in outline, and vary from seven to nine microns in diameter.

The most peculiar and characteristic element present, however, are the numerous cells filled with granules. The granules are about one and a half microns in diameter, and are very nearly the same in size as the melanin granules in the pigment cells. Where a pigment cell and a mass of granules have been cut in close proximity and both types of granules slightly scattered, it is difficult to distinguish them except by color. As stated above, they stain a very deep red with eosin, suggesting eosinophile granules in leucocytes, but they are larger and occupy the cell more completely, as figured by Rawitz (1900). With piero-fuchsin, on the other hand, they stain a deep yellow, similar to the color given to muscle fibres. In many of the vessels of the gland, especially in the larger ones, there are areas filled with a substance which strongly suggests granules in process of disintegration, and occasional scattered granules still intact may be

found in these areas. It is further significant that the substance stains exactly the same as the granules, red with eosin, and yellow with picro-fuchsin. I cannot say that the granules do pass into the vessels and disintegrate, but there is no positive evidence against it, and there are some facts in favor of it. Although the granules appear to be normal, the possibility exists that they may be due to parasitism, or some other abnormality.

The masses of granules, from ten to twelve microns in diameter, are held together by some membrane, probably a very thin cell wall, but it is not evident in the sections. The granules are sometimes scattered somewhat when the mass is cut across just as are the melanin granules in the pigment cells. Associated with each mass there is a fairly large nucleus which shows much more plainly in some cases than in others (pl. 11, fig. 2). Due to the lighter coloring of the granules with picro-fuchsin, the nuclei show best with that stain. These granular masses are found in varying density throughout the gland, except in the open connective tissue network immediately behind and around the cerebellum. In the main body of the gland they are scattered in approximately the density shown in plate 11, figure 2, though possibly on an average slightly more numerous. In the ear-like projections, however, they are far more dense, so dense, in fact, as to obscure all the other kinds of cells, and to conceal the reticular network entirely.

DEVELOPMENT

Thinking that the origin and development of this gland-like structure might throw some more definite light on its nature and function, I sectioned a series of embryos ranging from 6 mm. to 22 mm. in length. The heads of these embryos were stained in toto in Delafield's haematoxylin, imbedded in paraffin, and cut in sections 10 microns in thickness.

As the eosin counter-stain was the most effective with the adult, this was likewise used for the embryos.

Up to the 18 mm. stage there could be found no indication whatever of any structure lying over the myelencephalon, the ependymal lining of the brain cavity coming in close juxtaposition to the cartilaginous roof of the skull, or lying immediately

beneath the skin in the very young specimens in which the cartilaginous roof is not yet developed.

In the 18 mm. specimen was found the first indication of any tissue intervening between the brain covering and the roof of the skull, in the form of a blood sinus, a space across the dorsum of the myelencephalon filled with blood corpuscles. In the 21.5 and 22 mm. stages the condition is very similar except that the blood sinus is larger and more conspicuous. A typical section through the myelencephalon of the 21.5 mm. embryo is shown in plate 12, figure 3. Between the band of columnar endothelial cells covering the fourth ventricle, and the cartilaginous roof the skull, there is a space largely filled with blood corpuscles, and with a few strands of reticular connective tissue. A few scattered pigment cells may also be seen. Immediately beneath the skin, even in those embryos where the roof of the skull is not yet developed, there is a dense layer of pigment cells. As the cartilage grows over the dorsum, it is easy to see how some of the pigment cells might be pinched off and left inside, there to multiply and cause the apparently useless pigmentation of the covering of the brain. At any rate, it seems highly probable that the pigment cells found there are derived from the pigmented layer of the integument.

The next embryo available for study was a 55 mm. specimen which was cut in sections 10 microns in thickness and stained with Delafield's haematoxylin and erythrosin. The condition there presented is extremely instructive, as it is in every way intermediate between the 22 mm. stage and the adult form. The space between the covering of the fourth ventricle and the skull is much widened, being at least as wide as the depth of the fourth ventricle. This space is largely empty, but is partially filled by a reticulum of connective tissue (pl. 12, fig. 4). In this reticulum are large blood sinuses, more or less densely crowded with corpuscles, and with a number of large, scattered pigment cells. The latter differ from those in the adult structure in that they are far more regular in outline, often nearly round, and without the dendritic branches displayed later. It will be noticed that in plate 12, figure 4, there are lateral outpocketings of the ependymal epithelium. Farther caudad these outpocket-

ings are larger and much more pronounced, curving forwards or backwards, so that in some sections they appear entirely separated from the ependyma, and look like cavities lined with epithelium and surrounded by the reticular connective tissue lying over the myelencephalon. Farther caudad than the region represented in plate 12, figure 4, also, the cavity over the brain is much extended laterally, and the lateral portions are largely filled in with embryonic connective tissue cells. These regions seem to be the centers of proliferation of the connective tissue, as here the cells are hardly differentiated, while towards the median line the reticular processes of the cells become more and more developed, and the cells themselves become fewer in number. At this stage the gland has not the definite outline which is apparent in the adult, and there is still no indication of the leucocytes or granular masses which form such a conspicuous part of the fully developed structure.

It has not been possible thus far to obtain a specimen intermediate between this 55 mm. stage, and a young adult of 250 mm., the brain of which is figured entire in figure A. This specimen has not yet been sectioned, but as it is adult in all characters except size, it is doubtful whether it will throw any more light on the development of the gland. It has, in this specimen, the characteristic shape, but is slightly smaller in proportion to the brain than in older individuals.

SUMMARY

1. A lymphoid, gland-like structure overlies the myelencephalon of *Lepisosteus*, and is closely associated with the pial covering of the fourth ventricle. Though large and conspicuous, it has been almost universally overlooked by workers on the brain of *Lepisosteus*.

2. The myelencephalic gland (provisionally so named) is present in both *Lepisosteus osseus* and *L. platystomus*, which are the only species of the genus examined by me. No indication of it has been found in other ganoids.

3. The size and form are very constant in all specimens dissected and are practically the same in both species. Topographically it seems to be a highly developed portion of the pia mater.

4. The microscopic structure is suggestive of a lymph gland. A reticulum of connective tissue underlies the whole, and in this a number of other histological elements are netted.

5. The most characteristic feature is the abundance of cells containing masses of granules staining deeply with eosin, of unknown nature, and resembling melanin granules in size and form.

6. There is some evidence that the granules mentioned above pass into the blood vessels and there disintegrate.

7. The earliest indication of the structure is in an 18 mm. embryo, where there is a blood sinus lying between the covering of the fourth ventricle and the cartilaginous roof of the skull. This sinus is larger and more conspicuous in embryos 21.5 and 22 mm. in length.

8. In a 55 mm. embryo the condition is directly intermediate between the 22 mm. stage and the adult. The connective tissue reticulum and pigment cells are well developed, and large blood sinuses are still present, but the granular masses so characteristic of the adult are not yet in evidence.

LITERATURE CITED

ALLEN, W. F.

1907. Distribution of the subcutaneous vessels in the head region of the Ganoids *Polyodon* and *Lepisosteus*. Wash. D. C., Proc. Acad. Sci., 9, 79-125, pls. 1-15.

BALFOUR, F. M., and PARKER, W. N.

1882. On the structure and development of *Lepisosteus*. Phil. Trans. R. Soc., London, 173, pt. 2, 359-442, pls. 21-29.

BURCKHARDT, R.

- 1892A. The central nervous system of *Protopterus annectens*, preliminary report. J. Comp. Neur., 2, 89-91, pl. 13.
1892B. Das Centralnervensystem von *Protopterus annectens*, 8vo., 64 pp., 5 pls. (Friedländer, Berlin).

BUSCH, W.

1848. De selachiorum et ganoideorum encephalo. (Berlin.)

HERRICK, C. L.

1891. Contribution to the comparative morphology of the central nervous system. III. Topography and histology of the brain of certain ganoid fishes. J. Comp. Neur., 1, 149-182, pls. 11-13.

HUXLEY, T. H.

1872. Manual of the comparative anatomy of vertebrates (New York, Appleton), 431 pp., 110 figs. in text.

KINGSBURY, B. F.

1897. Encephalic evaginations in ganoids. J. Comp. Neur., 7, 37-44, pl. 6.

MAYER, F. J. C.

1864. Ueber den Bau des Gehirns der Fische in Beziehung auf eine darauf gegruudete Eintheilung dieser Thierklasse. Halle. Nova Acta Leop., 30, 40 pp., 7 pls.

OWEN, R.

- 1861-68. Comparative anatomy and physiology of vertebrates. (London, Longmans, Green). I. xlii, 650 pp., 452 figs. in text.

RAWITZ, B.

1900. Ueber die Blutkörperchen einiger Fische. II. Ganoiden und Teleostier. *Arch. f. mikr. Anat.*, **56**, 149-168, pl. 6.

WIEDERSHEIM, R.

1909. Vergleichende Anatomie der Wirbelthiere. (Ed. 7, Jena, Fischer), xx, 935, 1 pl., 476 figs. in text. Trans. of 6th ed. by W. N. Parker (New York, Macmillan), 1907, xii, 576, 372 figs. in text.

WILDER, B. G.

1875. On the brains of *Amia*, *Lepisosteus*, *Acipenser*, and *Polyodon*. *Proc. Amer. Assn. Adv. Sci.*, **24**, 168-193, pls. 2-3.
1891. The morphologic importance of the membranous or other thin portions of the parietes of the encephalic cavities. *J. Comp. Neur.*, **1**, 201-203.

EXPLANATION OF PLATE 10

Fig. 1. Cross-section of the myelencephalon and myelencephalic gland of a *Lepisosteus osseus*, 250 mm. in length. Section taken slightly cephalad of middle of gland, and cut farther cephalad on right than on left. $\times 12$.

ABBREVIATIONS

- A*—region where tissue of gland extends into pia mater.
- 4th vent.*—fourth ventricle.
- bl. ves.*—blood vessel.
- col. c.*—columnar epithelial cells.
- conn. tiss. n.*—connective tissue network.
- d. gran. m.*—region dense with granular masses.
- endol.*—endolymph.
- fl. c.*—flattened epithelial cells.
- inf. pl.*—infolding of choroid plexus.
- med.*—medulla oblongata.
- nerv.*—nerve.
- pig.*—pigment cell.
- p. m.*—pia mater.

PLATE 11

Fig. 2. Highly magnified portion of myelencephalic gland of *Lepisto-
steus osseus* from region marked with circle in figure 1. $\times 764$.

ABBREVIATIONS

conn. tiss. n.—connective tissue network.

eryth.—erythrocyte?

gran. m.—granular masses.

leuc.—leucocyte?

PLATE 12

Fig. 3. Cross-section of myelencephalon of 21.5 mm. embryo of *Lepisosteus osseus*, showing "anlage" of myelencephalic gland. $\times 112$.

Fig. 4. Cross-section of myelencephalon of 55 mm. embryo of *Lepisosteus osseus*, showing further development of myelencephalic gland. $\times 85$.

ABBREVIATIONS

- bas. art.*.—basilar artery.
- bl. sin.*.—blood sinus.
- cart.*.—cartilage.
- conn. tiss. n.*.—connective tissue network.
- epend.*.—ependyma.
- eryth.*.—erythrocytes.
- med.*.—medulla oblongata.
- pig.*.—pigment cell.
- 4th vent.*.—fourth ventricle.

NON-CIRCULATING BOOK

U.C. BERKELEY LIBRARIES
C040002002

Chandler

244474

UNIV