Such warming and cooling can be repeated a couple of times, whilst in proportion to this the number of reflections continues varying, provided the muscle be not for too long a time exposed to too high a temperature, as in this case a clearly perceptible permanent hardness will show itself.

Physiology. — "*On the structure of the ganglion-cells in the central nervous system of Branchiostoma lanc.*" (Second communic) By Dr. J. BOEKE. (Communicated by Prof. G. C. J. VOSMAER).

(Communicated in the Meeting of May 31, 1908).

a. *The infundibular organ.*

The cells of the differentiated part of the ventral cerebral wall of Branchiostoma, which I described some years ago in these Proceedings [1]), and which was then called the *infundibular organ* on account of its place and the homology that could be drawn from that, are quite different in their structure from the other cells, of which I gave a description in my former paper [2]).

Among the authors, who in recent years have published researches on the central nervous system of amphioxus, KUPFFER [3]) gives the same description of the cells as I gave in my paper in 1902, and only mentions the organ as consisting of long cylindrical cells with curved cilia and a clear hyaline protoplasm. KUPFFER homologises the differentiated epithelium with the tuberculum posterius of the craniote embryos. JOSEPH [4]) only mentions the organ without adding anything to the description. EDINGER [5]) who examined preparations stained after the method of BIELSCHOWSKY, calls it "das aüs grossen Flimmer- und Sinneszellen bestehende Infundibularorgan", without mentioning on what is founded the opinion, that there are two kinds of cells to be found. In the drawings reproduced in his paper nothing is to be seen but a faint striation of the ventral wall of the brain at the place of the infundibular organ. According to WOLFF [6]) there is a striking resemblance between the differentiated epithelium of the infundibular organ and the gelatinous tissue that we find in the

1) Proc. Roy. Acad of Sc. of Amsterdam, Math. Phys. Cl. Meeting of April '07 p. 86.

2) Proc. Roy. Acad. of Sc. of Amsterdam, Math Phys. Cl. Meeting of April '02 p. 695.

3) Handbuch der Entwickelingslehre (HERTWIG), Vol. 2, 3d part.

4) Verhandl. d. Anat. Gesellsch. 18. Vers. 1904.

5) Anat. Anzeiger, Bd. 28, 1906

6) Biol. Centralblatt. Bd. 27, 1907.

central nervous system of the higher vertebrate animals, but evidently he has not seen much of the real structure of the tissue. Besides these statements nothing more is to be found about this part of the brain of amphioxus, and so we seem to be justified in giving an exact description of it here.

To do this it is necessary to study first of all very thin carefully orientated median sections, as well as frontal and cross sections; the statements by EDINGER made it necessary to examine a great many BIELSCHOWSKY-preparations to form a correct opinion in this matter, hence we took so long for our research.

From the very early period at which the infundibular organ is regularly found and the constancy with which it appears, always in exactly the same form and structure, it is evident that it must play a distinct and important part in the animal's life. Already in larvae with only three primary gill clefts the differentiated epithelium is very obvious. Just where the narrow central canal opens into the wider part of the brain-ventricle, we see the ventral limit of the central canal rise slightly and sink again to the former niveau immediately after. This elevation is caused by the cells in the ventral wall growing out into long cylindrical elements, each cell bearing a long hair or cilium curving backwards, the cells lying regularly one beside the other.

It is an important feature in the development of the infundibular organ that the elongation of the cells first shows itself not in the median line but at the left side of the median plane; afterwards the cylindrical cells are also found at the right side. It is only at a much later stage that the long cells fuse in the median line and become one single mass. This and peculiarities in the course of the nerve-fibres springing from the cells in the full-grown organ, point to a bilateral origin of it.

The cylindrical elongation of the cells is the only change we find. There is no indentation at all of the wall of the brain in front of the organ to be found.

Already in very young animals we see in well-preserved and well-stained preparations that the cilia of the long cells point backward with a slight curve, the cilia of all the surrounding cells pointing forward, to the anterior neuroporus.

In older specimens we find the same state of things, but the cells get still more elongated, and the nucleus, now being small and spherical, is lying near the basis of the cell. All cells are directed backwards, that is to say, their free surface being turned craniad. (fig. 8).

For the topographical relations of the differentiated epithelium to

the other parts of the brain I refer to my former paper (1902).

I can only state here in reference to the contradictory statement of EDINGER, that even in more developed and in full-grown specimens I never found another kind of cells in the organ nor an indentation of the brainwall in front of the infundibular organ (KUPFFER). In fig. 1 is drawn a median section of the full-grown organ, and here we see that the cells are not slanting any more, but are directed perpendicularly to the longitudinal axis of the body. In slightly younger animals one often finds the greater part of the cell still curved backward, while the upper part of the cell has assumed the perpendicular direction already (fig. 4*a*). The cause of this change must be sought in the different rate of growth of the surrounding tissue, the whole cerebrum becoming shorter, and changing from an oblong into a more rounded form.

As I mentioned before, the cells of the infundibular organ have all a backwards curved cilium; these cilia form a plume reaching to the narrow part of the central canal. In young animals being examined alive under the microscope the transparent tissue all round the brain ventricle allows the course of these cilia to be very clearly visible, and then the cilia of all the surrounding cells, pointing forward to the anterior neuroporus, appear as straight hairs forming a compact bundle which runs towards the neuropore, into which the hairs can be traced as long as it is open. The back end of this bundle of cilia is crossed by the cilia of the infundibular-cells.

The form of the cells in the full-grown animal is shown in fig. 4*b*. The neurofibrillar differentiation in the protoplasm of the cell, as I described it already in my former paper, the neurofibrillar network round the nucleus and the way, in which the neurofibrilla leaves the cell is in Fig. 3 clearly to be seen. The course of the nerve-fibres after they left the cell-body I could not trace much farther with a sufficient amount of certainty. They all seem to curve backwards (caudal), and from the study of frontal sections it was possible to draw the conclusion that the nerve-fibres springing from the cells form two bundles, each at one side of the median plane, running backwards, but getting lost to view between the other fibres of the medulla very soon after.

I never succeeded in finding an indentation of the ventral cerebral wall in front of the infundibular organ, as described by KUPFFER, although a large number of serial sections were examined. It is true, that, as I mentioned before, often the nuclei in the ventral wall in front of and behind the differentiated epithelium lie closer together than in the other regions and in a few cases the arrangement of

these nuclei made the impression of a solid indentation. But upon closer examination I always found that this was only an apparent and no real indentation (infundibulum). Here one must be very careful not to draw any conclusions from a few series of sections. In a median section through the infundibular organ from one of my longitudinal series of a 47 mm. long Branchiostoma one would be inclined to draw the conclusion that there exists a groove-shaped indentation of the brainwall behind the organ, no trace of any indentation being found in front of it. So I think it dangerous to found a homology on this indentation, as KUPFFER did, and I adhere to the denomination "infundibular organ", as its structure and development have more resemblance with the epithelium in the saccus vasculosus of the ichthyopsidae, to which I gave the same name, than with the tuberculum posterius, which is still somewhat problematical.

b. *Shape and development of the brain-ventricle.*

I will here only mention those facts that are important for the comparison between the Branchiostoma cerebrum and that of the craniotes, and for the question whether the differentiated epithelium mentioned above may be homologised with the infundibular epithelium, or with the tuberculum posterius.

The second homology might be concluded from the drawing published by KUPFFER in 1894 and 1903, representing a median section through the cerebrum of a 2 c.m. long amphioxus. But this drawing seems to me not to represent the real state of things. Neither exactly orientated median sections (fig. 8) nor the median sections reconstructed from series of cross-sections (fig. 7) ever gave me anything like this drawing.

And yet it is in this case that the reconstruction-method must give an absolutely certain result. By this method we are able to correct entirely the deviations of the cerebral axis from the longitudinal axis of the body as they are found in almost every specimen. And as the cerebral vesicle has such a simple uncomplicated form this method gives us in every case an exact reproduction of the median section (which is certainly of the high value for the comparison of the brain KUPFFER ascribes to it), and at the same time allows us to get a sure knowledge of the width of the cerebral cavity. I give here three drawings of the median sections reconstructed from the cross-sections, one of a very young larva of 3,4 m.m. (fig. 5), one of a young amphioxus of 10 m.m. (fig. 6) and another of a specimen 21 m.m. long (fig. 7). All these are.

reconstructed from cross-sections of 5 μ, magnified 800 to 1600 times, and afterwards reduced by means of photography. To fig. 5 I added the cross-sections lying on the spots indicated with *a*, *b*, *c*, *d*, to show the width and form of the cavity at the different spots. In fig. 8 I give the reproduction of a real median section through the brain of a specimen of about the same age as the one the reconstruction of which is given in fig. 7, to show how much they are like each other.

The reconstruction of fig. 5 shows, that even in very young larvae, (larvae of 1.5 to 2 m.m. give about the same picture), in which the brain is still larger in diametre than the spinal cord, there exists a dorsal dilatation of the cerebral cavity, which may be compared with the fourth ventricle of craniote embryos (fig. 5, 6, *VQ*). It represents a dorsal dilatation of the central canal (fig. 5*c*) and is connected with the anterior vesicle by a narrow part (fig. 5*b*). In all my specimens this connection of the ventriculus quartus with the anterior vesicle could be stated with absolute certainty, contrary to the well-known observations by HATSCHEK. Even in very young larvae the connection was very conspicuous. In the caudal part of the dorsal dilatation (fig. 5*d*) the midpart of the narrow fissure-like central canal is obliterated, so that this part of the fourth ventricle is separated from the ventral central canal which remains open. In older animals this obliteration proceeds craniad. The dorsal wall of the fourth ventricle is very thin consisting of one layer of flattened cells, but it is always visible even in very young larvae, if only the specimens are well-preserved.

In much older individuals, which passed through the metamorphosis long ago (fig. 6), the fourth ventricle is still very conspicuous and connected with the anterior vesicle by a narrow dorsal canal. The dorsal wall is still thin and membranous. The large dorsal ganglion-cells (vide my former paper) that are now developed to a certain extent, are still only visible at both sides of the median plane and do therefore not appear in the median section through the brain. Afterwards this peculiar group of cells is developed to such an extent (fig. 7, fig. 8), that they occupy the entire dorsal part of this region of the central nervous system, and so appear also in the median section. It is only then that the distinct fourth ventricle becomes indistinct, irregular, flattened, alters its shape and even disappears here and there. Then we find the peculiar irregular dilatations of the central cavity, described by KUPFFER as "quere Schenkel" and "blasenförmige Erweiterungen". They are not segmental, are only of secondary importance, and are not to be com-

pared with special parts and stages of development of the brain of craniote embryos.

After these statements I will add a few words concerning the cranial or rostral part of the cerebrum and the adjacent organs.

In his paper of 1906 EDINGER describes a new organ, the "frontal organ", lying in front of the brain and being innervated by a special nerve. I regret to say that I (no more than WOLFF in his paper of 1907) could find no trace of a frontal organ. Even after a most careful study of a number of individuals I can only find in the rostrum the often queerly shaped irregular mucous canals (Schleimcanäle) lying ventrally and dorsally of the chorda. They are never connected with the epidermis, but all receive very thin nerve-fibres from the first cerebral nerve.

Although the existence of a distinct nerve connecting the olfactory groove of KÖLLIKER with the brain, is denied by EDINGER, I could find it in my preparations as a bundle of fine nerve-fibres, connecting the sensory cells of the groove with the dorsal part of the brain. In all respects I could affirm the exact observations of DOGIEL (1903) [1]) both concerning the sensory cells in the olfactory groove and the nervous connection of them with the brain.

In the dorsal part of the cerebral wall I find a distinct commissural system, wherefrom bundles of nerve-fibres curve backwards (much like the fasciculus retroflexus of the commissura posterior of the craniotes) and a few fibres curve round forward. There are more systems of fibres to be found in the wall of the cerebral vesicle, but they are rudimentary and composed of only a few fibres. This is not the place to enter into details about these things. But when we take all this into account I think it is not permissible to consider the amphioxus-cerebrum as an "archencephalon" (KUPFFER), that has remained on a very low stage of development, but we must regard it as a degenerated cerebral system, which has become rudimentary in many of its parts, a brain which has many of the features of the brain of the ichthyopsides, but there are entirely lacking the organs of the side-line system (lens of the eye, ear, side-line) and because of that and of the fact, that the head has not developed as in the higher vertebrates, it is degenerated and rudimentary. In connection with this and with the elongation of the chorda the foldings of the cerebral vesicle do not appear. Even a plica ventralis does not exist. The infundibular organ remains in the niveau of the ventral cerebral wall.

Leiden. Histological part of the Anat. Kabinet.

[1]) Anatomische Hefte 21. Bd. 1903.

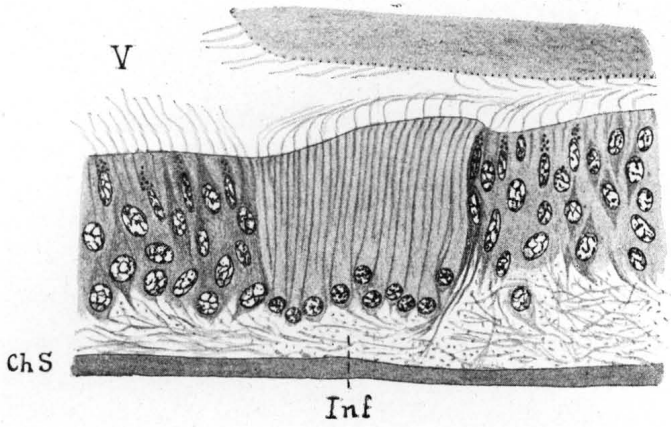
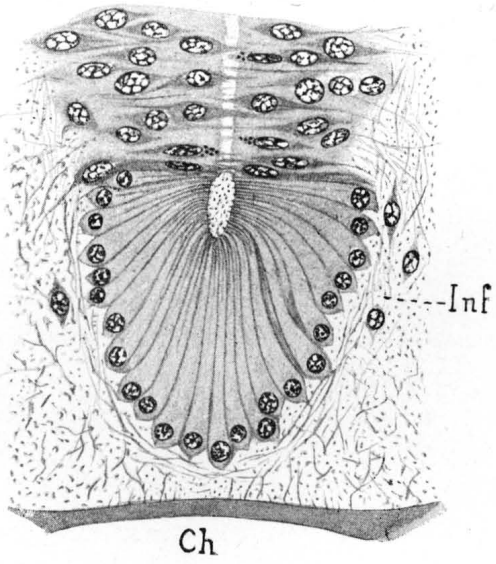


Fig. 1.



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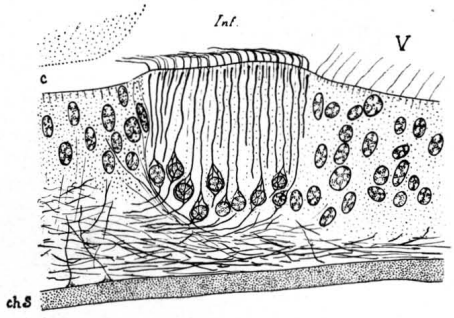

Fig. 3.

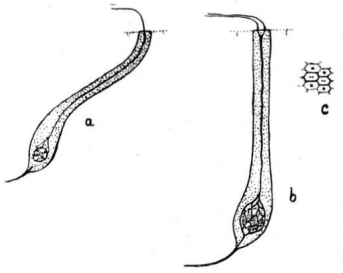

Fig. 4.

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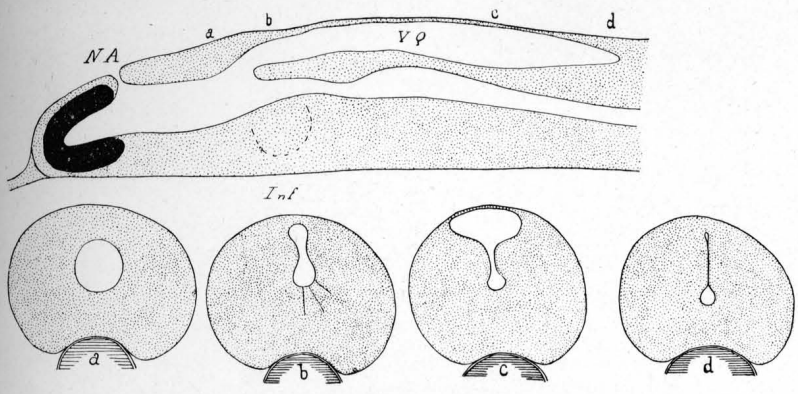

Fig. 5.

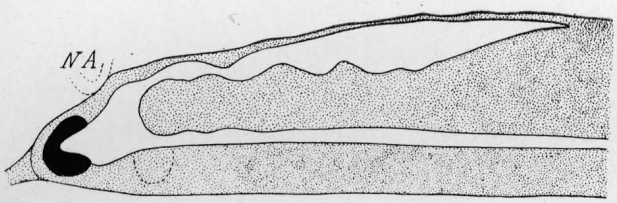

Fig. 6.

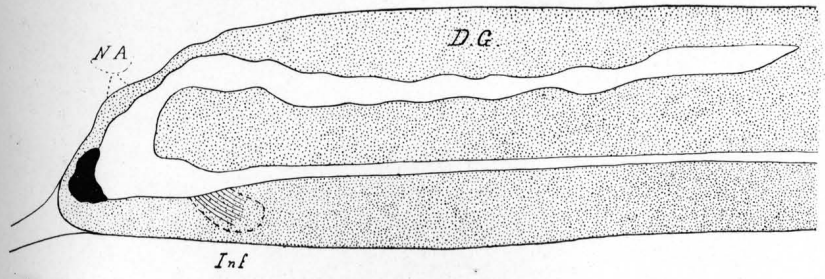

Fig. 7.

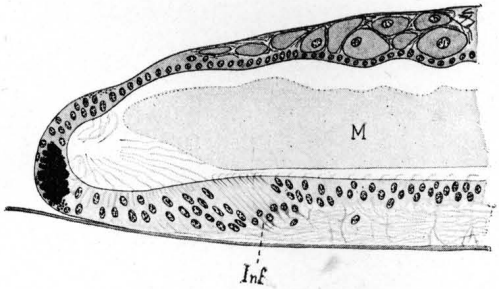

Fig. 8.

DESCRIPTION OF FIGURES.

Fig. 1. Median longitudinal section of the infundibular organ of a Branchiostoma of 52 m.M. in length, 600: 1.
Fig. 2. Cross section through the same of a Branchiostoma of 54 m.M in length, 600: 1.
Fig. 3. The same as Fig. 1. Neurofibrillae stained with chloride of gold.
Fig 4. Cells of the infundibular organ, *a* of a Branchiostoma of 22 m.M. in length, *b* of 50 m.M. in length, *c* cross-section of the upper ends of the cells.
Fig. 5. Median section of the brain of a Branchiostoma larva of 3,4 m.M., reconstructed from cross-sections.
Fig. 6. The same of a specimen of 10 m M. long.
Fig. 7. The same of a specimen of 21 m.M. long.
Fig. 8. Median section through the brain of a Branchiostoma of 28 m.M. in length.

Mathematics. — "*About difference quotients and differential quotients*". By Dr. L. E. J. BROUWER (Communicated by Prof. D. J. KORTEWEG).

(Communicated in the meeting of May 30, 1908).

Different investigations have been made which are very completely summed up in the work of DINI: "Grundlagen fur eine Theorie der Functionen einer veranderlichen reellen Grösse" Chapt. XI and XII, on the connection between difference quotients and differential quotients, particularly on the necessary and satisfactory properties which the difference quotients must possess in order that there be a differential quotient. One however always regards in the first place these different difference quotients in one and the same point x_0 together, forming as a function of the increase of x the *derivatory function in* x_0. The existence of a differential quotient means then, that that derivatory function has a single limiting point in x_0 i.o.w. that in x_0 the right as well as the left *derivatory oscillation* is equal to zero.

Other conditions for the existence of a differential quotient are found when in the first place the difference quotient for constant x-increase Δ is regarded as a function of x and then the set of these functions for varying Δ is investigated. Let $f(x)$ be the given function which we suppose to be finite and continuous and let $\varphi_\Delta(x)$ be the difference quotient for a constant x-increase Δ. The different functions $\varphi_\Delta(x)$ form an infinite set of functions, in which each function is continuous. We shall occupy ourselves with the