CONTRIBUTIONS TO THE STUDY OF THE MORPHOLOGY OF FISH.

By Frances M. Ballantyne, M.A., being a Thesis for the Degree of D.Sc..

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I beg to present the following record of research as my thesis for the degree of Doctor of Science. During the six years which I have spent in the Department of Zoology of the University of Glasgow, I have been working at various problems relating to the morphology of fishes, and this thesis contains the results of three separate pieces of research, the first two of which have already been published in the Transactions of the Royal Society of Edinburgh (VOL.LIII. pt.III. and VOL. LV. pt.II.), while the third* has been submitted to that Society for publication. Since the three pieces of research deal with different parts of the subject, I have divided my thesis into three chapters, the first of which is on the continuity of the nervous system of Lepidosiren, the second on the development and comparative morphology of the air-bladder in some of the primitive fishes, and the third on the development of the armoured Siluroid, Callichthys littorale.

* Now published in the Transactions of the Royal Society of Edinburgh Vol. LVI. Pt. II.

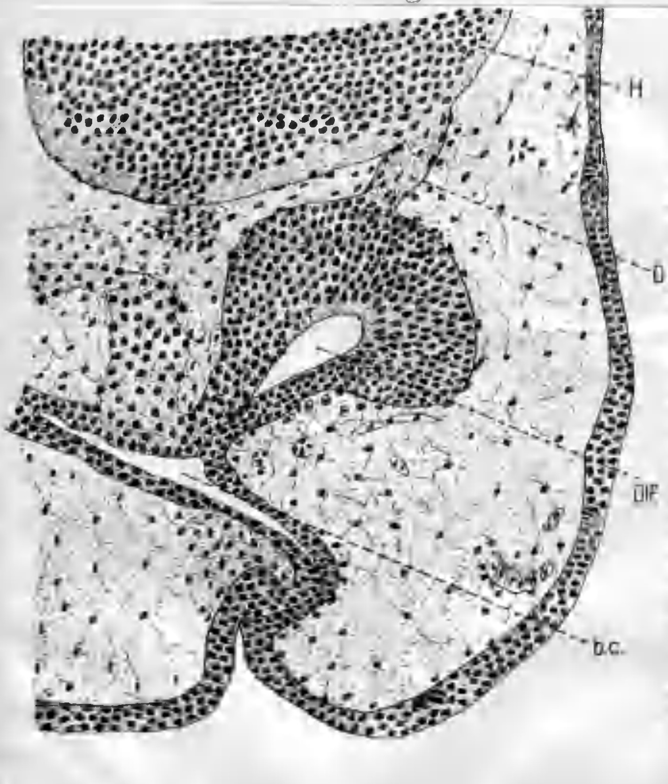
I

THE CONTINUITY OF THE NERVOUS SYSTEM IN LEPIDOSIREN.

As is well known, one of the chief problems relating to the vertebrate nervous system is the question of its continuity or discontinuity both in the adult and in the embryo. The upholders of the latter theory believe that the neurones of the adult are not in continuity but only come into close contact by means of the synapses, while in the embryo the young nerve grows out through the tissues with a free end, and only secondarily unites with its particular end-organ. Much experimental, physiological, and pathological evidence has been brought forward in support of this view, and its upholders include most of the neurologists of the day, whose knowledge, however, relates chiefly to the higher vertebrates and who have made no, or at most very little, detailed study of the phenomena presented by the more primitive forms.

Although the great mass of observational evidence is in favour of the theory of discontinuity, there have been some zoologists who have questioned it on general grounds. One or two of them have described continuity between the neurones in the adult, while several theories of nerve development have been suggested which are based on the idea that continuity is physiologically/

Fig.1. Olfactory nerve at stage 38.
b.c. buccal cavity; H.hemisphere; O.C.olfactory capsule; O.olfactory nerve; Olf.olfactory organ.



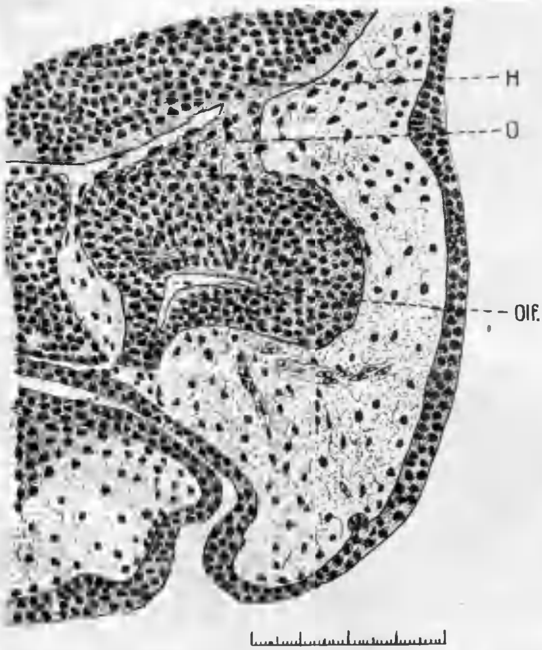


Fig.3. Olfactory nerve at stage 32

Fig.2. Olfactory nerve at stage 4.
Letters as in fig.1.

physiologically the more reasonable hypothesis.

A study of the development of the sensory nerves in Lepidosiren and also of the neurones of the spinal cord of the adult, brings out the following evidence in support of the theory of continuity.

DEVELOPMENT OF SENSORY NERVES.

Olfactory Nerve (figs.1 - 5). - Working back from the later larval stages, where the nerve has attained its adult form, to the stage where the olfactory rudiment is in contact with the brain-wall, the nerve can be seen at each stage joining the brain to the rudimentary organ.

At stage 38 (fig.1) the olfactory sac (Olf.) is well developed within its cartilaginous capsule (O.C.), and from its upper surface the nerve (O) passes back through the capsule-wall to enter the brain (H) at the dorso-lateral border of the hemisphere. The nerve is a broad band of fibres, with its nucleated sheath fully developed.

At stage 34 (fig.2) the olfactory organ (Olf.) is much closer to the brain and therefore the nerve (O) is shorter. It appears to run along the surface of the hemisphere for some little distance before entering the brain-tissue, but otherwise it has the same appearance as at stage 38. The organ itself is considerably less advanced, being simply a thick-walled sac opening externally.

At stages 32 (fig.3) and 31 the organ shows little difference

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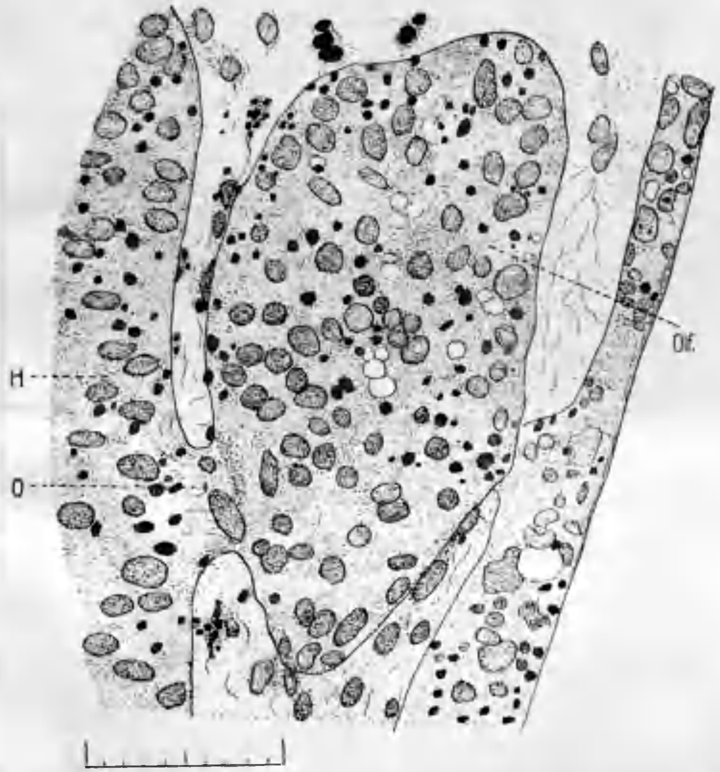

Fig.4. Olfactory nerve at stage 28.

Fig.5. Olfactory nerve at stage 27.

and the nerve has the same course, though at each earlier stage the distance it traverses is less.

At stage 30, the stage figured by ELLIOT SMITH (21), the organ is a large ingrowth of the deep layer of the ectoderm, in which the cavity is quite distinct though it is small. The nerve is a thick strand of protoplasm which brings the cells of the brain into organic continuity with those of the olfactory sac.

This condition can be traced back through earlier stages (figs.4 and 5) as far back as stage 25 where the organ is a thickening of the ectoderm at either side of the fore-brain, and a broad strand of protoplasm already unites it to the brain. At earlier stages than this the organ rudiment is in actual contact with the brain-wall so that it is no longer possible to demonstrate actual continuity of substance.

The figures which illustrate this paper have been chosen from a much more extensive series but are, I hope, sufficient to demonstrate the essential facts. The figures are all camera-lucida drawings of actual sections, the magnification being given in each case by a camera drawing, made at the same time, of a 0.01 mm. stage-micrometer scale. The drawings of the olfactory organ are taken from sagittal sections for the later developmental stages, from transverse sections for the earlier stages.

It will be seen from the figures that my observations entirely confirm those of ELLIOT SMITH who, in his paper on the Cerebral/

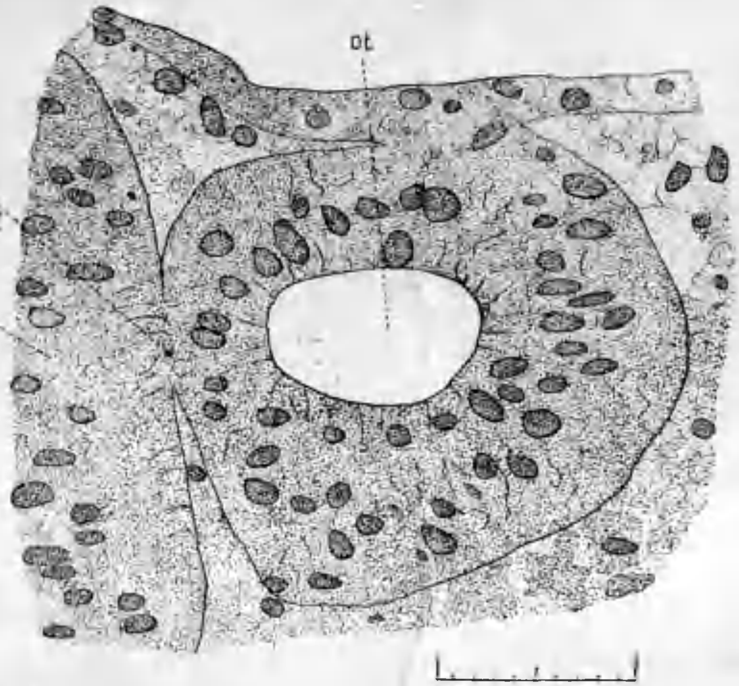

Fig.6. Auditory nerve at stage 24. M.medulla oblongata; ot.otocyst.

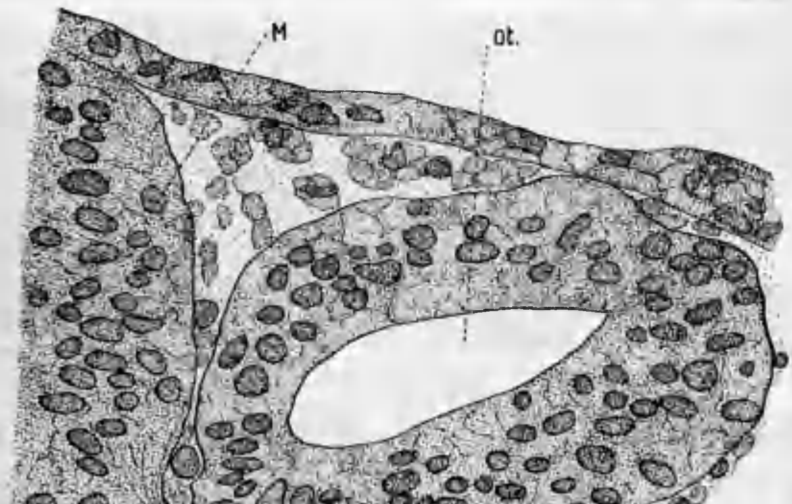

Fig. 7. Auditory nerve at stage 26. Letters as in fig.6.

Cerebral Cortex of Lepidosiren, describes the olfactory nerve as developing from a bridge of protoplasm when the organ and the brain are in close contact.

Auditory Nerve (figs.6-10) - The auditory nerve developes in a similar way. As has been shown by Professor GRAHAM KERR, the otocyst developes as a solid down-growth of the deep layer of the ectoderm at either side of, and in contact with, the brain. At first the rudiment is solid, but after a time a cavity appears in its centre. At stage 24 this cavity (fig.6, Ot.) is well marked, but the otocyst is still in contact both with the ectoderm and with the brain-wall (M). The rudiment gradually is separated from the brain, but at stage 25, where this separation has just begun, the nerve is represented by a wide band of protoplasm uniting the brain to the otocyst just where it bulges out ventrally. This bridge gradually elongates (figs.7 and 8, VIII) and becomes fibrillated as the later stages of development are reached. It is to be seen at every stage without a break.

The roots of the VIIth and the Vth cranial nerves arise very close to the auditory nerve, and in some sections appear to be actually merged with it, but at every stage it is possible to trace the fibres of the auditory nerve from the brain to the otocyst walls. At stage 34 and after that the otocyst is surrounded on its outer side by a cartilaginous capsule (fig.10,a.c.) This, however, is widely open on the side where the nerve enters the/

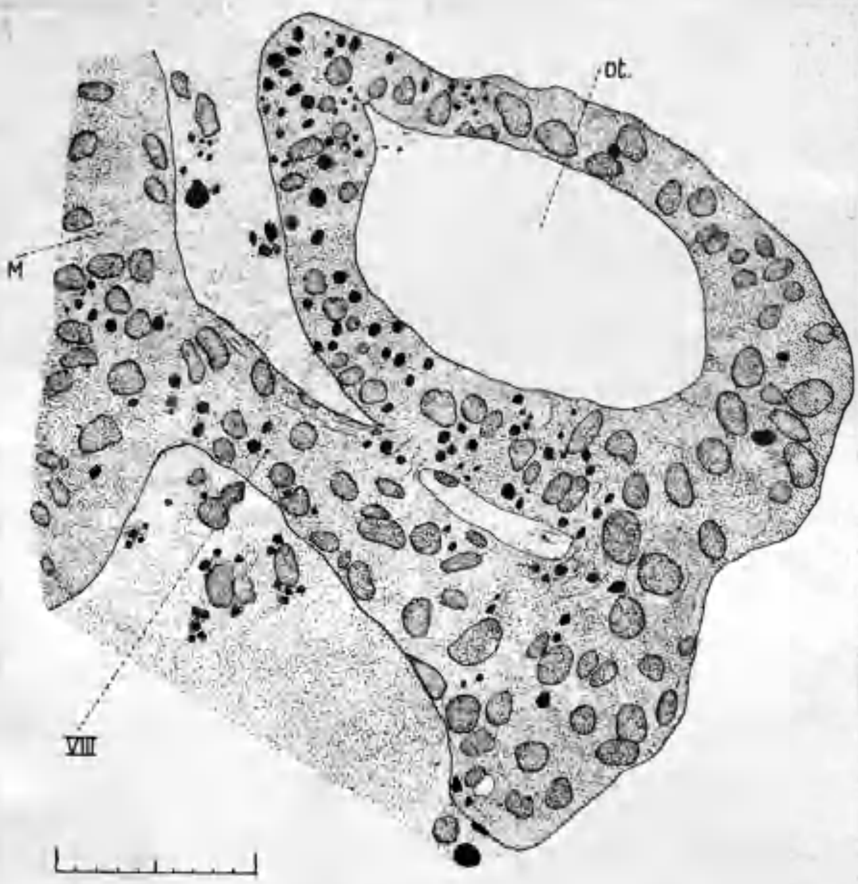

Fig.8. Auditory nerve at stage 28.

Fig.9. Auditory nerve at stage 31.

the otocyst. The ganglion of VII (fig.10, VII g.) lies between the anterior end of the capsule and the brain, though its roots enter the brain almost at the same place as those of the auditory nerve.

From the facts just enumerated it seems clear that the auditory nerve, like the olfactory and motor nerves, arises from a primitive bridge of protoplasm in existence while the organ and the brain are still in contact. My observations therefore agree completely with those of CAMERON and MILLIGAN (7, 8) as to the way in which olfactory and auditory nerves develop in other vertebrates.

Spinal Ganglia (figs.11-15). - The spinal ganglia and the sensory spinal nerves also develop in this way. At stage 38 (cf. fig.11) the ganglion (s.g.) is fully developed. It lies outside the cartilaginous vertebra and a thick strand of nerve fibres passes from it into the upper side of the cord. The sensory trunk comes away from the ventro-lateral border of the ganglion and, passing ventrally and outwards, it is joined by the motor nerve trunk. This condition is already patent at stage 31 (figs.12 and 13) though the ganglion is much smaller and the nerve less easy to trace. At this and later stages there is distinctly visible a small dorsal nerve which leaves the ganglion and runs up beside the muscle to the skin.

At stage 30 (fig.14) the ganglion is much less definite, being/

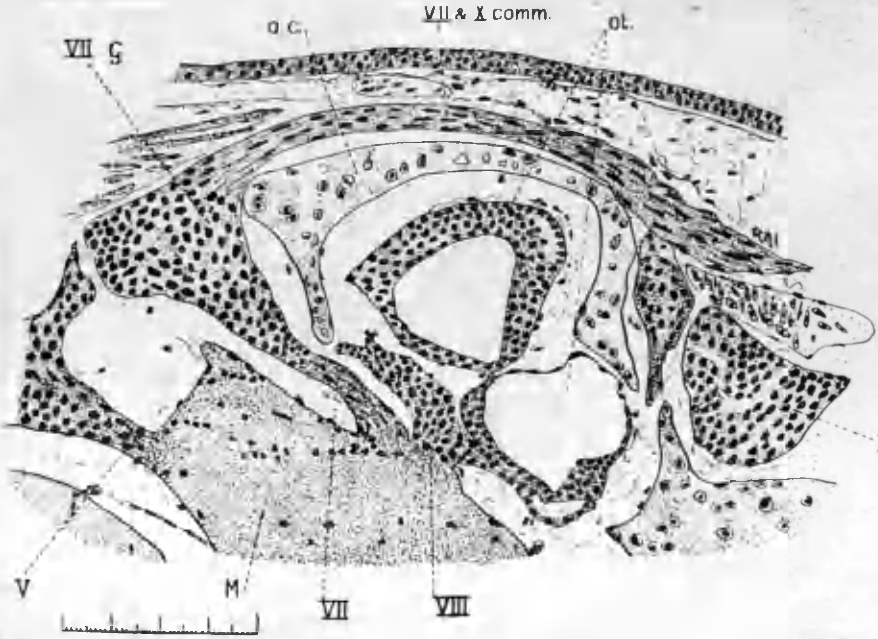

Fig.10. Auditory nerve at stage 36. a.c.auditory capsule; V,VII,VIII,X, cranial nerves; VIIg. ganglion of VII.

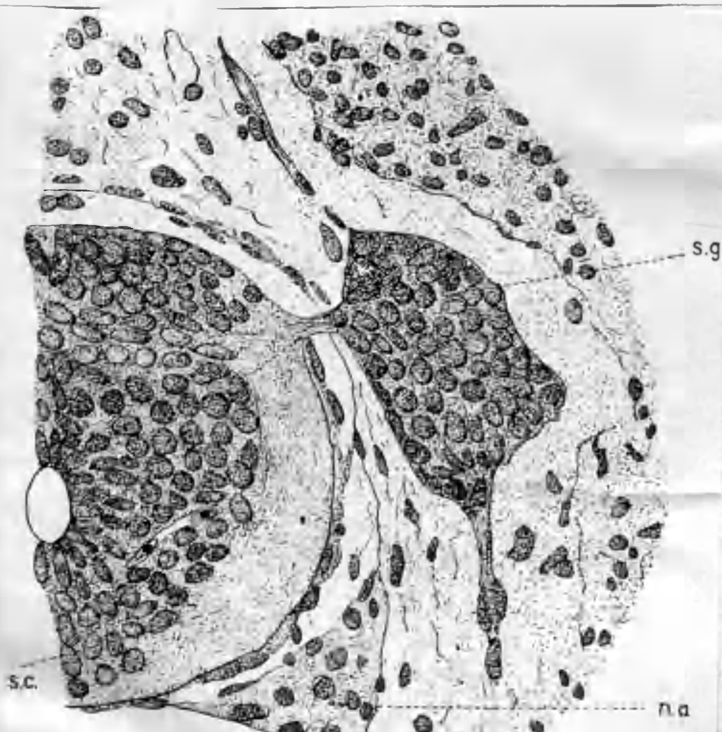

Fig.11. Spinal ganglion at stage 35. n.a.neural arch; s.c.spinal cord; s.g.spinal ganglion.

being represented by a somewhat scattered mass of cells among which there is a lot of yolky material. At this stage it is already possible to trace a connection between the ganglion and the motor nerve, though, as at all stages, in transverse sections the connection between the ganglion and the spinal cord does not appear in the same section as the connection between ganglion and motor nerve.

At stage 29 the ganglion is even more diffuse, but an aggregation of yolky material can be traced joining it to the motor nerve, and its connection with the cord is quite distinct. This condition continues back as far as stage 25 (fig.15) where the cord is still continuous with the ectoderm.

The generally accepted theory of the origin of the spinal ganglia is that they arise from a neural crest along the dorsal wall of the spinal cord. This crest, which corresponds morphologically to the marginal portions of the inturned medullary plate, becomes segmented, and its cells pass down the sides of the cord, between it and the myotome, where they develop into definitive ganglia by outgrowth of their processes towards the cord and to the myotome. I do not see any reasons from my observations to disagree with the general opinion as to the origin of the cells of the ganglia, but in Lepidosiren the developing ganglion in passing ventrally to its adult position never loses its continuity with the cord. The, at first, protoplasmic bridge, which is a continuous/

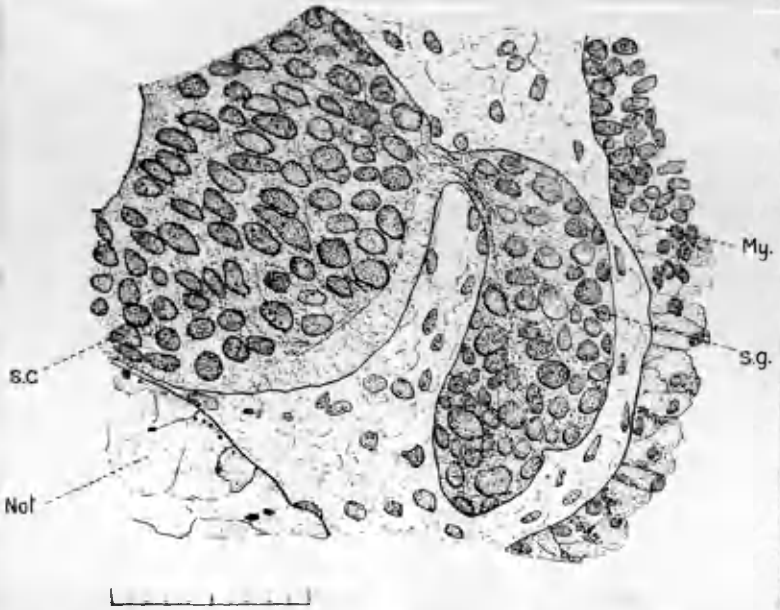

Fig.12. Spinal ganglion at stage 31+.
My.myotome; Not.notochord; other letters as in fig.11.

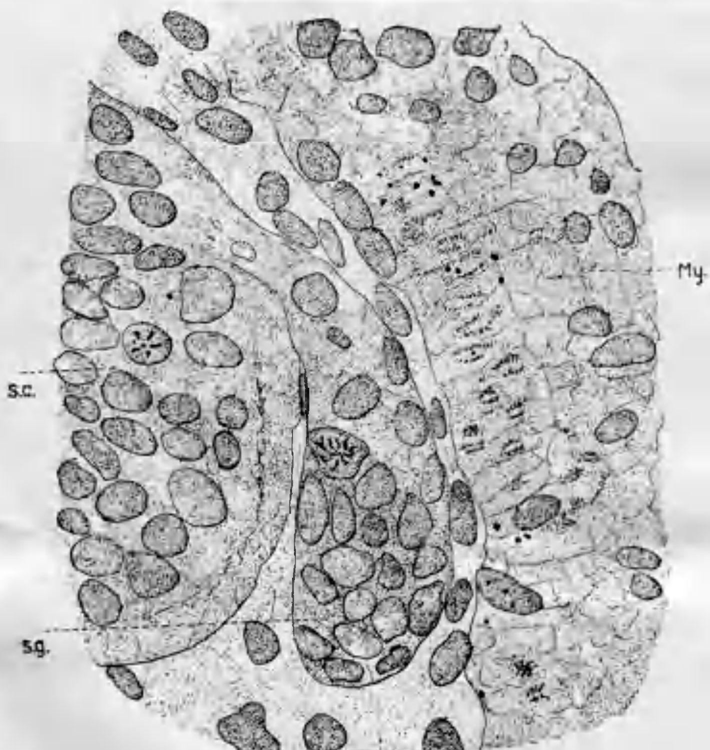

Fig.13. Spinal ganglion at stage 31.

continuous strand, not a chain of discrete cells, becomes fibrillated and lengthens out as the embryo grows in size, but it is quite distinct at each stage.

The motor nerve appears before the ganglion is recognisable, but, as I have said, at stage 25 where the myotome is first beginning to recede from the cord a definite, though yolk-containing connection can be traced between the rudimentary ganglion and the outer end of the ventral nerve trunk. Even here then in the case of the spinal sensory nerves I see no more evidence of discontinuity than in the case of the other nerves in Lepidosiren.

Lateral Line Organs (figs.16-18). - For further evidence on the method of development of sensory nerves I have looked into the development of the lateral line organs and their nerves in Lepidosiren. In the adult the sense organs are found, as GOELDI (10) has shown, in a dorsal and a ventral line along the body as well as in the main lateral lines. I have confined my attention to the latter as their nerves are more easily traced than those of the other sense organs.

The individual sense organ at stage 38 is a cup-shaped hollow in the ectoderm, the floor and sides of which are lined by long-shaped cells. These sense-cells have their free ends drawn out into sensory hairs which project from the mouth of the cup as a little tuft. The cup is filled with mucus. At this stage the organs lie in a slight longitudinal furrow of the ectoderm/

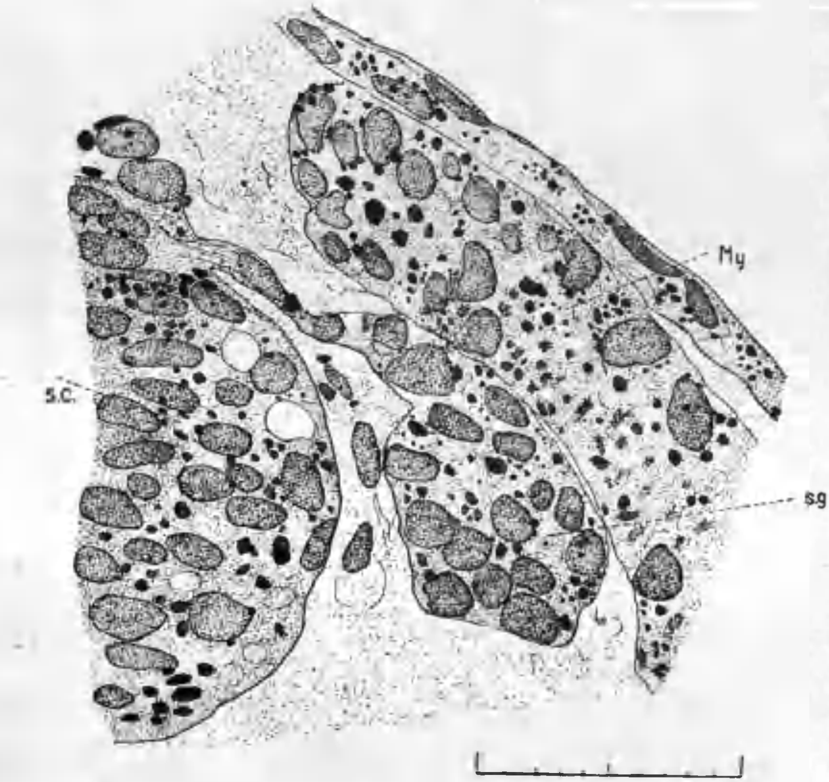

Fig.14. Spinal ganglion at stage 30.

Fig.15. Spinal ganglion at stage 25.

ectoderm, and in the lateral line are approximately segmentally arranged. Horizontal sections (fig.16) show this segmental arrangement quite clearly, the organs being opposite the myosepta in nearly every case.

By comparing the position and the number of the sense organs in the lateral line with those of the spinal ganglia at stages 38, 36, 35, and 34 further proof that the sense organs are segmental is obtained, for at each of these stages the two series tally fairly closely.

The lateral line nerve at stage 38 lies close to the spinal column, at the level of the junction of the neural arch and the centrum of the vertebrae. A branch nerve passes out from it through the myoseptum to each organ. The organs are more ventral than the main nerve trunk at this stage.

Working back from stage 38 the sense organs are seen to be progressively smaller, but they are quite distinct as early as stage 32. At stage 31 (figs.17 and 18) the neuromast rudiments are present as thickenings of the deep layer of the ectoderm, but the sensory hairs are as yet not developed. The nerve is already well marked, and in the section shown in fig.18 it may be seen running from the lateralis ganglion to one of the sense organs. At this stage there is visible a slight continuous thickening of the ectoderm between successive neuromast rudiments.

At stage 30 the row of lateral line organs is represented by/

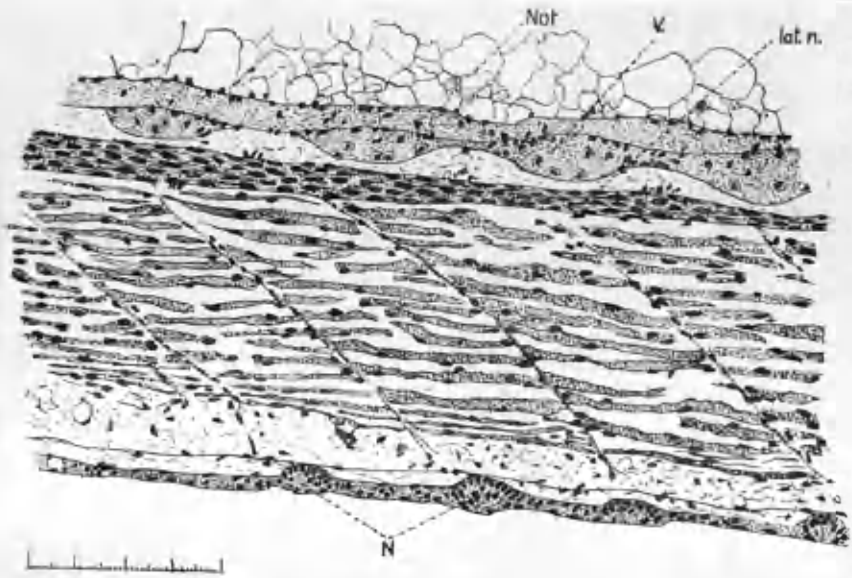

Fig.16. Horizontal section at stage 38,showing lateralis nerve(lat.n.) lying close to the vertebrae(V) and sending branches through the myo-septa to the neuromast organs(N) which are approximately segmental.

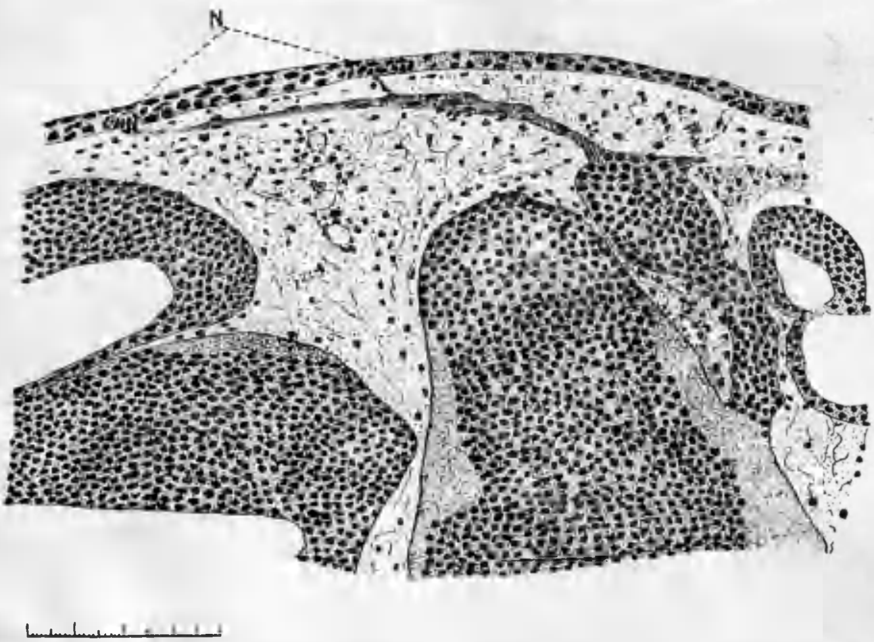

Fig.17. Two neuromast organs on the head,with their nerve and its ganglion at stage 31-.

by a continuous band of thickening in the deep layer of the ectoderm, reaching from the ear to well behind the heart. The longitudinal nerve trunk lies within a distance of 10μ from the sense organ rudiment, instead of about 65μ as it is at stage 38, and at intervals a branch joins the main nerve to the thickening at points where the neuromast organs will develop, and where the rudiment is becoming more pronounced than in the intervening parts.

At stage 29 the rudiment is a thick rod of cells in the deep layer of the ectoderm in the region of the gills, though it only extends back to about the level of the posterior end of the heart. It is in continuity with the lateralis ganglion at the anterior end where the first sense organs are beginning to develop and where the nerve is first separating from them.

As far back as stage 23 the rudiment appears to be already present as a thickening of the ectoderm in the region where the otocyst is developing, and in some sections this thickening appears to be actually continuous with the rudiment of the otocyst. At stage 23 the ectodermal rudiment appears to be united to the brain by a nucleated mass of protoplasm, which represents the ganglion and the nerve. At stage 24 the connection between the organ and the brain is recognisably a nerve, and at stage 25 it has become quite distinct. Owing, however, to the fading of the stain and a very large quantity of yolk it is difficult to satisfy oneself/

Fig.18. Neuromast organ with its nerve coming from the lateralis ganglion at stage 31.

Fig.19. Transverse section of the spinal cord of adult Lepidosiren, from preparation stained by Bielschowsky's silver nitrate method.

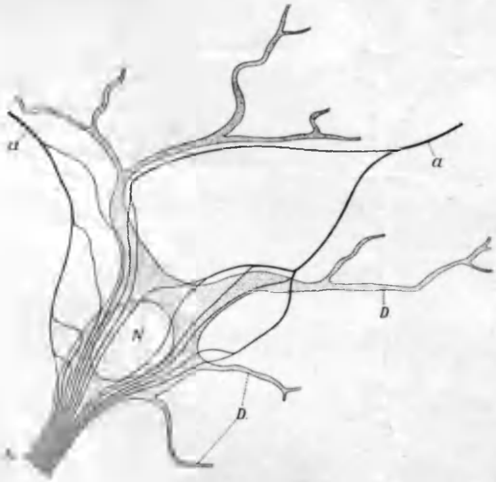

Fig.20. Diagram of a large neurone to illustrate conclusions as to continuity of neurofibrils.

oneself altogether regarding these early stages.

On the whole, however, I am disposed to conclude that the neuromast organs develop, as ALLIS has described for Amia, from a rod of cells in the deep layer of the ectoderm. This rod is from the first in continuity with the ganglion and brain. As the organs develop, the main nerve sinks inward from them, but in each case remains in connection with the sense organ by a branch nerve which is present from the beginning.

I think it will be clear from what I have said that I conclude in favour of all these nerves developing out of early protoplasmic bridges, which later become fibrillated, just as has been described in the case of the motor nerves, and that thus in these primitive vertebrates there is continuity in the nervous system from an early stage in development.

CONTINUITY BETWEEN NEURONES IN THE ADULT (Figs.19-27).

Turning now to the adult condition in Lepidosiren, the continuity of the neurones one with another is, as I believe, made clear by a study of the neurofibrillae within them. As in all branches of neurology, most of the research in this subject has been done on the higher vertebrates in which the cells are relatively very small, so it is difficult, if not impossible, to be certain about the internal structure of the individual cells. This difficulty is minimised by using Lepidosiren material, for the cells of this animal are enormous and relatively few in number.

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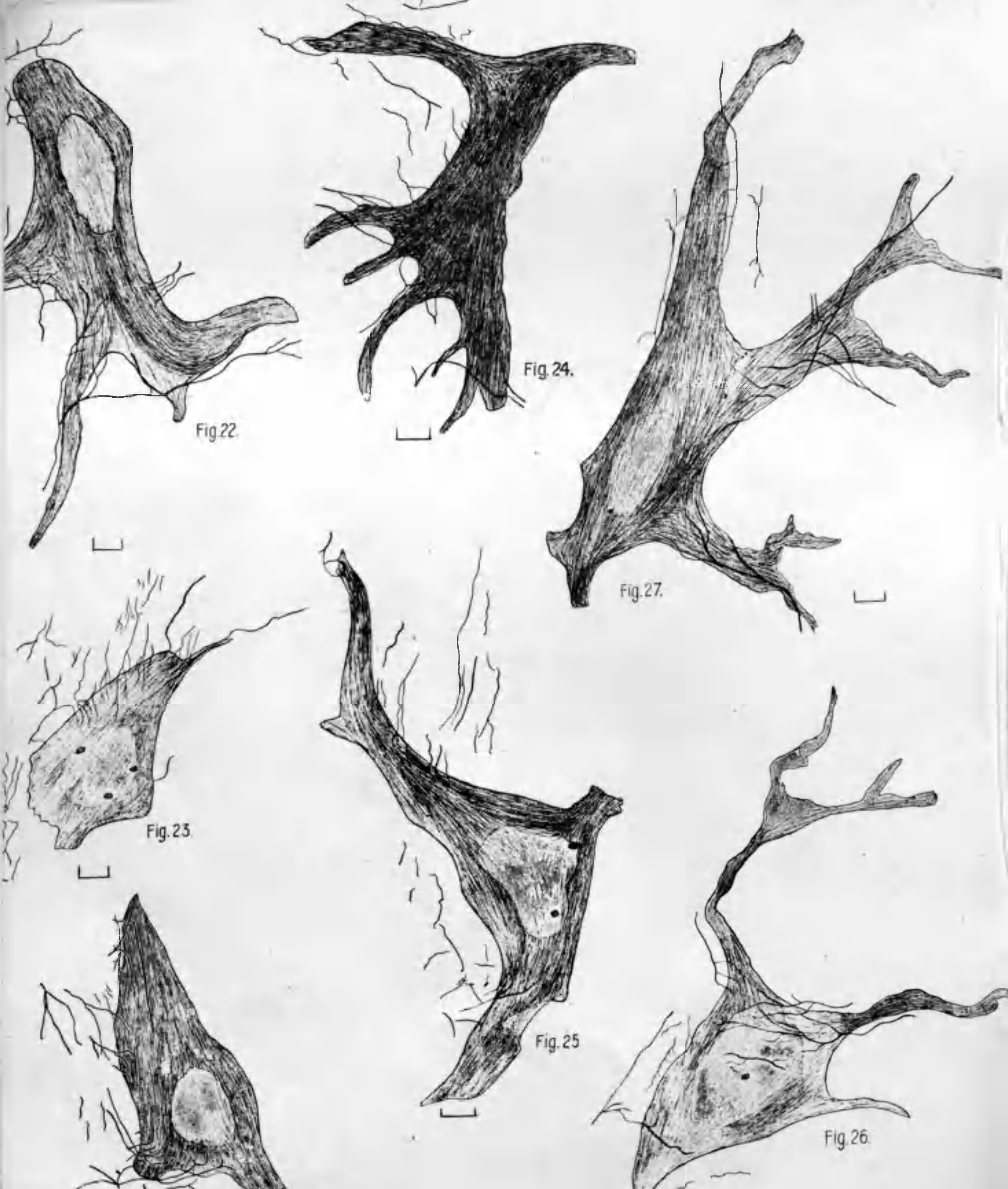

Fig. 22. Fig. 24. Fig. 27. Fig. 23. Fig. 25. Fig. 26. Fig. 21.

Figs. 21 to 27. Camera-lucida drawings of large motor neurones.

I believe that another important cause of error is the use of the monocular microscope, since it reduces several planes to one. Thus my observations lead me to believe that the idea of neurofibrillar networks, and the theory of end-clubs on the terminal branches of an axon, are both based on optical illusions due to the use of the monocular microscope. Under the monocular microscope the axon end-branch, whether near a neurone or not, appears to end in a little knob or button. Even under the monocular, with very careful focusing, the end-branch may sometimes be traced beyond this button, as it is only the blurred image of the axon where it goes out of focus. With the binocular this appearance is not produced at all, the axon ending either in a clean cut as it passes out of the plane of the section, or else tapering off into a cell, where it disappears.

While working out the arrangement of the neurofibrils in <u>Lepidosiren</u> I have used BIELSCHOWSKY'S silver nitrate method for pieces, as given by Da FANO in BOLLES LEE'S <u>Microscopist's Vade-mecum</u>, eighth edition. I find that the Pyridine process, using the maximum times advised, gives the best results.

In this research I have confined my attention to the neurofibrillae. I have not worked out the paths of the nerve fibres, nor the general relation of the dorsal and ventral roots of the spinal nerves. An account of the structure of the cord in the <u>Dipnoi</u> is given by BURCKHARDT in his paper "<u>Das Centralnervensystem von</u>/

von Protopterus annectens" (6). His drawings of the cord agree closely with the appearance of sections of the cord of Lepidosiren.

Fig.19 is a camera-lucida drawing of a whole transverse section of the cord, stained by BIELSCHOWSKY'S method and counter-stained with eosin. On the ventro-lateral boundary, between the white and the grey matter, there is a more or less continuous line of large neurones. In the figure there are two such cells on the left, and one on the right, of the section. It is these cells that I have chiefly examined.

Fig.20 is a diagrammatic drawing of one of these large motor neurones, to illustrate my conclusions as to the continuity of the neurofibrils from one cell to another. The terminals of the axons, instead of ending in a club or foot on the surface of the next neurone, penetrate into its substance; and their neuro-fibrillae pass directly and without a break through the cell and along its axon. I see no sign of a network either within or around the cell, and the neurofibrils are straight and unbranched within the cell.

The dendrites of the neurone are at first thick processes of the cell and are deeply stained in my silver nitrate prepar-ations. These thick processes, however, soon branch repeatedly and end in tapering threads of cytoplasm, which appear to be without neurofibrillae and are only visible in the preparations counterstained with eosin. The axon branches penetrate either into/

into the body of the cell or into the broad proximal part of the dendrites. This, I think, lends probability to the theory that the great development of the dendrites with their many branches is to increase the surface of the cell, in order to facilitate the metabolic processes; and that the function of the dendrites is rather nutritive than nervous.

Fig.21 is a camera-lucida drawing of a very large cell which shows quite distinctly the penetration of the axon branches into the cell-body. Towards the top of the neurone an axon end-branch may be seen passing over the surface of the cell and sending a long straight branch among the neurofibrils in the cell. This particular section is convincing in itself, but further proof of the penetration of the axon end-branch is given by a reconstruction of the whole cell. It is a cell of about 50μ diameter, and appears in five consecutive sections. It should be noted particularly that the section figured is a central one, as this does away with the possibility of the long straight neurofibril lying on the surface of the cell instead of in its interior. I made reconstructions, by the glass-plate method, of this and several other cells, and in each case was able to convince myself that the end-branch really penetrates into the cell-substance. In fig.21, besides the long branch at the top, there are numerous fibrils entering the cell at the left-hand basal corner, and reconstruction corroborates their penetration also.

In/

In all this work I have used a binocular eyepiece and, by very careful focusing alone, I am absolutely satisfied that the fibrils do penetrate from the axon end-branch directly into the cell. The fibrils within the cell are straight and unbranched, and run from one process to another without forming any network. The nucleus is usually approximately in the middle of the cell, and the fibrils pass over and round it, more or less filling the cytoplasm. The cytoplasm, however, can be seen in some sections as a faintly stained foam among the fibrils. In the section from which fig.21 is drawn it is distinctly visible round the nucleus under high powers.

Fig.22 is another typical nerve-cell, with end branches penetrating its substance. This drawing was also made under the camera lucida. The fibrils of the end-branches are at first quite distinct outside the cell; but gradually, as they penetrate into the cytoplasm, they disappear among the intracellular fibrils.

The naked end-branches of the axon are much more deeply stained than the intracellular fibrillae, so that the entering fibril gradually becomes paler as it penetrates into the cell-body. I suppose that this difference in staining is due to the greater amount of cytoplasm round the fibrillae within the cell. Sometimes I think there are two or three fibrils together in an axon end-branch; these separate on entering the cell-body.

This/

This, however, is hypothetical, as it is impossible to follow the end-branch once it penetrates among the fibrils of the cell.

Figs.23-27 are all camera-lucida drawings of typical neurones; each one shows numerous cases of neurofibrils from the end-branches of the axon penetrating into the cell-body.

BETHE describes the intracellular neurofibrils as straight and unbranched; but he, with HELD and others, holds that the neurofibrils from the branches of the axon form a pericellular network, from which fibrils pass into the cell. I have seen no convincing evidence of such a network, and I think that the belief in its existence is the result of using the monocular microscope. Crossing fibrils, which seem to be in the same plane under the monocular microscope, thus giving the appearance of a network, are seen under the binocular to be at different levels.

J. B. JOHNSTON suggests that the fibrils are the colloid substances in the cytoplasm of the cell, forming the walls and filling the solid angles of the protoplasmic foam. I do not at all agree with this, as the cytoplasmic foam is sometimes visible amongst the fibrils; and they are threads of uniform diameter all the way through the cell, which they would not be if JOHNSTON'S theory were correct.

As pointed out at the beginning of this chapter, though the vast majority of neurologists support the theory of discontinuity both in the adult and in the embryo, there are some, such as BETHE,/

BETHE, HELD, and MARUI, who accept the less fashionable but intrinsically more probable theory of continuity. To the actual evidence of such continuity must be added the facts here given relating to Lepidosiren.

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II

THE DEVELOPMENT AND COMPARATIVE MORPHOLOGY OF THE AIR-BLADDER IN SOME OF THE PRIMITIVE FISHES.

In view of the frequent appearance, even in technical literature, of the old-fashioned idea that the lung of the tetrapod has evolved out of the air-bladder of fish, further work on the morphology of the air-bladder seemed clearly necessary. The theory of an air-bladder origin of the lung was brought forward by BOAS, who based his view on purely theoretical arguments, which have, however, not found support in the facts brought to light by the advance of our knowledge of the embryology of the more archaic vertebrates.

It is generally accepted that there is no air-bladder in the Elasmobranchs, though, according to the observations of MIKLUCHO-MACLAY (25), there is a very transitory rudiment in the young stages of Galeus, Mustelus, and Acanthias. PAUL MAYER (24), in dealing with MIKLUCHO-MACLAY'S paper, states that the supposed air-bladder rudiment is simply the early stage of one of three large and distinct glands present in the adult, the other two of which are ventral in position. This seems conclusive, but in regard to his criticisms of the rest of MIKLUCHO-MACLAY'S paper, I do not think his reasoning is sound. His only argument against the existence of the rudiment in Galeus and Acanthias is that/

that he has not found it in much older specimens. But if the air-bladder is only a vestigial organ in these fish, one would not expect to find it persisting until the later stages of development. Further investigation might be of value, especially in view of the widespread tendency of the air-bladder to become reduced even in such primitive fish as Acipenser and Scaphirhynchus, not to mention the more highly specialised benthotic Teleosts.

The air-bladder is a well-developed organ in most Teleosts and in the Dipnoi, though its adult form varies very considerably in different groups. In the Dipnoan Lepidosiren and Protopterus, and in the Crossopterygian Polypterus, there are two well-developed lungs opening ventrally into the alimentary canal. In Ceratodus there is a single lung, which, though lying dorsal to the alimentary canal, yet communicates with the ventral wall of the œsophagus, its pneumatic duct passing round the right side of the canal. In the Teleosts, the air-bladder is a dorsal sac, opening in the Physostomes, into the alimentary canal either dorsally or laterally, and in the Physoclisti, having in the adult no such communication with the alimentary canal.

As regards the evolutionary history of the organ, there have been two main theories; one of which regards the air-bladder as an organ sui generis, whereas the other traces the homology of the air-bladder with the lungs of other vertebrates. The first of these theories was supported by Wiedersheim (40). It was largely based/

based upon his observations upon Dipnoi which have been proved to be erroneous, and the view can no longer be entertained in the light of our further embryological knowledge.

The second of the two theories is due especially to SAGEMEHL (36), who based his view on the great variability in position of the glottis in different fishes, especially in the Characinidae. Professor GRAHAM KERR'S (19) results, on Polypterus and the Dipnoi, make it impossible to avoid accepting this view. The early stages of development of the organ in Polypterus and the Dipnoi, are identical with those of a typical lung. In Polypterus, which in this respect is more archaic than the Dipnoi, the two lungs are not of equal size, the larger right lung assuming a dorsal position where it is not balanced by the left lung. This posterior part of the right lung is supplied by both the right and the left vagus nerves, the left nerve crossing over dorsal to the alimentary canal. This is very important, since it foreshadows the crossing of the nerves in the Dipnoi, a fact which caused great difficulty in understanding the arrangement of the lungs in this group.

In the Dipnoi the right nerve and the pulmonary arteries show clearly that the lungs have travelled round the right side of the alimentary canal to take up their adult position, and the apparent contradiction provided by the crossing of the left pulmonary nerve dorsal both to the alimentary canal and to the right/

right nerve, is explained when the crossing is found to be already present in an earlier evolutionary stage. Probably this crossing is due to short-circuiting of nerve impulses.

Another important fact established by Professor GRAHAM KERR'S (19) work on the Dipnoi, is that the symmetrical lungs of *Lepidosiren* and *Protopterus* pass through an asymmetrical "Polypterus stage" in the course of their development.

The object of the work recorded here was to review and extend observational knowledge bearing on the question, by studying in detail the air-bladder as it exists in the adult and the embryonic stages of the more primitive types of existing fish. The material used consisted of embryos and adults of *Amia*, *Lepidosteus*, *Acipenser*, and *Gymnarchus*, while adults of *Scaphirhynchus* and embryos of *Ceratodus* and *Callichthys* were also examined.

CERATODUS.

As has been stated above, in *Ceratodus* the whole lung lies dorsally, though the pneumatic duct passes round the right side of the alimentary canal to open into the ventral wall of the œsophagus. There is only one lung in the adult, and the left lung is only vestigial in the embryo. As NEUMAYER (28) has described, the lung first appears as a small, ventrally directed evagination of the ventral wall of the alimentary canal. This grows ventrally and caudally at first, and then begins to turn to the right and upwards round the alimentary canal. The lung rudiment/

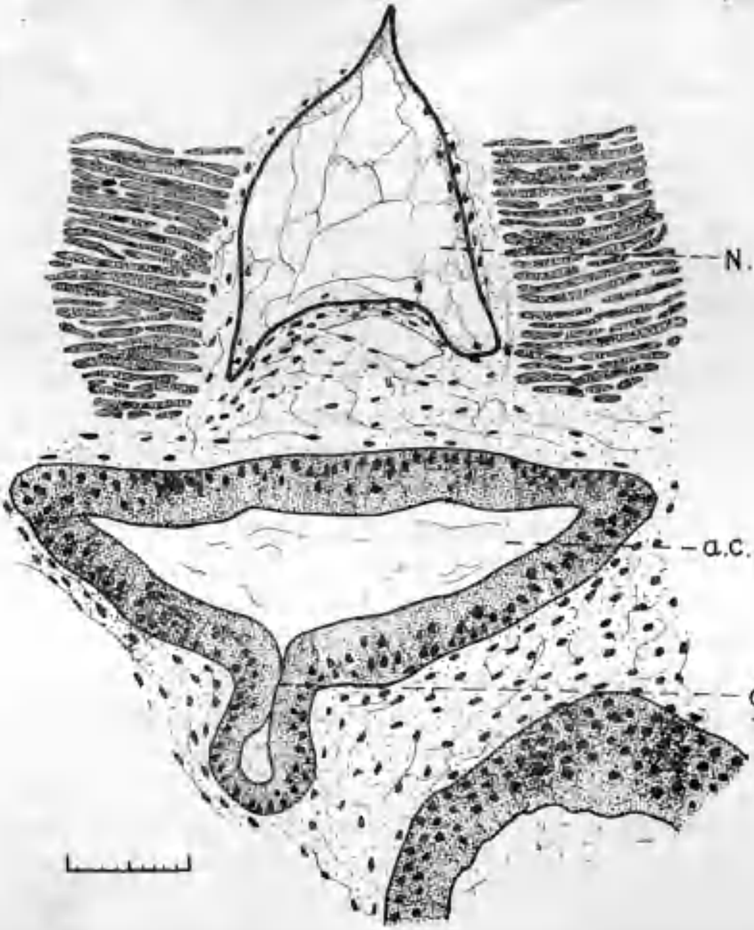


FIG. 28. Lung rudiment of *Ceratodus* at an early stage when the lung extends back and towards the right only.

Lettering for all Text-figs. : B, brain ; N, notochord ; *a.b*, air-bladder ; *a.c*, alimentary canal ; *a.h*, anterior horn of air-bladder ; *c.a*, cœliac artery ; *d.ao*, dorsal aorta ; *gl*, glottis ; *k*, kidney ; *l*, liver ; *l.l*, left lung ; *l.p.a*, left pulmonary artery ; *l.p.n*, left pulmonary nerve ; *p.a*, pulmonary artery ; *ph*, pharynx ; *r.l*, right lung ; *r.p.a*, right pulmonary artery ; *r.p.n*, right pulmonary nerve ; *sp.c*, spinal cord.
Each division of scale, except in Fig. 33 D, represents 0·01 mm.

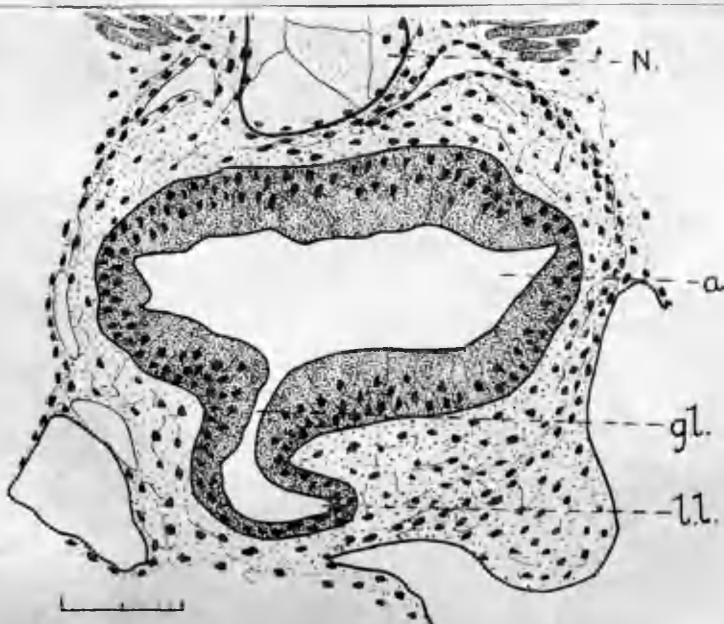


FIG. 29. Lung rudiment of *Ceratodus*, showing the vestigial left lung ; 15 mm. embryo.

rudiment at first passes straight down ventrally and then turns almost at a right angle and extends back caudally. At a slightly later stage the ventral end of the pneumatic duct, if we may so call the anterior vertical part of the lung rudiment, becomes broadened out towards the left side. This is figured both by GREGG WILSON (41) and by NEUMAYER, and the latter says that this left process of the widened-out end of the primitive lung cavity may represent the rudiment of the left lung.

In the sections which I have examined, there is no trace of this left process in the earliest stages (Fig.28).The tip of the rudiment turns backwards and upwards round the right side of the canal. It is just where the turn occurs, and when already the lung has grown some distance backwards and towards the right, that the minute left lung appears (fig.29). In an embryo of 15 mm. length the left lung has attained its maximum size, and after that rapidly becomes merged in the main or right lung (fig.30). As in the other Dipnoi, the lung of Ceratodus is supplied with blood from the sixth aortic arch by normal pulmonary arteries, which by their course show clearly how the lung has travelled round the oesophagus, the left artery passing round ventral to the alimentary canal to supply the ventral side of the lung. The nerves are typical pulmonary branches of the vagus of each side. The left nerve crosses dorsal to the alimentary canal and to the right nerve as in other Dipnoi, but, as shown by Professor GRAHAM KERR (18)/

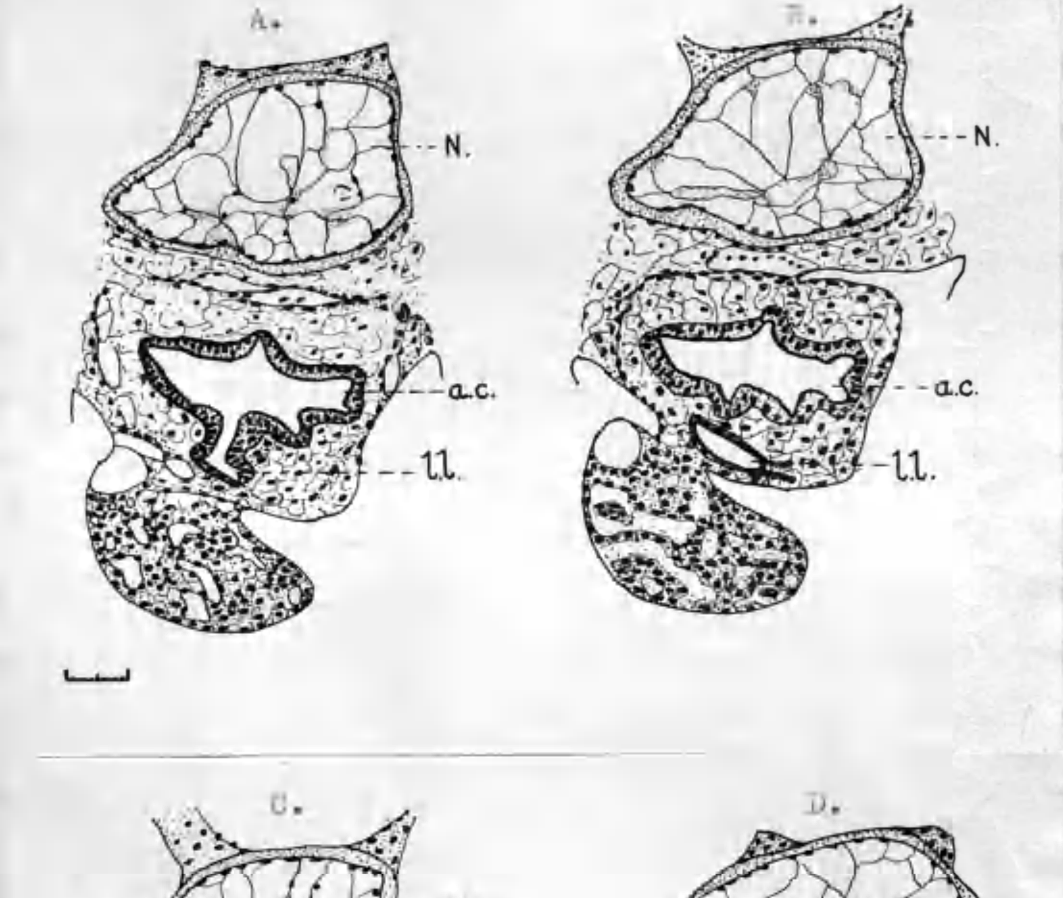


Fig.30. Lung rudiment of Ceratodus; 18mm embryo. A. showing glottis and vestigial left lung; B. left lung merging with main right lung; C.and D. showing rotation of the lung round the right side of the alimentary canal.

(18), this is to be regarded as a secondary arrangement.

AMIA.

The next probable step in the evolutionary history of the air-bladder is illustrated by Amia. In Amia (fig.31) the air-bladder of the adult is a large, very vascular sac, lying in the dorsal mesentery and extending from the level of the pectoral fins almost to the end of the body cavity. It is distinctly bilobed in front and tapers slightly towards the posterior end. The walls are membranous with a complicated network of blood-vessels, giving a spongy appearance to the organ. The short pneumatic duct leads from the front of the air-bladder to the glottis situated in the mid-dorsal wall of the alimentary canal, a little behind the last gill cleft. The blood supply is by typical pulmonary arteries from the sixth aortic arch of each side. This artery (r.p.a.) is given off at the bend of the gill arch, passes inwards under the cardiac branch of the vagus, and then turns tailwards along the outer side of the pulmonary branch (r.p.n.) of the vagus nerve. Just after entering the body cavity, the artery bends under the nerve so that it enters the air-bladder internal, and somewhat ventral, to the nerve. Both artery and nerve enter the bladder on its ventral side, on either side of the pneumatic duct. The coeliac artery (c.a.) crosses dorsal to the artery and nerve on the right side, as one would expect it to do if the air-bladder had been originally ventral. The veins run along-side/

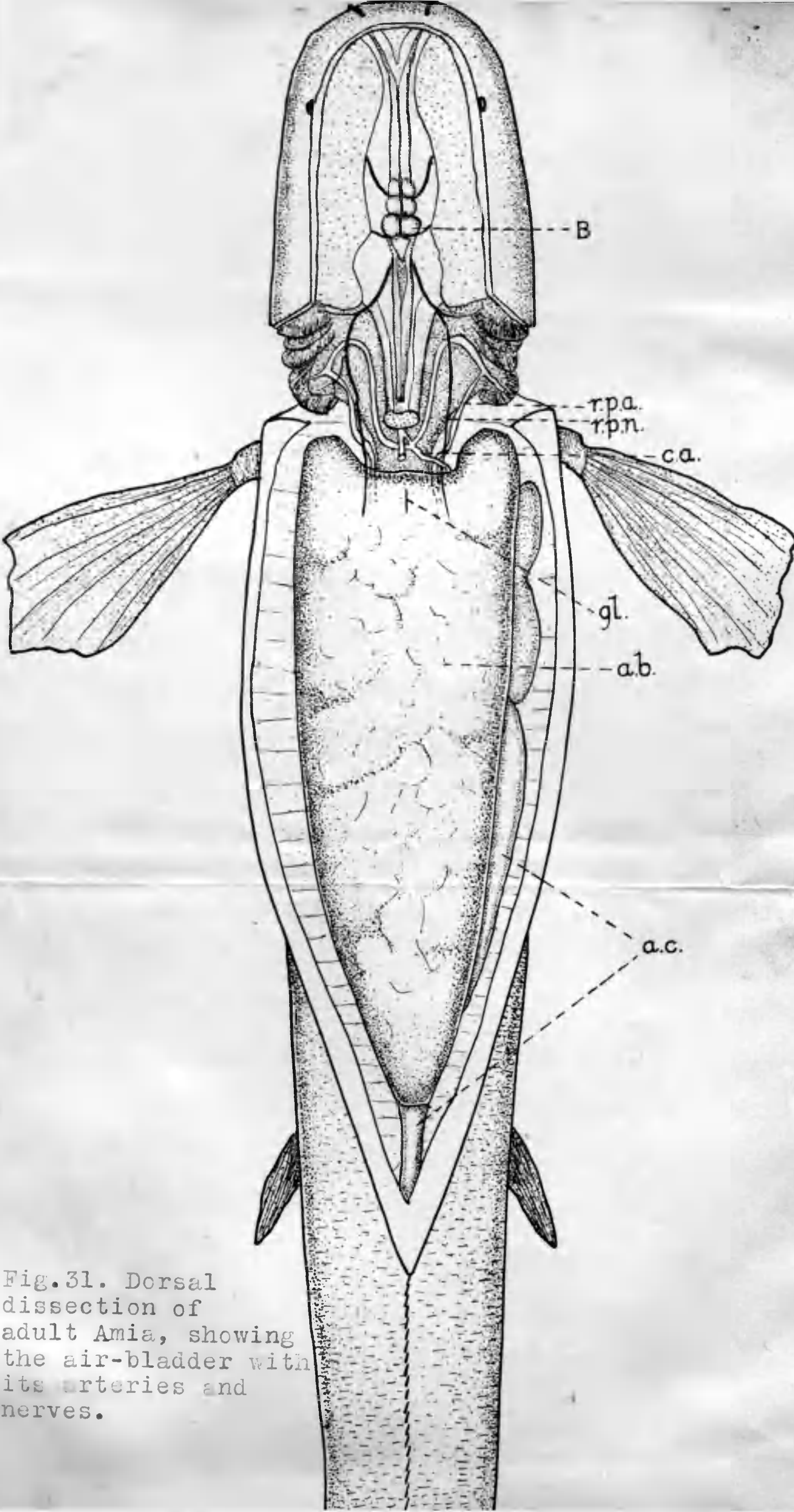


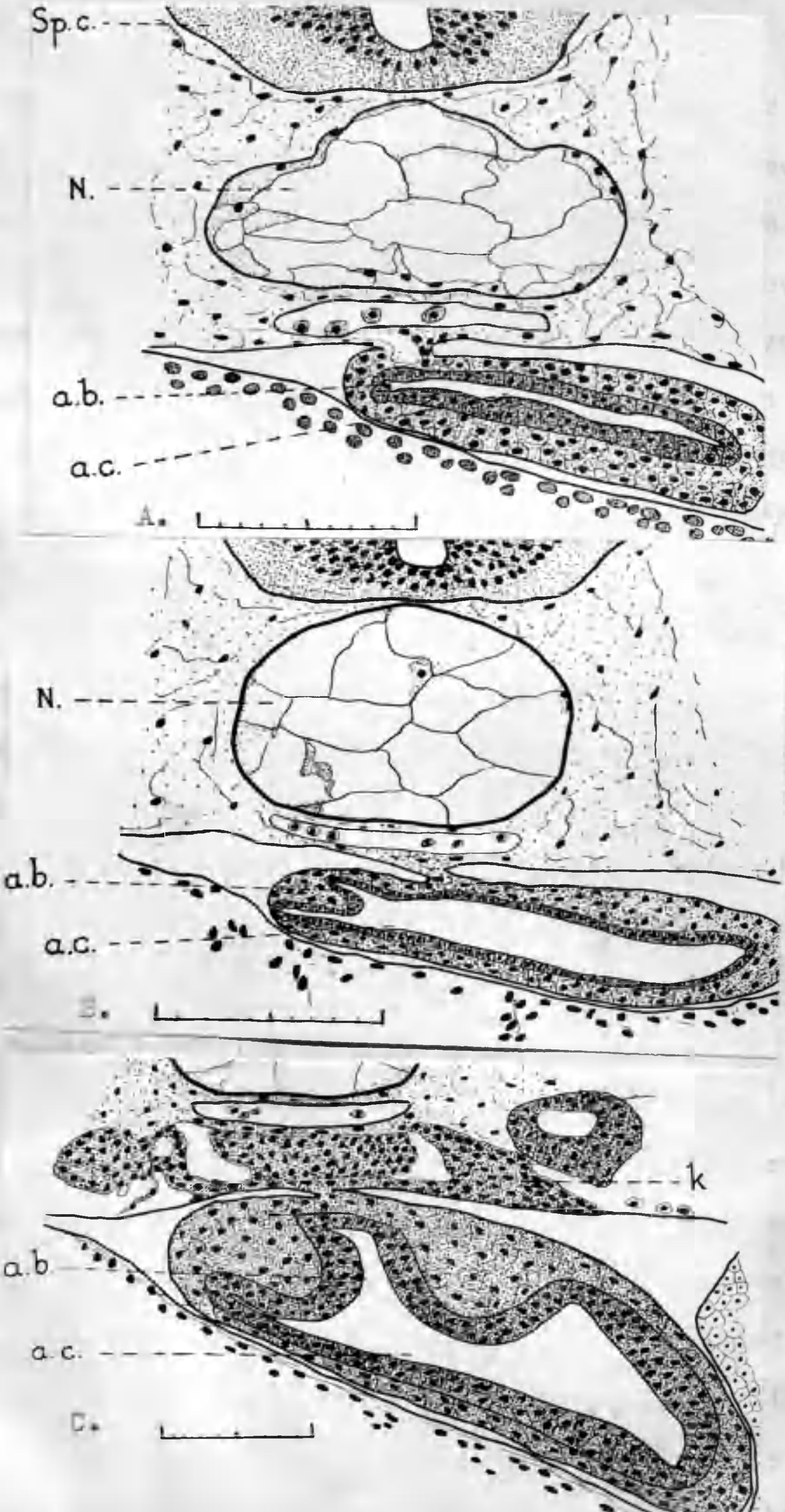
Fig.31. Dorsal dissection of adult Amia, showing the air-bladder with its arteries and nerves.

alongside the artery and nerve until they enter the posterior cardinal vein. In front of this, for some distance, the artery and nerve are closely pressed against the wall of the posterior cardinal vein.

In development the air-bladder of Amia first appears in an embryo of about 7 mm. length (fig.32,A), as a very minute pocket of the right side of the dorsal wall of the alimentary canal, well to the right of the mesentery. PIPER (29) describes the first rudiment as mid-dorsal, but his figure shows the opening a little to the right with the tip still further over to the right. This is a later stage than that represented by the 7 mm. embryo.

The rudiment gradually increases in length, becoming an open groove in the wall of the canal, and by rotation of the œsophagus, it tends to become mid-dorsal in position (fig.32,C). The simple groove becomes T-shaped, as seen in transverse section (fig.32,D,E), and the left branch of the T grows back as a tubular sac lying dorsal to the alimentary canal, and a little to the left side of the median plane.

Gradually the groove becomes closed off from the alimentary canal from behind forwards, and the cavity of the air-bladder becomes larger and the right branch of the T-shaped part merges with the left to form the long vascular sac of the adult, lying mid-dorsally in the body cavity (fig.33, A-D). This process is comparable with that by which the left lung rudiment in Ceratodus gradually/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gradually disappears into the general contour of the persistent right lung; for in comparing the dorsally placed sac in Amia with the typical, ventral lungs, it must be borne in mind that the left side of the air-bladder of Amia represents the morphologically right side of the lungs. Therefore in Amia, the air-bladder may be considered as the persistent right lung as in Ceratodus. Here, however, the rotation of the alimentary canal has brought the glottis round to the right side of the dorsal wall, when the rudiment first appears, and by further rotation to the mid-dorsal line in the adult. It is of interest to note that in the embryos of Amia and other Ganoids, the alimentary canal hangs free in the body cavity, and is therefore free to rotate; whereas in the embryo of Ceratodus, the œsophagus is firmly embedded in connective tissue.

Another point which emerges from a study of serial sections and which deserves to be emphasised is that the position of the stomach, well down on the left side, is such as would seriously interfere with the left lung were one present. In the absence of a similar handicap in the case of the right lung, we have a factor which probably played an important part in initiating the predominance of the right lung over the left, which is seen in the evolutionary history of the air-bladder.

The fact that the arteries are not crossed in Amia is explained when it is noted that the air-bladder is already a large/

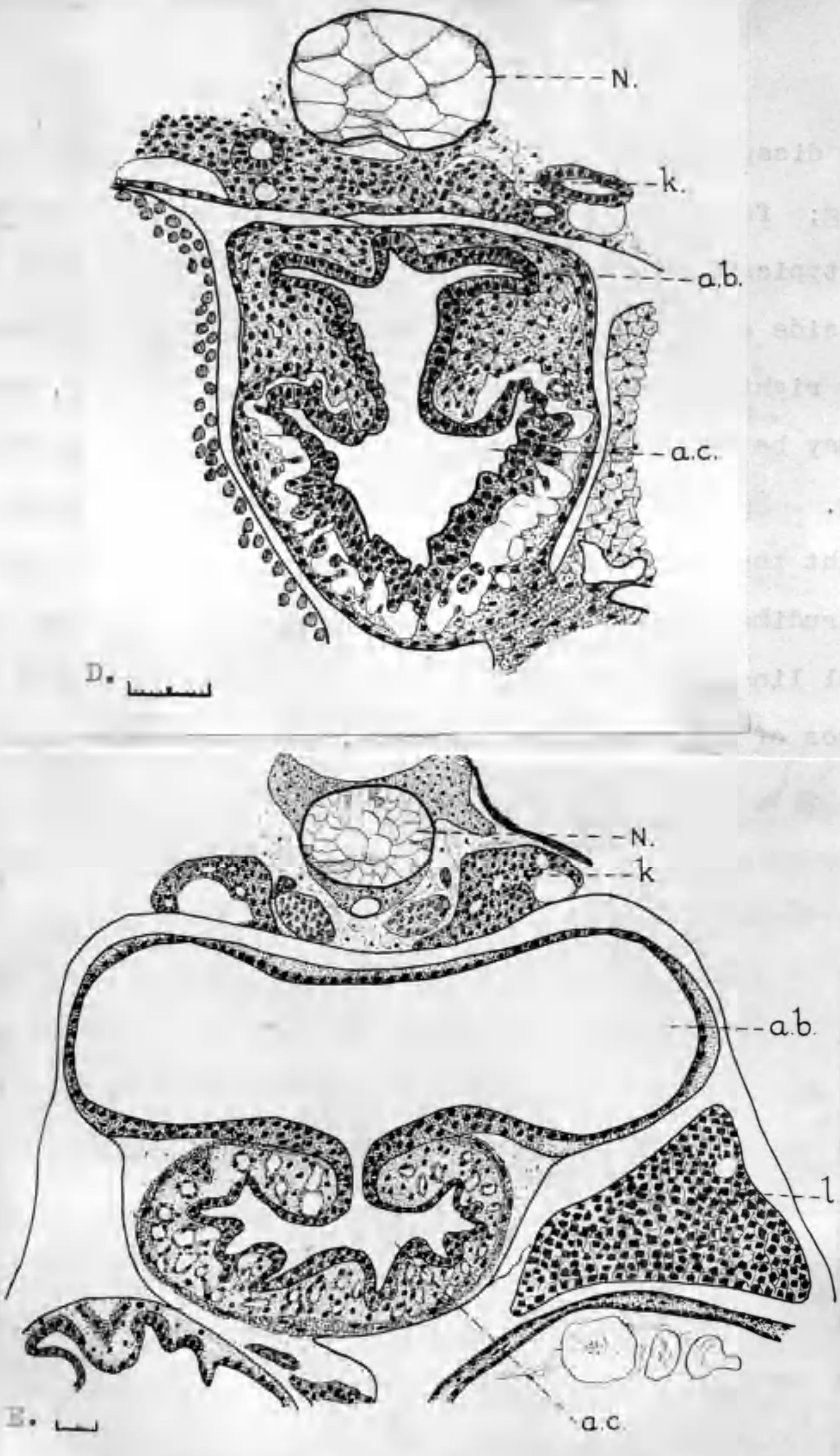


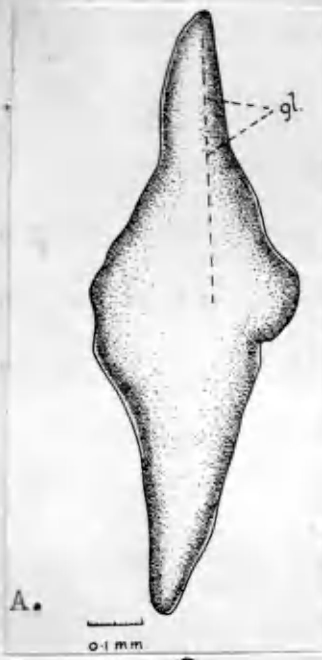
Fig.32. Transverse sections through the developing air-bladder in Amia. A,7mm embryo; B. slightly later stage, 7mm.embryo; C,9mm embryo; D, 12mm embryo; E,19mm embryo.

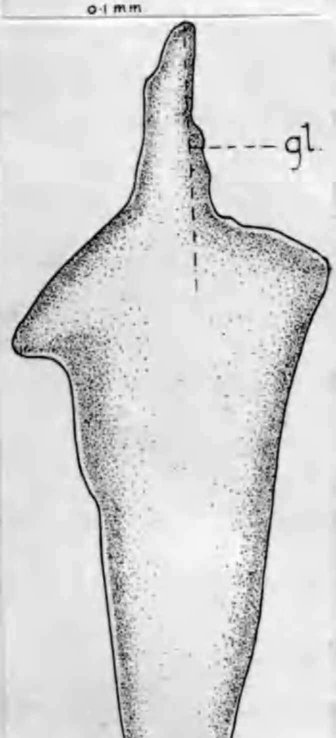
large, well-developed sac when the last gill cleft is first opened. The first appearance of the air-bladder is in a 7 mm. embryo, but the gill clefts are completely developed for the first time in a 12 mm. embryo, in which the air-bladder is already approximately 1 mm. long. This is the first stage at which the arteries can be traced, so that the principle of economy of tissue would naturally account for the left artery taking a short cut to the left side of the air-bladder.

The nerves (fig.34) also go straight to the same side of the air-bladder - left nerve to left side - but the condition in *Polypterus*, of the nerves to the posterior part of the right lung, foreshadows this arrangement. As already noted, the coeliac is dorsal to the right artery and nerve, thus showing a trace of a more primitive arrangement.

LEPIDOSTEUS.

Another step towards the Teleostean air-bladder is shown in *Lepidosteus*. As in *Amia*, the air-bladder is typically lung-like; the vascular network of the walls is very intricate, and is supplemented by blood-vessels running across the cavity of the sac and forming pockets at the sides of the main cavity. In the adult, the glottis is mid-dorsal, but somewhat further forward than in *Amia*, and opens immediately behind the pharynx. The nerves are pulmonary branches of the vagus on either side; the blood-supply, however, is not from the sixth aortic arch, but from numerous arteries coming straight from the dorsal aorta. The veins also/





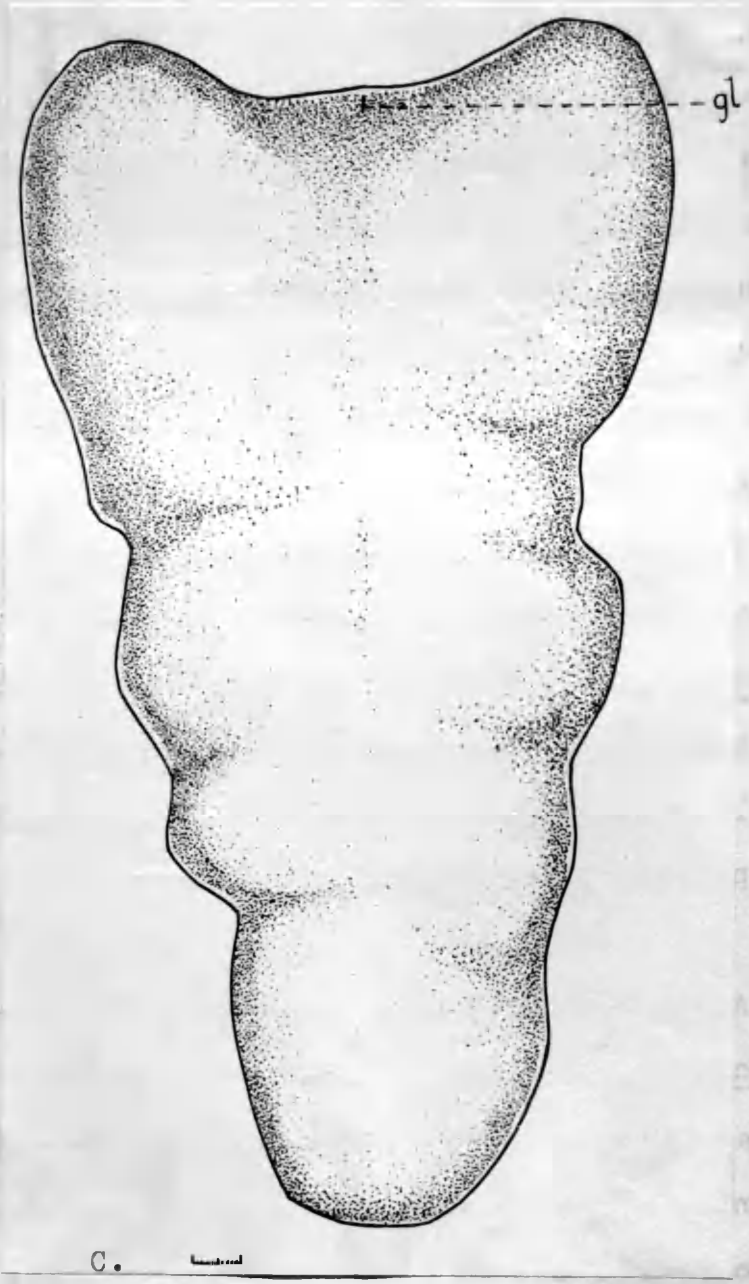


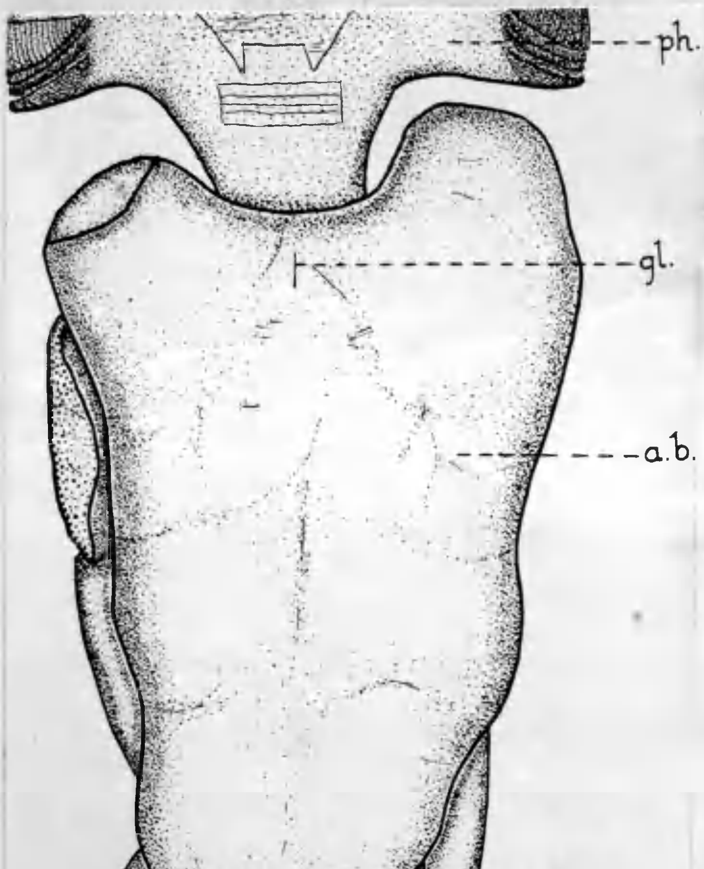
Fig.33. Reconstructions of the air-bladder of Amia, from horizontal sections. A. 10mm embryo; B. 11mm embryo; C. 20mm embryo; D. dissection of 45mm larva of Amia. Scale much smaller than in A,B,C.

also are numerous and enter the posterior cardinal veins

According to MAKUSCHOK (22), the air-bladder appears first in an embryo of about 8 mm. length, as a mid-dorsal diverticulum of the posterior part of the œsophagus. The, at first, small and circular glottis extends forwards, giving the appearance of an immediately post-pharyngeal origin for the air-bladder.

In embryos of 12, 15, and 18 mm. length, which I have examined, the lung is well developed and extends through almost the whole length of the body cavity. It has distinct processes to the right and left anteriorly, reminding one of the T-shaped part of the air-bladder of Amia. Reconstructions from horizontal sections of these three stages seem to indicate that the rudiment is at first a simple sac, and that the processes to right and left develop later. In the 12 mm. embryo there is a long, straight sac with, at the anterior end, a small process on the left side. In the 15 mm. specimen this process has become much larger, and another process to the right has appeared. In the 18 mm. embryo the left pocket is disappearing into the general contour of the lung, the right process being still distinct. MAKUSCHOK only figures earlier stages than this.

In embryos of 11 and 10 mm. length, transverse sections show that the air-bladder, at the anterior end near the pharynx, is a mid-dorsal groove in the wall of the apparently solid œsophagus. As the cavity of the œsophagus appears farther back, the cavity of/



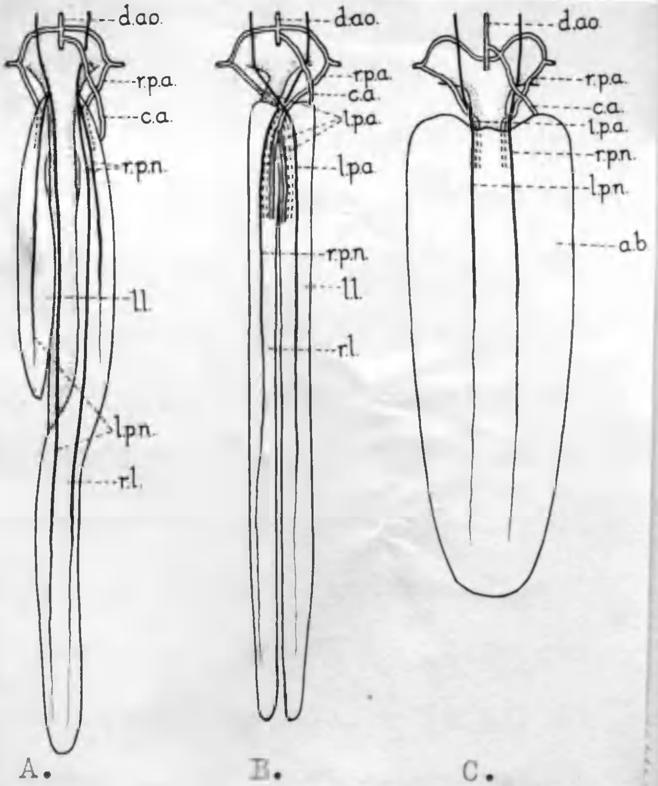


Fig.34. Diagram to show nerves and arteries of the lungs of:- A,Polypterus; B,Lepidosiren; C,Amia.

of the groove-like rudiment of the air-bladder also appears, and finally the groove becomes cut off from the alimentary canal and extends for some distance as a tubular sac, lying mid-dorsally in the body cavity. This is very like a corresponding stage in *Amia*.

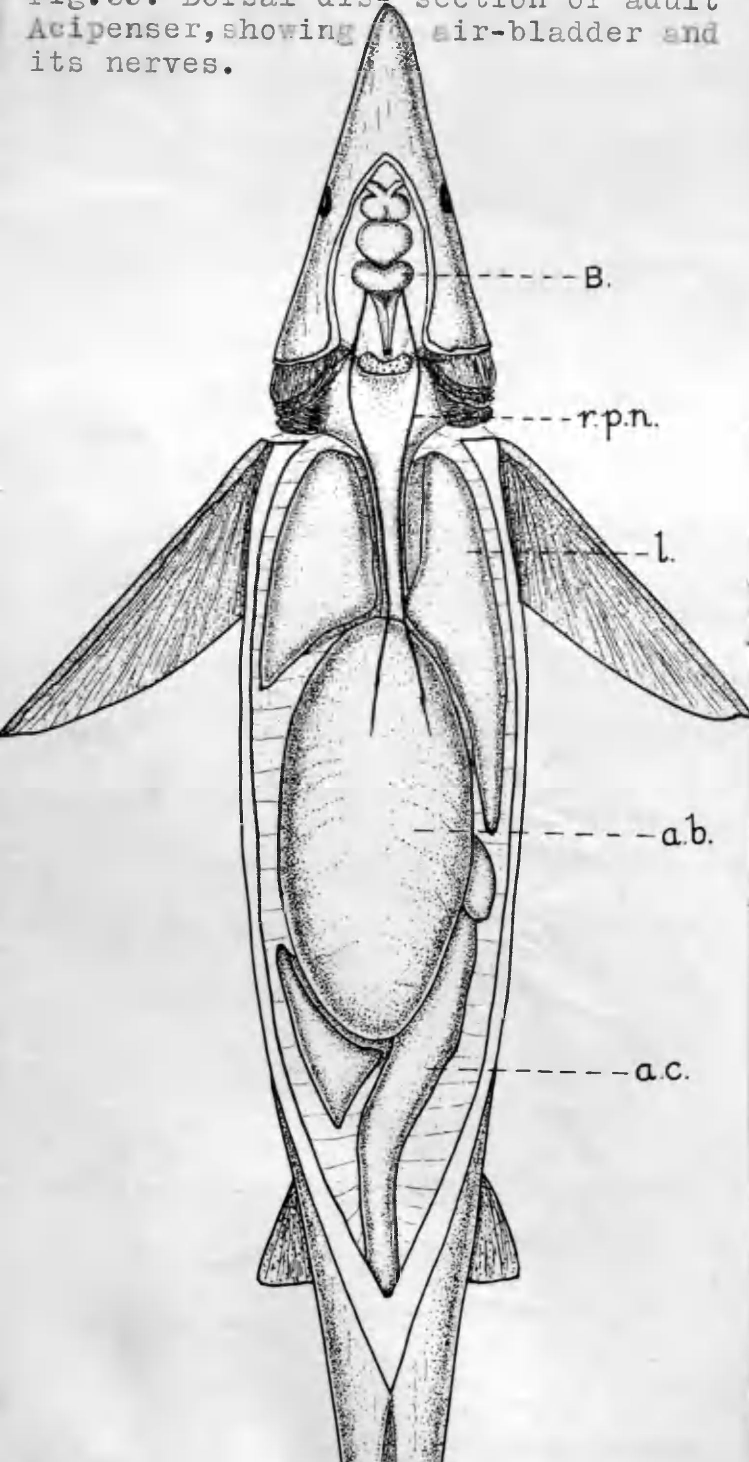
ACIPENSER and SCAPHIRHYNCHUS.

In the sturgeons, as we might expect from their ground-feeding habits, the air-bladder is in a very much reduced condition, and probably represents a stage when both the respiratory and the hydrostatic functions have degenerated owing to life at the bottom of the water. The air-bladder lies much farther back in the body cavity than in *Amia* and *Lepidosteus*, and the glottis opens into the anterior end of the stomach (fig.35).

MACALLUM (21), in describing the alimentary canal of *Acipenser*, states that, from the histological structure of the walls of the canal, the œsophagus may be said to extend just posterior to the opening of the pneumatic duct, so that here also the air-bladder is in reality a diverticulum of the œsophagus.

According to RAUTHER (30), the size and development of the air-bladder varies very much in different species of *Scaphirhynchus*. In *S. fedtschenkoi*, it is only a small sac with a rudimentary cavity opening into the hinder part of the œsophagus, although in *S. rafinesquei* it is a long sac with a large pneumatic duct. In *S. platyrhynchus* (fig.36), specimens of which I have dissected, it is a fairly large membranous sac with a curious, tough, fibrous lining/

Fig.35. Dorsal dis- section of adult Acipenser, showing [illegible] air-bladder and its nerves.



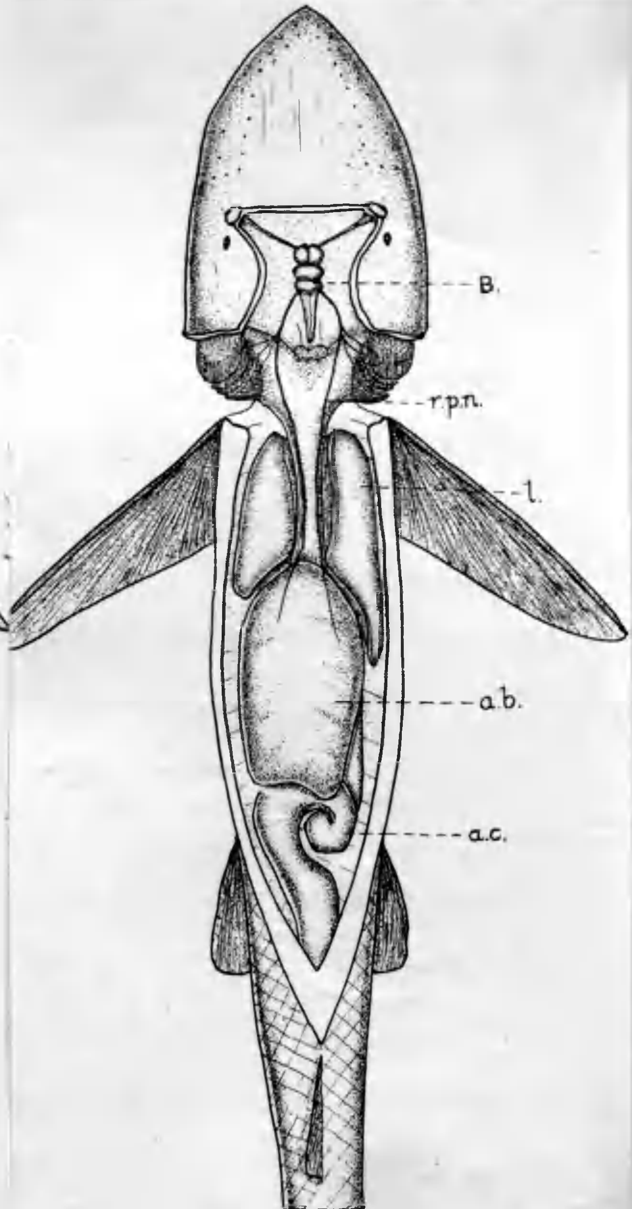


Fig.36, Dorsal dissection of Scaphirhynchus platyrhynchus showing air-bladder with its nerves.

lining to the dorsal and lateral walls. It lies dorsally in the middle of the body cavity.

In development the air-bladder in Acipenser does not appear until late - another step in the process of degeneration. The very first rudiment is seen in an embryo of 28 mm. length (fig.37), where it is a diverticulum of the lining of the alimentary canal. The opening is well over to the right side, and the tip of the diverticulum, which is directed towards the left, is distinctly bilobed. This diverticulum causes the outer wall of the alimentary canal to bulge very slightly. In an embryo of 30 mm. length (fig.38, A), the air-bladder rudiment is a very small pouch on the proximal part of the stomach. In a specimen of this size the alimentary canal is fully formed and has reached its adult condition except in size. The air-bladder-rudiment is nearly mid-dorsal and is a simple sac. In further stages it increases in size, especially in length, until it attains its adult structure (fig.38, B-C).

GYMNARCHUS.

The development of the air-bladder in Gymnarchus is of special interest for the light it throws upon the method by which the change has occurred from the normal pulmonary blood supply to that direct from the coeliac artery or the dorsal aorta. In the embryo the pulmonary artery originates from the fused fifth and sixth aortic arches of the left side, those of the right side forming/

lining to the dorsal and lateral walls. It lies dorsally in the middle of the body cavity.

In development the air-bladder in Acipenser does not appear until late - another step in the process of degeneration. The very first rudiment is seen in an embryo of 28 mm. length (Fig.37), where it is a mid-dorsal outgrowth of the lining of the alimentary canal.

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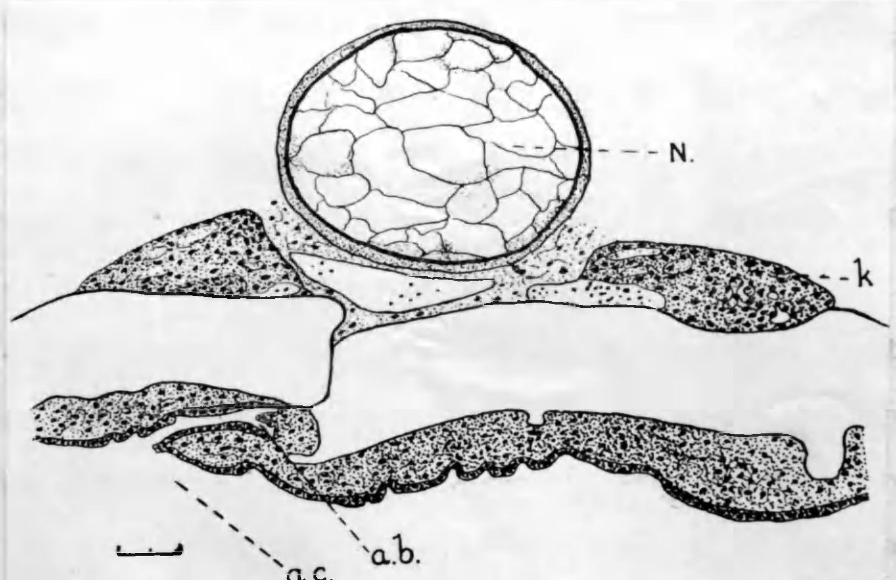

Fig.37. Earliest stage of development of air-bladder of Acipenser; 28mm embryo.

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The development of the air-bladder in Protopterus is of special interest for the light it throws upon the method by which the change has occurred from the ventral pulmonary blood supply to that direct from the coeliac artery or the dorsal aorta. In the embryo the pulmonary artery originates from the fused fifth and sixth aortic arches of the left side, those of the right side forming/

forming the coeliac artery. Later, the origin of the pulmonary artery becomes shifted on to the aorta just in front of the coeliac, the fused fifth and sixth arches of each side coming together into the dorsal aorta. In the adult the pulmonary and the coeliac arteries are anastomosed at their origin from the dorsal aorta. It may be noted that in Amia the coeliac comes off from the junction of the sixth arches before they enter the aorta. In Gymnarchus there is only one artery to the air-bladder, and one vein coming from it to enter the posterior cardinal vein.

In Gymnarchus the air-bladder rudiment first appears in an embryo five days old (fig.39, A) as a small diverticulum of the dorsal wall of the alimentary canal. The rudiment appears to be very slightly to the left of the mid-dorsal line, but I think this is probably due to the whole body of the embryo being twisted over to the right in the specimen sectioned. At this stage the embryo is still within the egg-membrane, and the whole of the head and anterior part of the body is lying on its right side. This twisting tends to obscure the exact topography of the organs, but the lung rudiment is, I think, approximately mid-dorsal. In a six-day embryo the rudiment is deeper and forms a short groove in the wall of the canal. It is mid-dorsal as regards its relations to the notochord and the nerve cord, but is now well over to the left of the dorsal wall of the somewhat flattened alimentary canal, and is tilted over to the right (Fig.39,B-E).

Reconstructions/

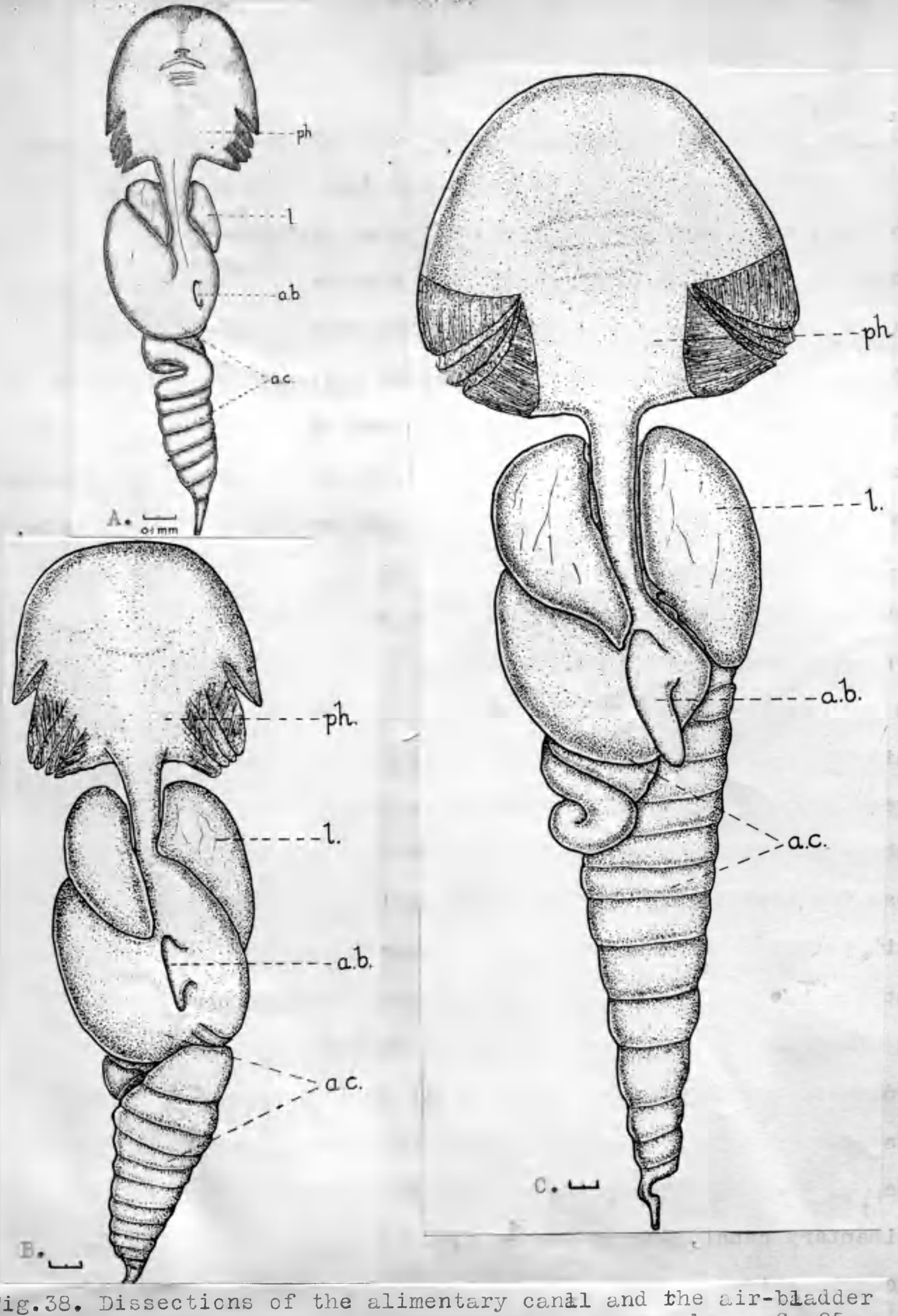
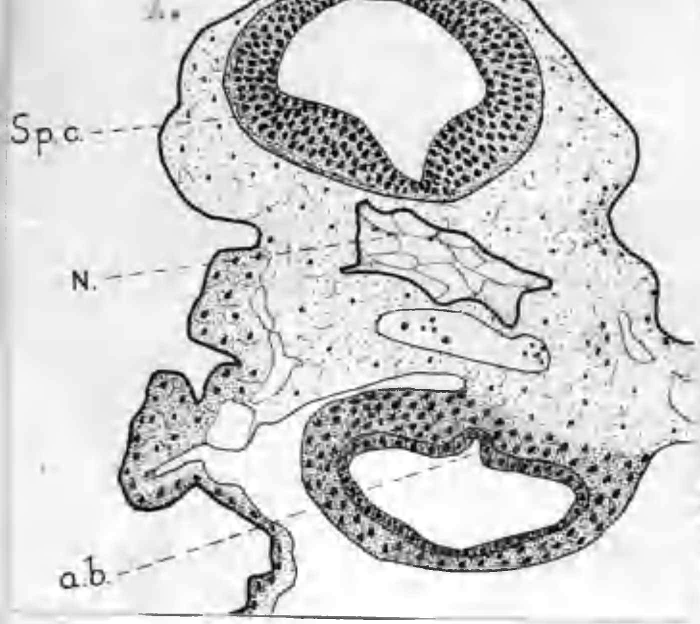


Fig.38. Dissections of the alimentary canal and the air-bladder rudiment of Acipenser; A, 30mm embryo; B, 50mm embryo; C, 95mm embryo.

Reconstructions of horizontal sections of 7-day embryos show that the rudimentary air-bladder bulges well over to the right from the pneumatic duct and extends forwards and towards the middle-line, and later, in a 15 mm. embryo, backwards also (fig.40). In this 15 mm. embryo the duct is bent well over to the right so that the whole organ is distinctly to the right of the body cavity. In an 18 mm. (8-day) embryo the organ has greatly developed. The anterior portion is, at its origin from the duct, well to the right, but further forwards it has assumed a central position and its anterior end is bifurcated, the two branches growing forward and somewhat to the sides. The portion posterior to the duct is very much enlarged and is also much nearer the mid-dorsal line than before. Already the cavity is becoming complicated by localised bulgings of the wall, so as to form intercommunicating chambers. In 9- and 10-day embryos the anterior horns of the air-bladder have reached the neighbourhood of the otocyst, and their stems are becoming reduced in diameter. The posterior or main portion of the air-bladder has a larger number of branching cavities than in earlier stages. The alimentary canal, which at earlier stages had a distinct and open cavity is now almost solid, though the lumen of the air-bladder and its duct are not closed.

A reconstruction from horizontal sections of the air-bladder in an 11-day embryo, 34 mm. long (fig.40, C), shows the curious arrangement of the cavities. The pneumatic duct now leads straight/



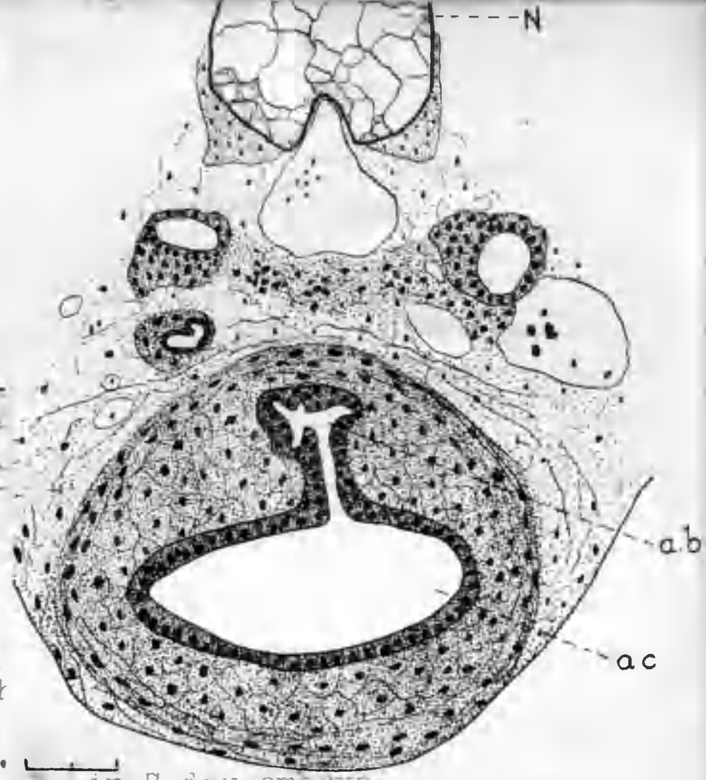
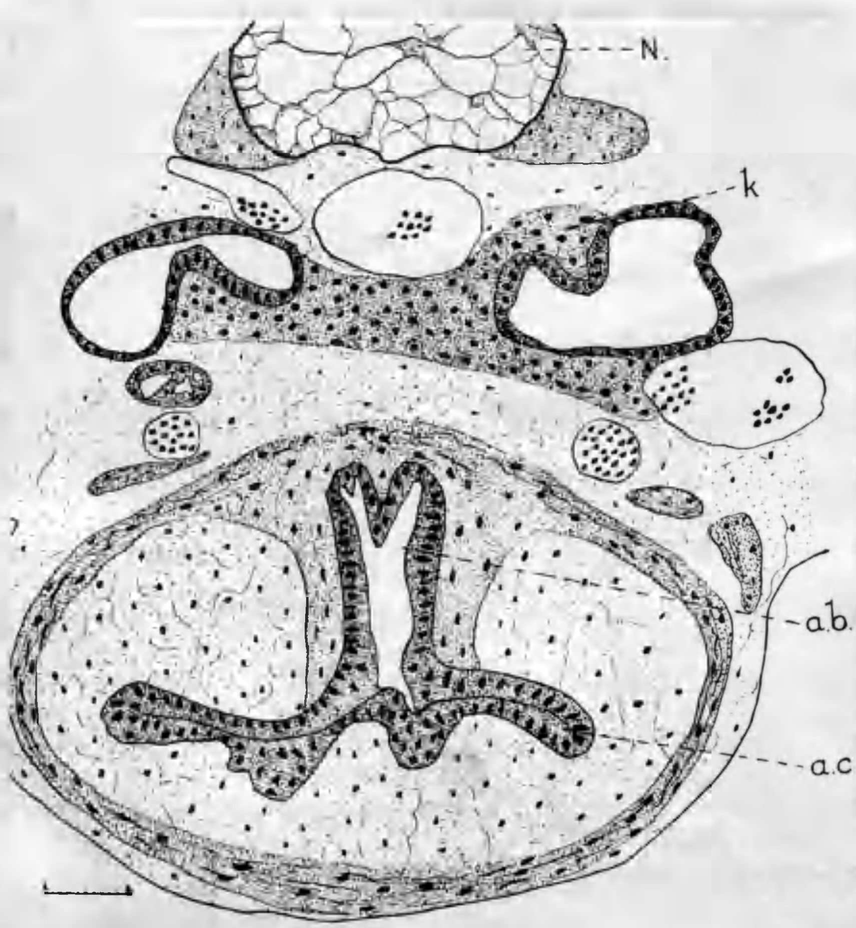


Fig.39. Transverse sections through the glottis of the developing lung of Gymnarchus. A. in 5-day embryo. B. in 7-day embryo.



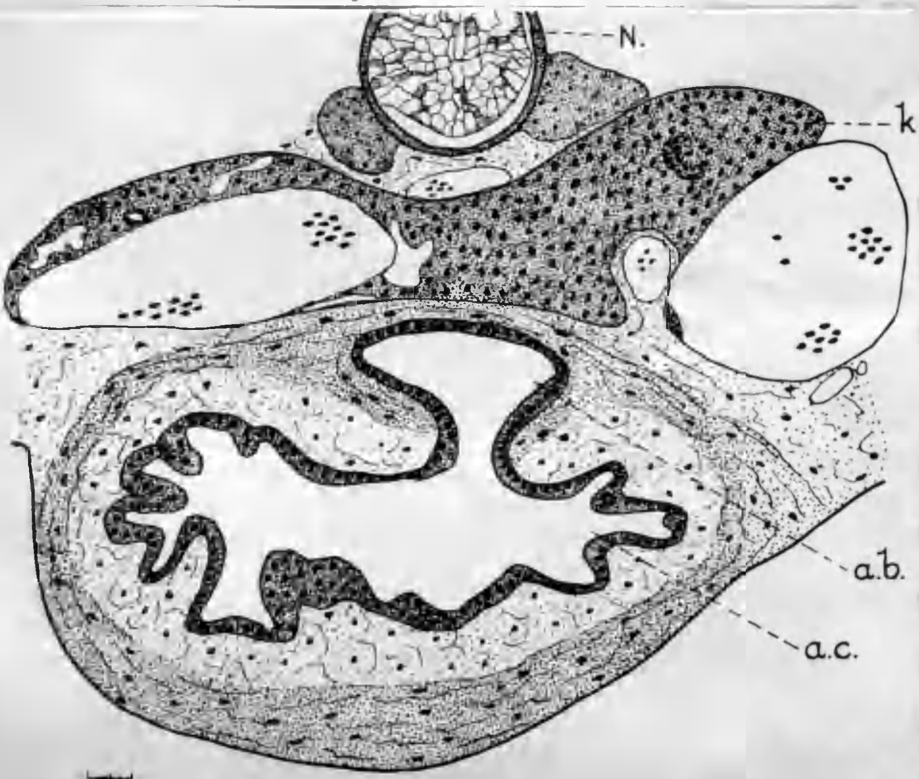
C, in 10-day embryo.

straight back into a long, tubular cavity which gives off side branches all round. This cavity extends the whole length of the air-bladder down the middle. Near the anterior end there is a large branch to the right side which ends in a short branch caudally and a much larger branch which runs forward into the stem of the anterior horns. In a 40 mm. embryo (fig.40, D), which I dissected, the air-bladder extends the whole length of the body cavity. Its posterior end is slightly to the left side, and the whole organ has a spongy appearance. The glottis is in the dorsal wall of the alimentary canal, a little behind the pharynx. The undivided duct of the anterior horns is well over to the right at its origin from the air-bladder, but turns towards the middle line before dividing into the branches leading to the vesicles near the otocyst. Both the single duct and the two branches are reduced to narrow, almost solid, strands of tissue. These branches are ventral to the kidneys, but anteriorly they bend upwards in front of the kidneys.

In transverse sections of a 43-day specimen, I see no trace of the stems of the vesicles, though they themselves are large. These observations agree closely with those of ASSHETON (2).

In this large embryo, the glottis is distinctly to the left of the dorsal wall of the alimentary canal, though it is nearly mid-dorsal in the body cavity. According to ROWNTREE (35), the glottis is well to the left in adults of the Mormyridae. It would/

D, in 12-day embryo.



E, in 43-day embryo.

would appear, therefore, that the glottis tends to move from an approximately mid-dorsal position towards the left.

ERLD(11) describes a roomy vacuity in the side of the wall of the skull of Gymnarchus, in close relation to the otocyst and covered over by a thin, oval shell-like bone. The presence of this thin bone may be the explanation of the loss of the air-bladder connection of the vesicles, since it will physiologically serve the purpose of a tympanic membrane. This same cavity in the skull is described by RIDEWOOD (33), and TATE REGAN (31) states that there is in the Mormyridae "on each side of the skull, superiorly, a lateral foramen lodging a vesicle which has lost its connection with the air-bladder". BUDGETT, in his diary of the expedition to Uganda, states that the sacs of the anterior horns of the air-bladder are blown out tight; that there is a stem with a hollow canal in the skull bone, but no apparent opening into the air-bladder. He also states that this bulbous horn derived from the air-bladder is present in all the Mormyridae which he has dissected.

In the specimen which I have dissected, there are present at the sides of the skull the two vesicles in close contact with the otocysts. Each has a short duct running caudally and towards the middle line, in the bone of the floor of the skull. These ducts end blindly just before they meet, and there is no trace of the connecting tube which at earlier stages united them to/

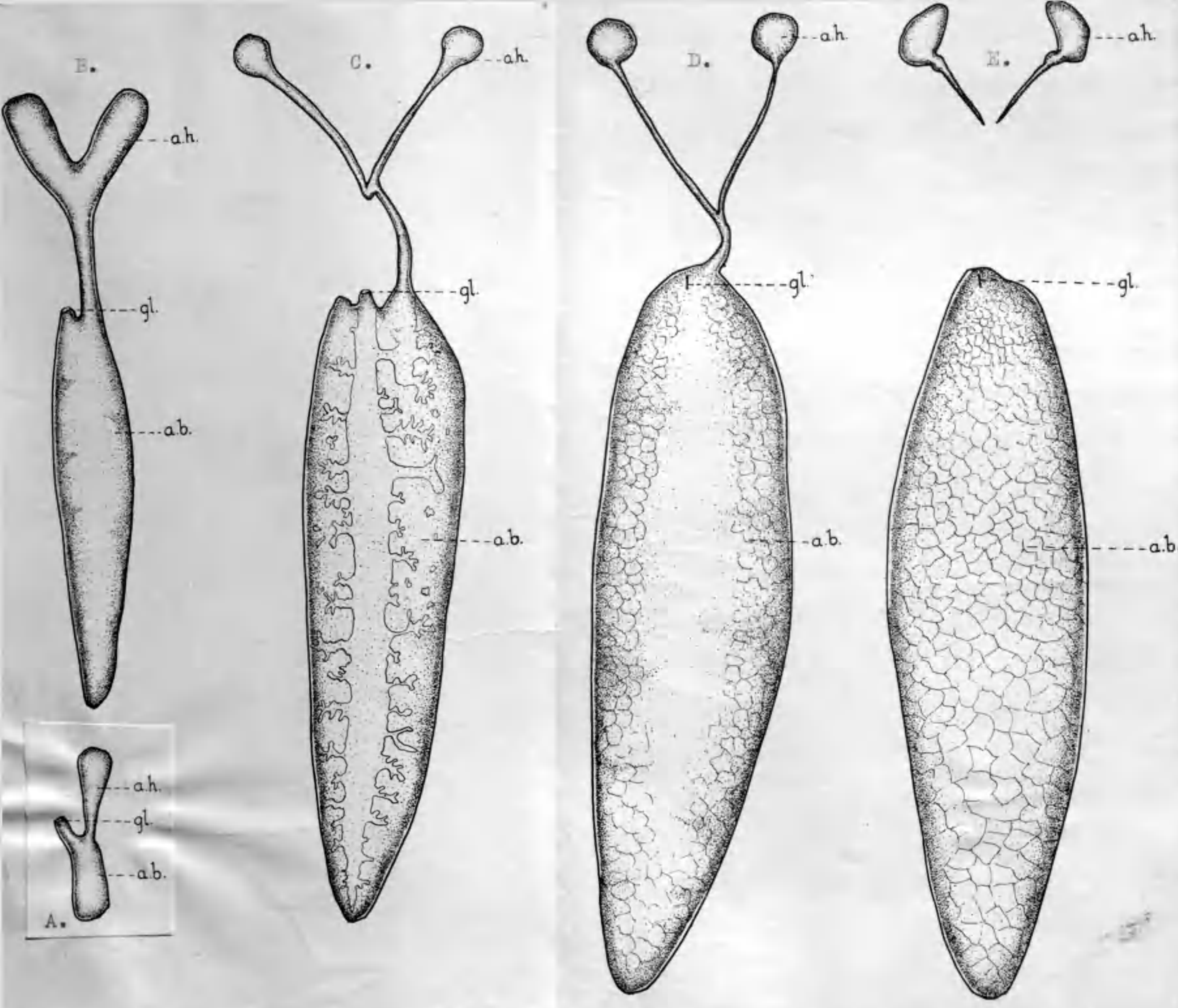


Fig.40. Drawings of different stages in the development of the lung of Gymnarchus.
A, from 5mm embryo; B, from 18mm embryo; C, from 34mm embryo; D, from 40mm embryo; E, from adult. Earlier stages more highly magnified than later stages.

to the anterior end of the air-bladder (fig.41).

It would appear, therefore, that in *Gymnarchus* the connection between the otocyst and the air-bladder is degenerating, though the air-bladder itself is to some extent a respiratory organ.

One point of interest in the development of the air-bladder in *Gymnarchus* is that, though when the rudiment first appears, the œsophagus is an open tube (fig.39, A-B), at a later stage the walls of the alimentary canal are approximated so as to give the appearance of a solid rod of cells (fig.39, C), although the cavity of the groove-like pneumatic duct remains open. I think that this may be the case with *Lepidosteus*, for in the 11 and 10 mm. embryos which I have examined, the anterior part of the air-bladder rudiment is a groove in the dorsal wall of the solid œsophagus. In MAKUSCHOK'S Fig.6 there is a distinct groove in the dorsal wall of the œsophagus, which looks remarkably like the air-bladder rudiment, though he says that it makes its appearance first in an embryo considerably older than the specimen represented in this figure.

CALLICHTHYS.

BRIDGE and HADDON (7) describe a widespread degeneracy of the air-bladder and the Weberian apparatus among the Siluridae ; and BRIDGE, in the *Cambridge Natural History* (5), states that the air-bladder is dumb-bell shaped in *Callichthys*, though SAGEMEHL (36) and others say that in some Siluridae, including *Callichthys*, there/

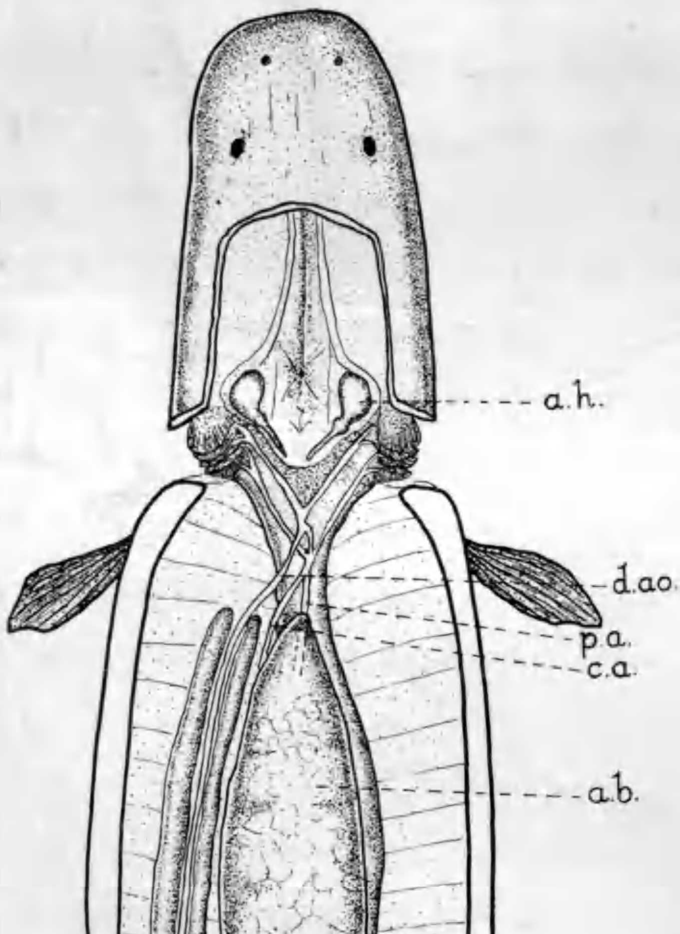


Fig. 41. Dissection of adult Gymnarchus. Kidneys displaced to the left to expose the air-bladder.

there is no air-bladder. In embryos of $10\frac{1}{2}$ and 12 mm. of this fish (fig.42 A-B), there is a large sac on either side of the head extending from the otocyst to a little distance beyond the gill clefts. In both specimens the two cavities, near their posterior end, are in communication with one another by a narrow duct lying ventral to the notochord and the aorta, but dorsal to the kidneys and alimentary canal. There are traces which indicate that there is a connection between this duct and the alimentary canal at earlier stages of development. These vesicles may, I think, be taken to be part of an air-bladder, which has degenerated except for the anterior horns.

EVOLUTIONARY HISTORY of the AIR-BLADDER.

The observations chronicled in this paper enable us to picture more fully the probable evolutionary history of the transition from the bilaterally symmetrical, ventrally placed, paired lungs to the dorsal and unpaired air-bladder of the modern fish. TRACY (39) has worked out the probable later stages of evolution from the simple air-bladder of Salmo, with its anteriorly placed pneumatic duct, to the complex organ present in Physoclistic fishes. The transition from the air-bladder as it is in such forms as Amia, to the condition shown by the air-bladder of Salmo is primarily a still further reduction of the respiratory, and a fuller development of the hydrostatic function, with the accompanying changes in the form and blood supply of the organ,

The

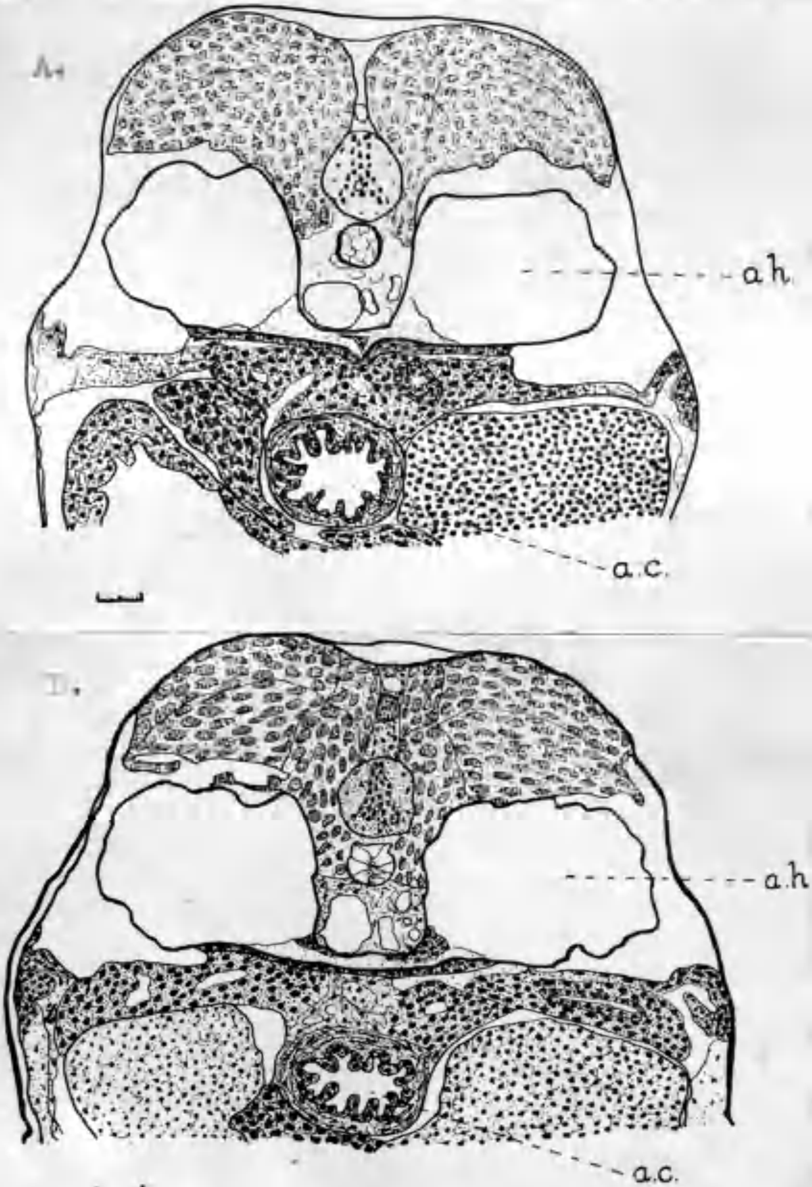


Fig. 42. Sections through air-bladder of Callichthys. A, in 10½mm embryo; B, in 12mm embryo.

The evolutionary stages, as far as they can be traced, seem to be: first, normal, ventral, and paired lungs with the usual pulmonary blood and nerve supply; secondly, the gradual reduction of the left and enlargement of the right lung, seen in its early stages in *Polypterus* and completed in the adult *Ceratodus*. This predominance of the right over the left lung is possibly due in large measure to the position of the stomach well down to the left side.

The next stage is illustrated by *Amia* with its "pulmonoid" air-bladder, which has the normal pulmonary nerve and blood supply, but opens dorsally into the alimentary canal. *Lepidosteus*, again, has a pulmonoid air-bladder with pulmonary nerves, but blood coming from the aorta. In *Acipenser* the reduction of the respiratory function is complete and the air-bladder is a simple membranous sac with its blood supply from the dorsal aorta. In *Salmo* the hydrostatic function has developed further and the air-bladder has now "red-glands" in its interior walls, but it is still a simple sac opening dorsally into the alimentary canal.

Ceratodus, *Amia*, and *Lepidosteus*, in their development, show transitory traces of the existence at one time of a left lung; and the change in position of the glottis from the ventral to the dorsal side may be reasonably interpreted as, at least partially, due to the rotation of the alimentary canal round by the right, as has already been shown by the work of MOSER (26,27) KERR (20), and others.

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III

DEVELOPMENT OF CALLICHTHYS LITTORALE.

Dr. G. S. CARTER, on his expedition to the Gran Chaco of South America in the year 1926-27, collected a fairly complete series of the developmental and adult stages of *Callichthys*. Several species were represented among the adults obtained, but the developmental stages are almost entirely those of *Callichthys* (now called *Hoplosternum*) *littorale*. *Callichthys* belongs to a small sub-family of fresh-water fishes belonging to the group Siluridae. The fish was described first under the name of *Callichthys littoralis* by HANCOCK (10). He gives a brief description of the external form and tells how the fish make a regular nest of leaves in which they lay the eggs, and which the male and female both guard most carefully.

The only other literature on the subject appears to be a note on the "parietal foramen" by von KLINCKOWSTRÖM (15), and a few papers on the breeding habits and the nest by various workers. BRIDGE and HADDON (4) give a brief description of the Weberian ossicles in their monograph on the subject, and RIDEWOOD (18) gives a figure of the dorsal blood system of *Callichthys littoralis* in his paper on the "*circulus cephalicus*" of fish.

As the armoured Siluroids are a group of extraordinary interest/

interest, this material offered a welcome opportunity of enquiring into their embryology, and the following observations were made on the eggs and larvae which Dr. CARTER brought back from South America.

DEVELOPMENT.

In the series of developmental stages there are some 58 tubes of eggs. In most cases where a nest of eggs was taken, one half of the eggs was fixed in Bouin, the other half in corrosive acetic, and then each preserved in 70% alcohol. There are, therefore, about thirty different nests represented in the series. About one half of the tubes contain eggs in the later stages of segmentation, but in the others there is a fairly complete series of the later embryonic stages.

The eggs are held together in bundles and are very numerous. Each egg, with its capsule, measures approximately 1 mm. in diameter and is spherical in form. The capsule is a thin, tough membrane which is quite free from the egg itself, from which it is separated by a coagulated mass of an albuminous substance in the preserved material. When the capsule is removed, this albuminous substance breaks away from the egg, from which it is quite free.

"Stage 5". - (Fig.43). The youngest specimen in the series of eggs is one where there is a little cap of whitish cells at the apical pole of the egg, while the remaining and larger part of/

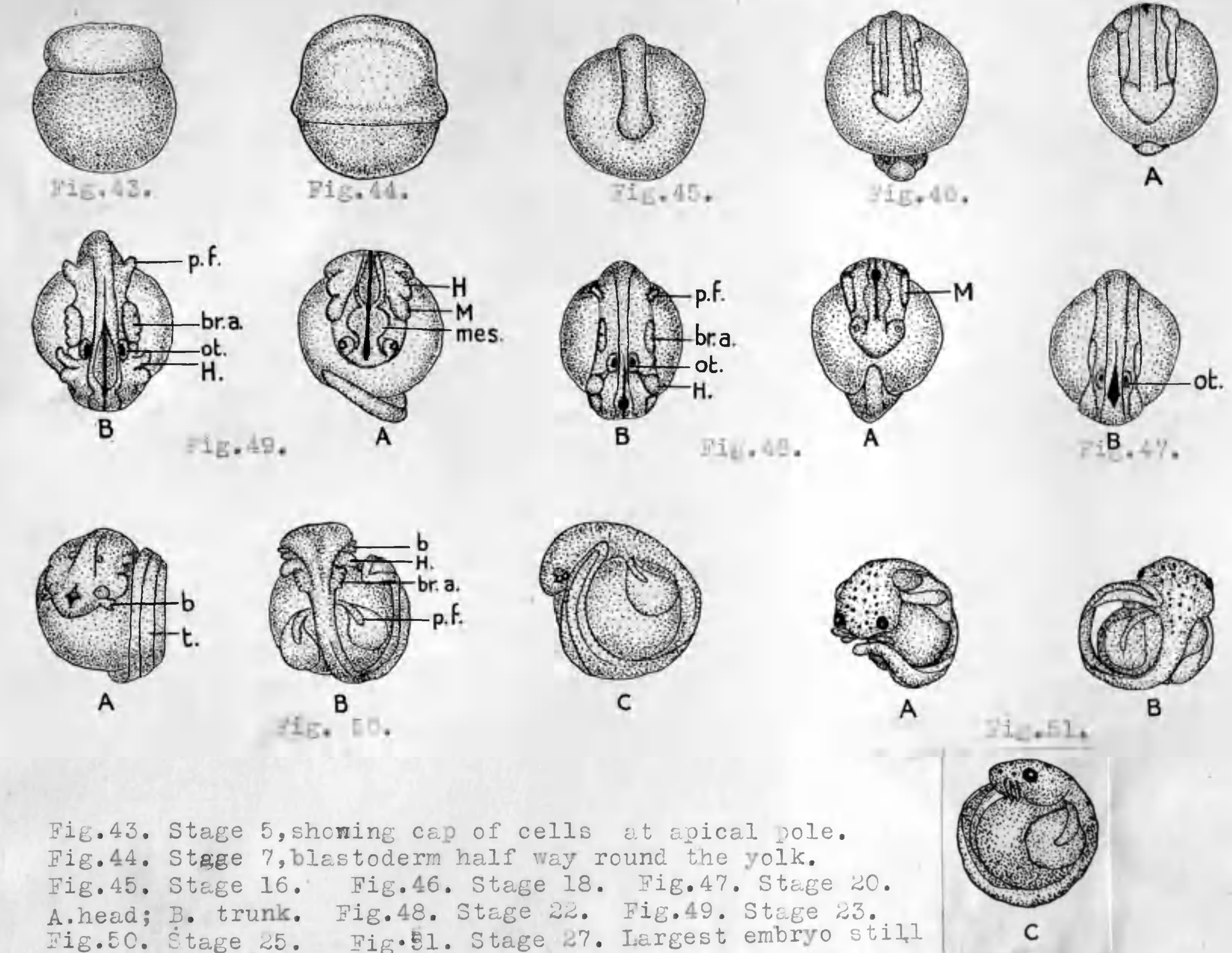

Fig.43. Stage 5,showing cap of cells at apical pole.
Fig.44. Stgge 7,blastoderm half way round the yolk.
Fig.45. Stage 16. Fig.46. Stage 18. Fig.47. Stage 20.
A.head; B. trunk. Fig.48. Stage 22. Fig.49. Stage 23.
Fig.50. Stage 25. Fig.51. Stage 27. Largest embryo still within the egg membrane.

b.barbels; br.a.branchial arches; H.hyoid; M.mandibular arch; ot.otocyst; p.f.pectoral fins; t.tail.

of the egg is composed of undivided, yellow yolk. This stage appears to correspond roughly to that described as stage 5 by TAYLOR (20) in her paper on the development of Symbranchus. Sections through this stage show that the cap is formed of very numerous, more or less round cells, with very distinct nuclei, many of which are in the process of dividing. These sections closely resemble those of the eggs of Salmo fario at the thirteenth segmentation division as figured by KOPSCH (17).

Between the blastoderm and the yolk there is a thin layer of syncytial protoplasm. The blastoderm is very easily separated from the yolk at this and subsequent stages. A very thin membrane surrounds the yolk which is very hard and is apt to tear badly when cut into sections. The yolk contains round droplets of oil and also in the middle some solid substance which stains differently and seems to form a more or less solid sphere with oil droplets in the centre and round the outside.

Stage 7 . - (Fig.44). The blastoderm gradually increases in area, spreading down the sides of the yolk, and at stage 7, it has reached the equator of the egg, ending there in a slightly raised rim. In a stained and cleared specimen, the top of the yolk can be seen quite distinctly through the blastoderm, there being a small cavity at the apical pole between the yolk and the blastoderm. Sections show that the blastoderm is approximately two cells deep. Round the margin of the blastoderm, there are scattered/

scattered and rounded cells between the blastoderm and the yolk, and continuous with the blastoderm peripherally.

Though the blastoderm is at this stage as large relatively as that of an egg of Symbranchus at stage 11, there is no sign of the formation of the embryo.

Stage 13 . - It is not until the blastoderm has almost completely enveloped the yolk that the first formation of the embryo is apparent. At this stage the greater part of the blastoderm is extremely thin, being simply an attenuated membrane surrounding, but free from, the yolk. Round its margin the blastoderm is greatly thickened, and at one part this thickening extends for some distance away from the margin. This thickening which is fairly wide but is not yet in any way differentiated into tissues, represents the first formation of the embryo. I have found only one or two eggs at this stage of development, and it is only by examining sections that it is possible to make out the thickening described.

Stage 16 . - (Fig.45). The embryonic rudiment rapidly increases in length and by stage 16, extends along about one quarter or one third of the circumference of the egg. The rudiment has now a solid and quite definite nerve cord which has a tendency to bulge out anteriorly into the two optic rudiments, but otherwise is not yet differentiated into brain and spinal cord. On either side of the nerve cord are the myotomes, closely pressed/

pressed up against it, and ventrally there is the rudimentary notochord, also closely apposed to the spinal cord. As yet there are no signs of the visceral clefts nor of the otocyst, The margins of the blastoderm have not quite met and just in front of the opening, at the posterior end of the embryo in some sections, there is for a short distance an oval cavity which is probably to be regarded at Kupffer's vesicle.

Of this stage and stage 18 there are only a comparatively few specimens in the series, stages 5 and 7 and the later embryonic stages being much the most numerous.

Stage 18 . - (Fig.46). By stage 18 the embryo stretches round about two-thirds of the circumference of the yolk. The greater part of the embryo is still attached to the yolk, but the tail has begun to separate from it. The optic rudiments are larger than at stage 16, and extend a little back from the top of the fore-brain, giving the front end of the embryo an arrow-head like appearance. The brain and spinal cord are still quite solid. The otocyst has been formed, and ventral to it, two large blood vessels, the vitelline veins, lie between the embryo and the yolk. In the trunk region the notochord is more distinctly formed than at earlier stages. As yet the branchial arches have not appeared.

Stage 20 . - (Fig.47). At stage 20 a cavity has been developed in the brain and spinal cord. Both optic and otocyst rudiments/

rudiments are larger and have a distinct cavity, that of the optic rudiment being continuous with the cavity of the fore-brain. The mandibular, hyoid and branchial arches are represented by a continuous line of thickening at either side of the head, from some distance behind the optic rudiments to just beyond the otocyst. As yet the pectoral fins have not been formed. The whole embryo almost completely encircles the yolk.

Stage 22. - (Fig.48). At this stage the mandibular arch is still represented by a long line of thickening, but the hyoid is becoming distinct from it posteriorly, and behind the level of the otocyst, the branchial arches, though still all fused together, can be distinguished from one another by the line of epithelium bounding each rudimentary cleft. The pectoral fin is present as a slight thickening of the deep layer of the ectoderm somewhat further back on the body than the last branchial arch. The olfactory organ is still simply a thickening of the ectoderm, the eye is similar to that of a 65-hour chick embryo, and the otocyst is larger than at stage 20 but otherwise has much the same appearance.

Stage 23. - (Fig.49). In a very slightly older embryo, the eyes are more developed, the lens being now free from the ectoderm and the optic stalk considerably narrower than at earlier stages. In places the very much flattened out fore-gut is beginning to develop a cavity. The branchial arteries are formed/

formed and the heart is bulging out to the left, between the embryonic body and the yolk. The operculum is beginning to grow back from the hyoid arch, and the branchial arches, though still undivided, are more distinct. Behind them the solid alimentary canal extends back for some distance under the notochord. The pectoral fin is more prominent than at stage 22. The brain is now distinctly divided into the thalamencephalon with the optic rudiments still in open continuity with it, the mesencephalon and the rhombencephalon. The mesencephalon is marked off from the latter by a constriction which is visible externally. The roof of both the mid- and hind-brain is very thin and closely underlies the outer ectoderm. Opisthonephric tubules are present about the level of the pectoral fins, on either side of, and ventral to the notochord.

Stage 25 . - (Fig.50). The next big step forward in development is represented by stage 25. The whole animal shows a considerable advance. The head is more definitely formed, and at either side of it anteriorly there are three little projections. The first two of these projections are barbels developed from the mandibular arch, and the third is the rapidly growing operculum, which extends back over the first branchial arch. As yet neither the mouth nor the gill clefts are open, but the four gill arches are slightly separated from one another externally. Each has its own blood vessel running through it. Anteriorly the pharynx is/

is still solid, but towards its posterior end, a cavity is forming, and a short distance behind the last branchial arch, where the cavity of the alimentary canal is at its largest, a tiny little diverticulum from the right side of the dorsal wall represents the very first rudiment of the air-bladder. Just anterior to the air-bladder rudiment and immediately dorsal to the alimentary canal, there is a curious little organ lying just below the dorsal aorta, which I believe to be the pronephros. Posteriorly the alimentary canal bulges out to the left and then stretches straight back to the anus, with the kidney duct lying alongside it.

The heart has increased in size and the ventricle has already got a muscular wall with trabeculae beginning to form in it. The pectoral fins are very much more developed than at stage 23. Round the free end of the solid axis, the fin has a membranous fold in which are numerous blood vessels. The median fins are beginning to form as a continuous fold of skin round the free end of the tail. The eye shows a considerable advance from that of stage 23. The optic stalk is now quite closed and is becoming drawn out into the definitive optic nerve. The lens is becoming crystalline in structure. The semi-circular canals of the otocyst are formed though their cavities are still very large.

Stage 27 . - (Fig.51). This is the stage at which the embryo hatches.

Some/

Some of the tubes of eggs in which this stage is found, contain newly hatched larvae as well. The yolk is now considerably reduced in size. The whole tail behind the anus, is free from the yolk and has a well developed, continuous fold of fin round it. The tail is bent laterally so that one side is next the yolk, round which it is curved so that its tip overlaps the head. The pectoral fins are well developed. The barbels which made their first appearance at stage 25, are now quite long.

The buccal cavity is now open for a short distance, but the greater part of the pharynx is solid though the branchial arches are well marked externally, so that each arch projects freely from the solid central part into the branchial chamber which is now completely covered in by the operculum at each side. Behind the pharynx the alimentary canal has a distinct cavity, and it is now recognisable right back to the anus, bulging out to the left in the region of the stomach. The air-bladder is at this stage a very small, flattened sac lying median and dorsal to the alimentary canal with which it is connected by a narrow duct, just in front of the stomach rudiment.

The olfactory organ has now developed a slight concavity and is connected with the fore-brain by a broad strand of nerve. The eye is surrounded with black pigment which is visible externally. The retina is beginning to form, though the lens almost completely fills the cavity of the eye. The optic nerves are quite/

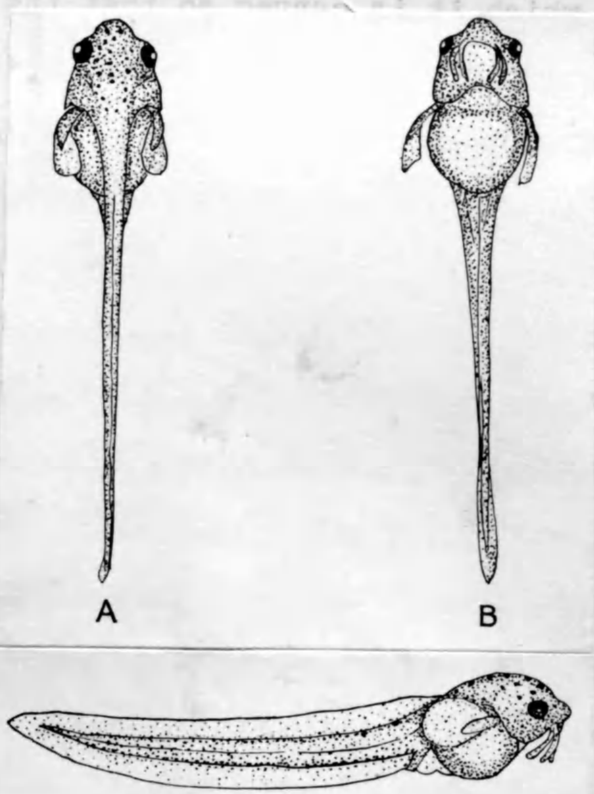

Fig.52. Stage 28. Newly hatched larva. A. dorsal; B. ventral; C. side view.

quite definite. The semi-circular canals and the sacculus are present in the otocyst. The brain is rapidly developing. At stage 25, the tissue of the brain and spinal cord had begun to differentiate into gray and white matter, and by stage 27, this has advanced considerably. The infundibulum is present and the pineal is formed, while the optic lobes have increased in size and thickness.

The blood system is rapidly assuming its adult form. Anterior and posterior cardinal veins are present. The atrium appears to be partially in front of the ventricle. The ventricle has already got its typical, trabecular wall. The conus (i.e. that portion of the heart between the ventricle and the anterior end of the pericardiac cavity) is extremely short, as is also the ventral aorta. The cartilage of the chondrocranium has begun to make its appearance and the notochord is large and well developed.

Stage 28 . - (Fig.52). In the newly hatched larva, conditions are much the same as at stage 27. The tail has increased in length and is now straightened out. There is still a continuous fin along its dorsal side, round the tip and along the ventral side. The yolk is even smaller than at stage 27, and is confined to the trunk region. It is embraced laterally by the pectoral fins which have the same form as at stage 27. There are spots of brown pigment on the head and the dorsal side of the trunk.

The stage of development at which hatching actually takes place/

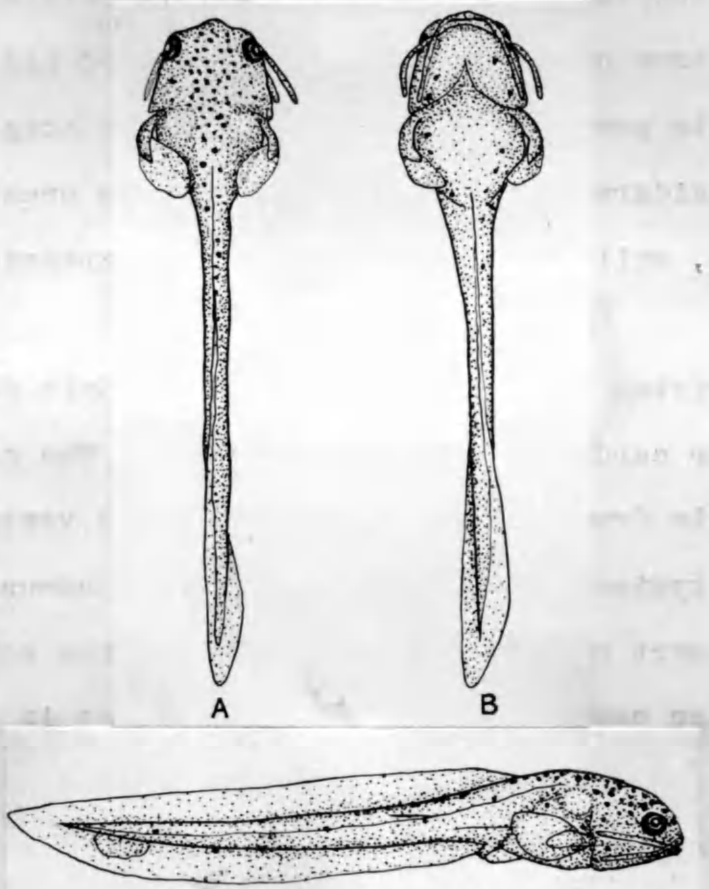

Fig.53. Stage 30. Larva of approx. 5mm length.

place,seems to vary considerably, for in some of the eggs the alimentary canal is open throughout its length, while in some of the newly hatched larvae the greater part of the pharynx is still closed. In the larva described as stage 28, the first branchial cleft is open, but the other clefts are still closed and the pharynx is just beginning to form a cavity down the centre.

Stage 29 . - At stage 29, the conditions are much the same as at stage 28, except that the alimentary canal is completely open, as are also the gill clefts. The air-bladder has increased in size especially at the sides and there is a solid strand of condensed connective tissue uniting the two sides dorsal to the notochord.

Stage 30 . - (Fig.53). Stage 30 represents larvae of about 36 hours after hatching. The caudal fin is becoming distinguishable from the continuous dorsal and anal fin and is marked by a thickening of the membranous fold, ventral and slightly anterior to the tip of the tail, which is, however, still quite straight. In the pectoral fins the anterior part of the membranous fold is tending to become reduced. The fins still project out almost at right angles and clasp the sides of the rapidly diminishing yolk.

At this stage all the gill clefts are open and the branchial arches have developed numerous membranous folds which are richly supplied with blood. The alimentary canal is becoming coiled and/

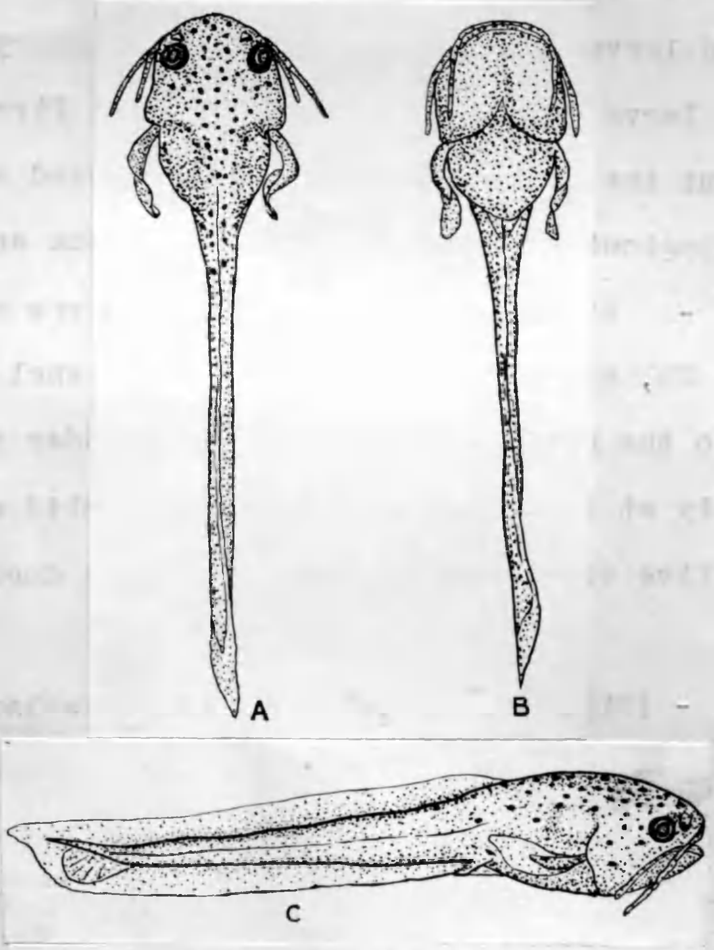

Fig.54. Stage 31. Larva of approx. $5\frac{1}{2}$mm length.

and is deeply embedded in the yolk, instead of lying on its dorsal surface as at earlier stages. The liver, which made its first appearance as a definite organ at stage 27, is now large and very vascular.

The sense organs are rapidly assuming their adult form. The olfactory organ has still a single wide opening to the exterior, but it is now quite a deep pit with thick walls. In the eye, the retina is well developed and the cavity is larger, though the lens still occupies the main part of it. The otocyst is rapidly assuming its adult form and the air-sacs are very much the same as they are in the adult, except that they are still connected with one another by a wide open channel, which is connected with the alimentary canal by a narrow, but open, pneumatic duct.

Stage 31. - (Fig.54). In three- and two-day larvae, the tail is still quite straight, tapering off to a point, but there is a marked increase in the depth of the fin just ventral to the tip of the tail and posterior to the group of fin-rays. The head is much rounder and more compact and the yolk is smaller. The pectoral fins are beginning to form fin-rays. Internally the structures are much the same as at stage 30, the main difference being in the reduction of the quantity of the yolk.

Stage 32. - (Fig.55). This stage is represented by larvae from five to seven days old. The head is still large in comparison/

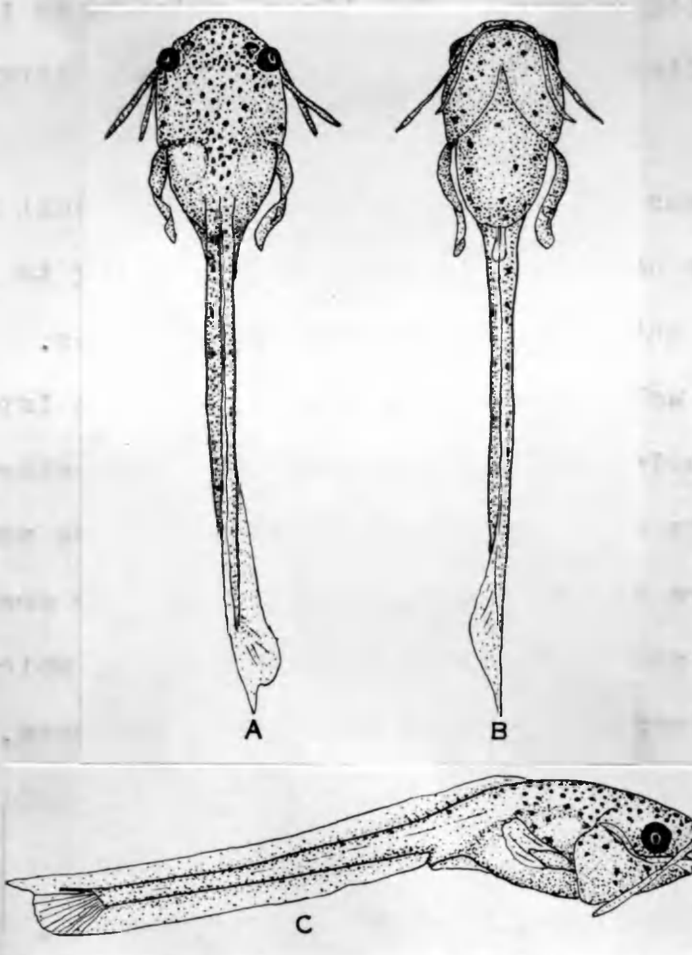

Fig.55. Stage 32. Larva of approx. 6mm length.

comparison with the tail, which is long and thin. The tail is now bent up slightly so that the group of fin-rays is practically terminal and forms a definite caudal fin which is continuous with the anal fin. The fin round the tip of the tail projects out in a point dorsal to the caudal fin, and is directly continuous with the dorsal fin.

On the head, as at earlier stages, the position of the air-sacs is visible externally. The yolk, though still present, is very much reduced in size.

Stage 35 . - (Fig.56). At this point in the series of larvae, there is a considerable gap and the next specimen is a single larva of 10 mm. length, which I describe as stage 35. Fortunately, there are also series of transverse, horizontal and sagittal sections of larvae of about the same size, prepared from specimens in Professor GRAHAM KERR'S collection. The single specimen in Dr. CARTER'S series is very much more like the adult fish than any of the younger larvae. The head, trunk and tail merge into one another more gradually than at stage 32, though the trunk is still distended ventrally and laterally.

The skull has developed considerably so that the position of the air-sacs is no longer visible externally, but the roof of the skull is not yet complete and a large opening in it can be seen through the skin. The pectoral fins have a thick muscular base and fin-rays running out fan-wise from it, as in the adult fin/

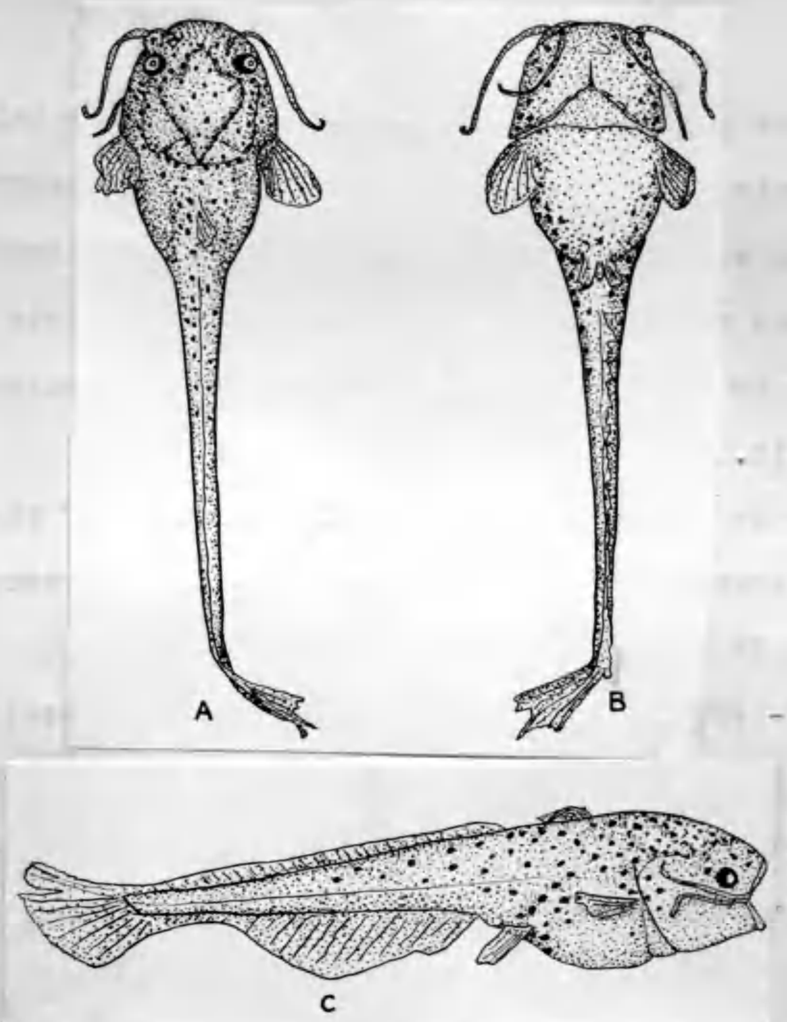

Fig.56. Stage 35. Larva of approx. $10\frac{1}{2}$mm length.

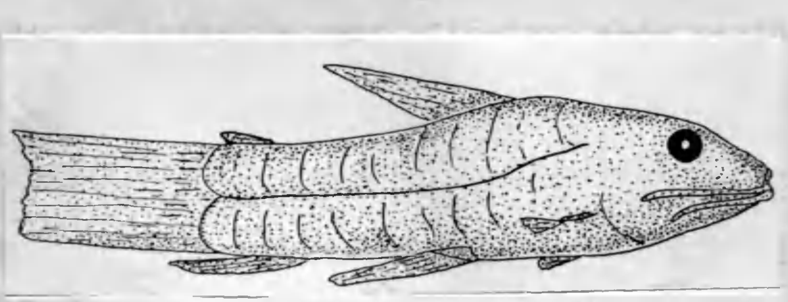

Fig.57. Stage 39. Larva of 22mm length.

fin, though the anterior strong spine has not appeared. The pelvic fins are quite large, though they had not appeared at stage 32. The continuous dorsal fin is now divided into a short, but deep, anterior part and a long, low, posterior part. It is quite separate now from the caudal fin which has lost its dorsal part externally. The anal fin still extends along the greater part of the ventral wall of the tail from the anus to the caudal fin, but it is quite separate from the latter. The whole of the animal is still covered with large and numerous spots of brown pigment.

Stage 39 . - (Fig.57). The only remaining specimens in the series of developmental stages are larvae of from about 20 mm. length, upwards. In a specimen of 20 mm. length, which I describe as stage 39, the adult external features are all well marked except that the bony plates and the skull are not completely ossified. At stage 35 these plates are not visible, but at stage 39 they cover the whole animal laterally and tend to stick out from the surface, giving the fish a rough appearance. The head has developed bone just under the outer skin so that it is to a large extent encased in bone. On the surface of the head and along the lateral line are sense organs more or less segmentally arranged, with several rows running in different directions on the head. Even in this late larva the gonad is not visible.

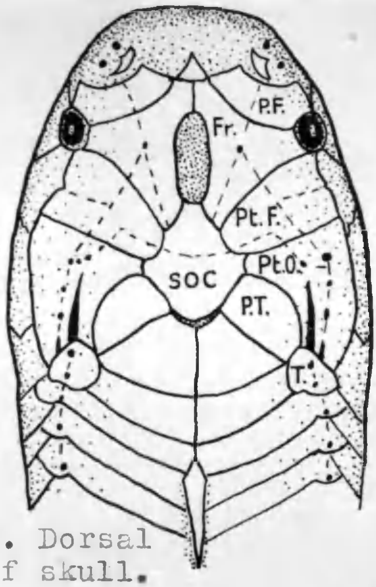

Fig.58. Dorsal view of skull.

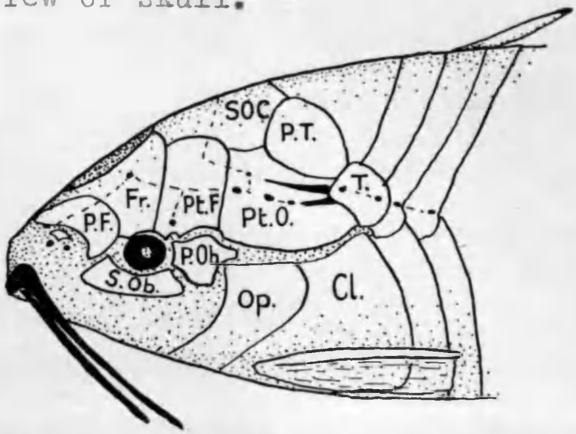

Fig.59. Lateral view of skull. Cl.cleithrum; Fr.frontal; Op.operculum; P.F.prefrontal; P.Ob.postorbital; P.T.post-temporal; Pt.F. postfrontal; Pt.O. pterotic; S.Ob suborbital; SOC.supraoccipital; T. tabulare.

SKELETON.

Skull.

In the adult the head is very completely enclosed with bones which are easily seen through the thin, superficial skin. Above and between the eyes, are two large frontal bones (Fig.58, Fr.), with an oval foramen between them. This "parietal foramen" has been described by KLINCKOWSTRÖM (15) who states that, though the foramen is large and well developed, the pineal organ associated with it, is very rudimentary and strongly resembles the condition of the pineal in the Anura.

Comparing the bones of the skull with the figures of the skulls of *Clarias* and *Synodontis* given by GOODRICH in Lankester's Treatise of Zoology, there appears to be a little diamond-shaped ethmoid in the middle, in front of the frontal bones. On either side of this and coming down to the antero-dorsal side of the eye, is a prefrontal (PF) with a small nasal bone just above the olfactory openings at each side.

Behind the frontals at either side, is the postfrontal (Pt.F.), bounded behind by the pterotic (Pt.O.), along which is a narrow groove leading down to the outer end of the air-sac capsule, and below this groove, a well marked row of sensory tubes. In the mid-dorsal line between the postfrontals and pterotics, there is a comparatively large supraoccipital (SOC), an oval posttemporal (PT) being wedged between the posterior ends of the supraoccipital and the pterotic.

Laterally/

Laterally (Fig.59), below the eye but coming up to meet the prefrontals, is a suborbital (S.Ob.) and behind this, forming the posterior part of the circle of bones round the eye, is a post-orbital (P.Ob.). The postorbital is ventral to the postfrontal and the anterior end of the pterotic. Behind it, and running down to meet its fellow in the mid-ventral line, is the operculum, which overlaps the anterior end of the very large plate of bone, the cleithrum, through which the pectoral fin passes to its attachment to the pectoral girdle.

Behind the supraoccipital are the much enlarged first dorsal plates of the body. On either side there is a small, more or less circular bone, bounded dorsally by the posttemporal and first two dorsal plates, ventrally by the cleithrum, posteriorly by the third dorsal plate, and anteriorly by the pterotic. This bone does not appear to be present in either Clarias or Synodontis. It overlies the widest part of the opening of the air-sac capsule. The line of sensory tubes crosses this bone, as it bends down from the head to the ordinary lateral line of the body. This bone probably corresponds to the bone called the tabulare by GOODRICH (9) in the skull of the fossil fish Osteolepis macrolepidotus. The skull of Callichthys bears a general resemblance to that of Osteolepis both in the arrangement of the bones and in the position of the lines of sense organs.

When the supraoccipital and the posttemporals are removed, the/

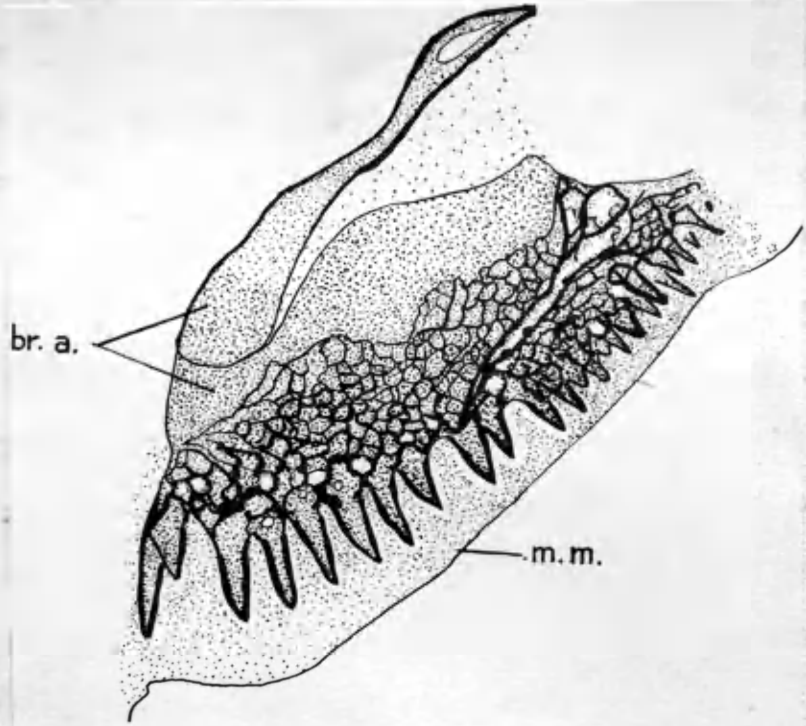

Fig.60. Horizontal section through the pharynx of young adult, showing teeth connected to a network of bone. br.a. branchial arch; mm.mucous membrane of the roof of the pharynx.

the posterior part of the brain is exposed, with the semicircular canals of the otocyst passing outwards under the postfrontal and pterotic bones. Immediately behind the auditory capsule and under the posttemporal bone, but separated from it by muscles, is the air-sac capsule, which is a sort of flask-shaped structure of thin bone lying on its side with the opening lateral and immediately above the top of the pectoral girdle.

There are little conical teeth on the upper and lower jaw right at the front of the mouth, and also at the posterior end of the pharynx on both roof and floor. The little teeth on the roof of the pharynx are attached to a kind of network of bone on the lower side of the cartilage of the dorsal part of the last branchial arch (Fig.60). In the adult there is quite a large cushion on either side of the posterior part of the roof of the pharynx on which these denticles occur.

In development bone begins to appear in a 12 mm. larva, stage 35, and is further advanced in a 20 mm. larva, stage 39. A large part of the floor and lateral walls of the cranium are preformed in cartilage, but almost the whole of the roof, that is to say all the bones visible through the skin, are not so preceded by cartilage. Some of the cartilage of the chondrocranium is already becoming invested with bone at stage 35, but a large part of it never becomes completely replaced by bone.

In the adult the outermost layer of bone on the head is pigmented/

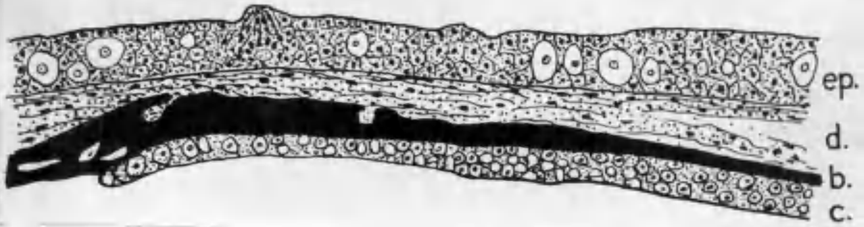

Fig.61. Transverse section of stage 39, showing bone forming in the dermal layer over the surface of the cartilage of the skull. b.bone; c.cartilage; d.dermis; ep.epidermis.

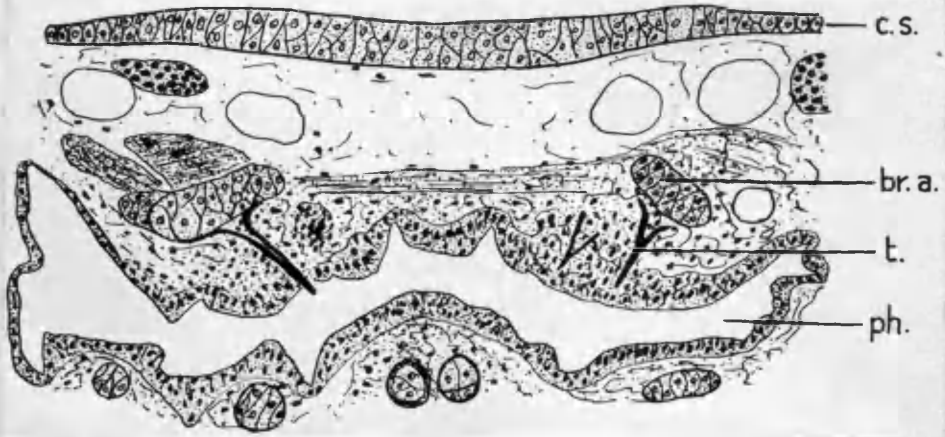

Fig.62. Teeth on the roof of the pharynx at stage 31. br.a.branchial arch; c.s.cartilage of skull; ph.pharynx; t.denticle.

pigmented and is only covered by a thin skin. The deeper bone is more compact and solid and is not pigmented. It seems probable that this outer layer of bone is of dermal origin both from the fact that it is so superficial in the adult and from the appearances during development. In a 22 mm. larva the epidermis is fairly thick, with numerous large round cells in it. Immediately below the epidermis is a dense layer of dermis, with many small nuclei scattered through it. Down the sides of the body, the bony plates are beginning to form in this layer, and over the surface of the head, the bone of the skull is forming just beneath it and in some places apparently actually in the dermis.(Fig.61). Where the superficial bone appears to be beneath the dermal layer, I think that there is no doubt that this separation is due to the shrinkage of the tissues in sections prepared by the paraffin method, especially since the bony plates of the body are quite definitely formed in the dermal layer.

The teeth which occur on the roof of the pharynx attached to a network of bone in the adult, make their first appearance at stage 30, where there is one large denticle on each side and one or two smaller teeth beside it. The base of the large tooth reaches back through the mucous membrane to the cartilage of the dorsal part of the last branchial arch. By stage 31 there are several teeth which are still separate from one another (Fig.62). The bases of one or two of the teeth reach the cartilage. By stage/

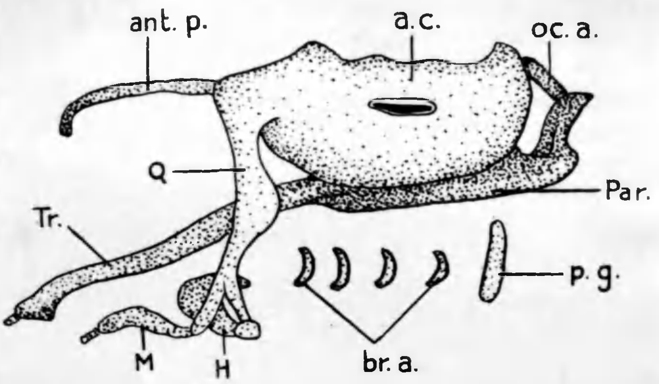

Fig.63. Reconstruction of the chondrocranium of a larva of stage 29, from sagittal sections. a.c.auditory capsule; ant.p.anterior process of cartilage meeting in front of optic lobes; br.a. branchial arches; H.hyoid; M.mandibular arch; n.c.nasal capsule; oc.a.occipital arch; Par.parachordals; p.g.pectoral girdle; Q.quadrate; T.trabeculae.

stage 32 the bases of the denticles are becoming joined up with one another and the number has increased. In a larva of stage 35 the trabeculae of bone are well developed and the denticles are much more numerous, and by stage 39 the adult condition has been attained.

Chondrocranium.

At stage 27 there are localised patches of condensed connective tissue where chondrification is taking place, but cartilage is not definitely formed until stage 29 when the chondrocranium (Fig.63) is already formed and resembles roughly that described by AGAR in Lepidosiren. It is clearly of the primitive, platybasic type, the trabeculae extending forwards between the eyes and meeting anteriorly to form the front part of the floor of the cranium. They appear to be continuous behind with the parachordals. This supports the view held by some workers that the trabeculae and parachordals were not originally separate, but that the break is secondary, and correlated with the cerebral flexure of many embryos.

The parachordals meet in the middle to form a broad floor to the posterior end of the cranium. There is a very large space in the floor between the part formed by the parachordals behind and that formed by the trabeculae in front. At either side of the parachordals are the auditory capsules, already well developed by stage 29, though still open to the exterior by an oval/

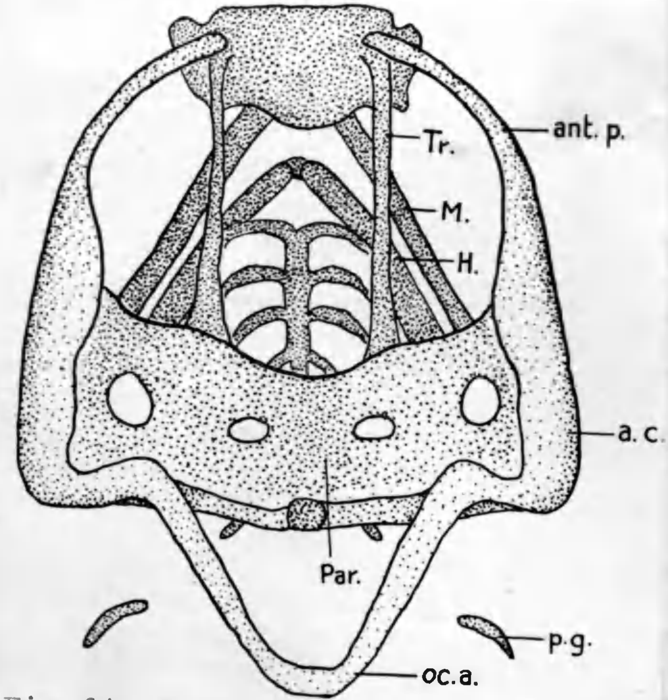

Fig.64. Reconstruction of the chondrocranium of a larva of stage 30, from horizontal sections. Lettering as in fig.63.

oval opening on the outer side and with a large vacuity on the dorsal surface. The capsule is widely open on the inner side where it is in communication with the cranial cavity. It seems probable that this is the condition from the beginning, as stage 29 is the first stage at which the chondrocranium is definitely recognisable. Two of the recesses for the semicircular canals are beginning to be marked off. From the outer, anterior border of the capsule, a long quadrate cartilage is given off and extends down the side of the head to some distance below the trabeculae. The outer ends of the mandibular and hyoid arches are continuous with its lower extremity. From the posterior ends of the para-chordals, where they embrace the anterior end of the notochord, a little curved rod (occipital arch) extends up on either side to the inner, posterior border of the auditory capsule. Little rods of cartilage from the barbels of the upper lip appear to be attached to the lateral borders of the fused trabeculae and those from the barbels of the lower lip to the anterior ends of the mandibular cartilages. The rods of cartilage in the barbels are first visible in embryos of stage 27, before the cartilage of the chondrocranium is definitely formed.

At stage 30 (Fig.64) the anterior outer border of the auditory capsule is continuous with a half hoop of cartilage (ant.p.) which extends round the outside of the brain at each side and a little way towards the middle line in front of the optic/

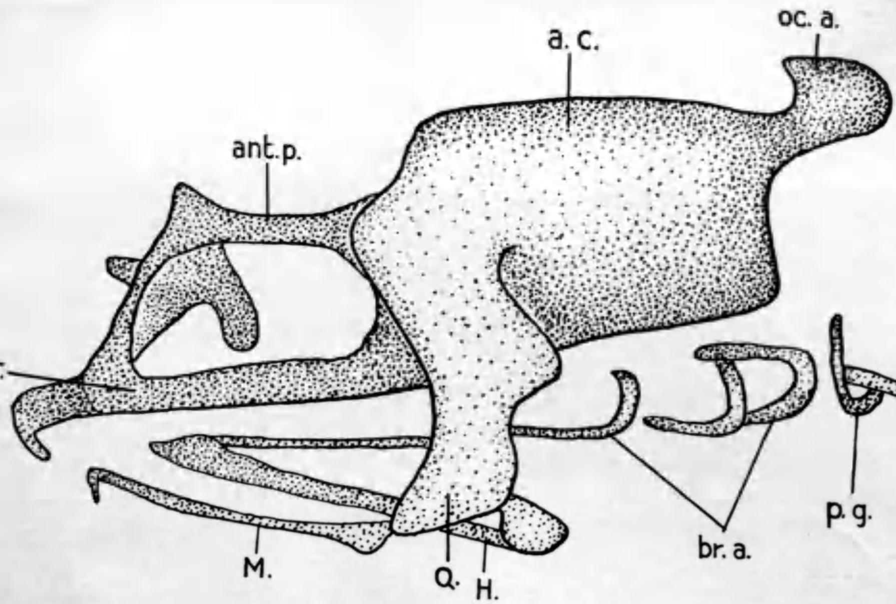

Fig.65. Reconstruction of the chondrocranium of a larva of stage 32, from sagittal sections. Lettering as in fig.63.

optic lobes. The auditory capsule is more complete and is beginning to form pockets for the semicircular canals. From the postero-dorsal inner border of the auditory capsule an arch of cartilage extends somewhat backwards over the top of the hind-brain - a development of the occipital arch of stage 29. Just above the tip of the notochord, there is a recess in the cartilage of the basioccipital for the sinus endolymphaticus. The rudiments of a nasal capsule have appeared at either side of the anterior floor of the cranium. Near the posterior end of the auditory capsule and behind the branchial arches, there is a curved rod of cartilage extending out laterally and ventrally, - the rudiment of the pectoral girdle. At this stage the resemblance of the pectoral girdle to the cartilaginous branchial arches is very remarkable. The rudiment appears to be actually in contact with the fused ventral ends of the arches and looks exactly like the branchial arches. This agrees with the view that the pectoral girdle is serially homologous with the skeleton of the visceral arches.

At stage 32 (Fig.65), the main developments are the completion of the hoop of cartilage in front of the optic lobes, the greater development of the nasal and auditory capsules and the broadening of the occipital arch. The lateral walls of the anterior part of the cranium are also much more complete, though there is still a large vacuity in them. This part of the wall of the cranium forms a shallow cup for the eye on its inner and posterior sides. There/

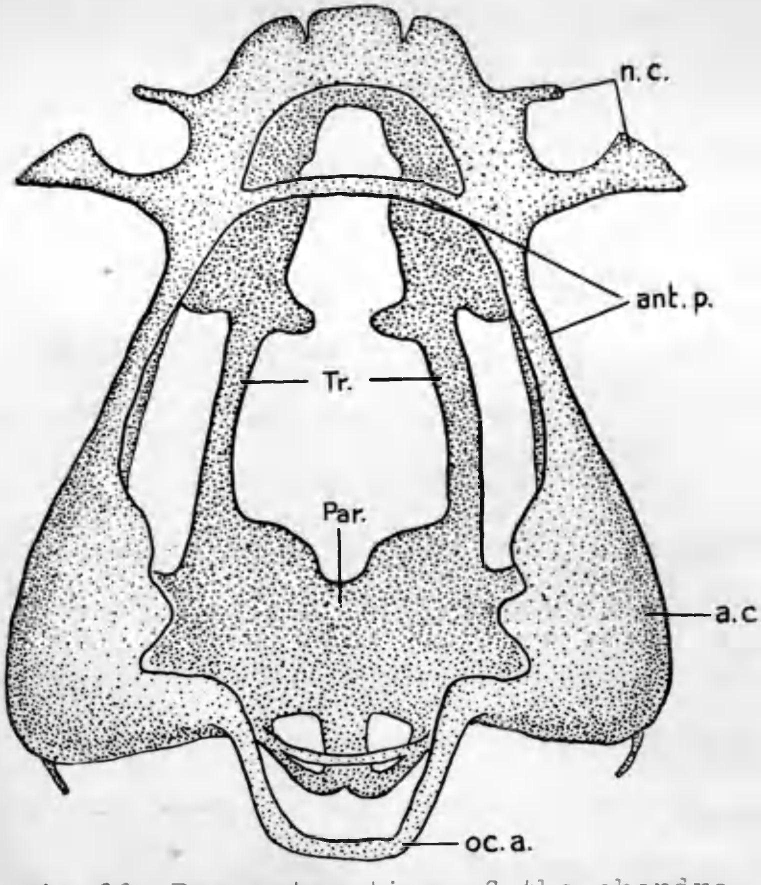

Fig.66. Reconstruction of the chondro-cranium of a larva of stage 35, from horizontal sections. Lettering as in fig.63.

There is a tendency for the median part of the anterior floor of the cranium to extend forwards into an internasal cartilage. The branchial arches are all united to a median rod of cartilage extending back from the hyoid, to the last branchial arch. The posterior part of the quadrate is growing back over the bases of the branchial arches.

In a 12 mm. larva, stage 35 (Fig.66), the floor of the cranium still has a large fontanelle. The nasal capsule is well developed and its posterior wall is extended out laterally into a preorbital process. The roof of the cranium is only represented by the narrow bridge of cartilage in front of the optic lobes, and by the occipital arch above the posterior end of the medulla. The three cavities for the semicircular canals in the auditory capsule are all formed. Anteriorly the sides and front wall of the cranium are complete and the bar of cartilage which unites the anterior bridge with the anterior, lateral wall of the auditory capsule is much deeper than at earlier stages, so that it may be said that there is now a large vacuity in the lateral walls between this cartilage and the trabeculae. The pectoral girdle is larger and longer than at earlier stages.

Vertebral Column.

The account given here of the vertebral column is incomplete as there is a gap in the series of larvae between stage 35 and 39, and I have therefore been unable to work out satisfactorily the/

the development of the vertebrae and whether it is the A or B elements or both which form the adult vertebra.

As described in speaking of the skull, the air-sac capsule lies close up against the auditory capsule and below the post-temporal bone. It is continuous with what looks like the first vertebra and, therefore, would appear to agree with the description given for other allied forms by BRIDGE and HADDON (4). This first vertebra would, then, represent the "complex" vertebra of BRIDGE and HADDON, made up of the second, third and fourth vertebrae; and the capsule would be the modified transverse process of the fourth. The neural spine of this "complex" vertebra is represented by a continuous and very thin sheet of bone. The whole thing is firmly attached to the skull. At least anteriorly, from sections of a 22 mm. larva and of earlier stages, the air-sac capsules seem to be in contact with the basioccipital. The first normal looking vertebra (probably the fifth) has a very well developed transverse process to which a large, thick rib is attached. This rib extends well down the side of the body just behind the pectoral girdle under the first pair of ventral bony plates. As already stated, it is the rib of what is probably the fifth vertebra, so that it does not exactly correspond with the cranial rib of the Dipnoi, which is attached to the skull, though it is similar to it.

The neural spine of this fifth vertebra, along with that of the/

the next vertebra, leads up to the spine of the first dorsal fin. This first dorsal spine has on either side a stout process of bone projecting from it laterally. The next eight or nine vertebrae have no transverse processes and very little in the way of haemal arches, but the neural spines are long and are connected with the fin-rays of the first dorsal fin. Behind this each vertebra has a well developed neural arch and spine and also a haemal arch and spine, some of which are connected with the fin-rays of the second dorsal or of the anal fin. The vertebral column stops short in the tail in front of the two triangular bones which support the tail fin-rays, the upwardly bent part of the spinal cord and notochord disappearing before the adult condition is reached. Associated with the two triangular blocks of bone at the base of the tail are the last neural and haemal spines which are somewhat thicker than the rest.

In development at stage 35, the 12 mm. larva, the vertebrae are beginning to form, and the notochord is still persistent behind the last vertebra, extending up between the upper block of cartilage and the last neural spine and out beyond, into the top of the tail fin. The neural spines which support the dorsal fin-rays seem to be very much stouter and thicker than they are in the adult and are cartilaginous, though the neural arches themselves are becoming bony. In the 22 mm. larva, stage 39, the notochord is still persistent in the centra of the vertebrae and in/

in fact is not completely cut into blocks, being only deeply constricted in each vertebra.

Pectoral and Pelvic Fins.

In the adult the paired fins bear a strong resemblance to the unpaired. They are armed with a stout, strong spine anteriorly and the rest of the fin is supported by paired and segmented lepidotrichia, as in the other fins. In development however, the paired fins are at first represented by a symmetrical fold of skin round the tip of a solid central axis, as they are in *Polypterus*. The fin gradually loses its symmetry, becoming reduced along the anterior, morphologically post-axial, border and extended on the pre-axial side; and finally lepidotrichia develop in the membranous fold. The pelvic fins make their appearance, first, in a larva of about stage 32, though they are not well developed until stage 35. The pectoral fins on the other hand, are already well developed at stage 27, in an embryo just about to hatch, and as early as stage 22, they are represented by little stumps behind the rudiments of the gill arches. The cartilage of the fin first appears as a slight condensation of tissue up the centre of the solid axis of the fin rudiment at stage 23, and by stage 25 there is a definite bar of cartilage up the middle of the fin. This bar of cartilage appears to become flattened out into a more or less symmetrical, spade-shaped structure with the narrow handle part attached to the pectoral girdle/

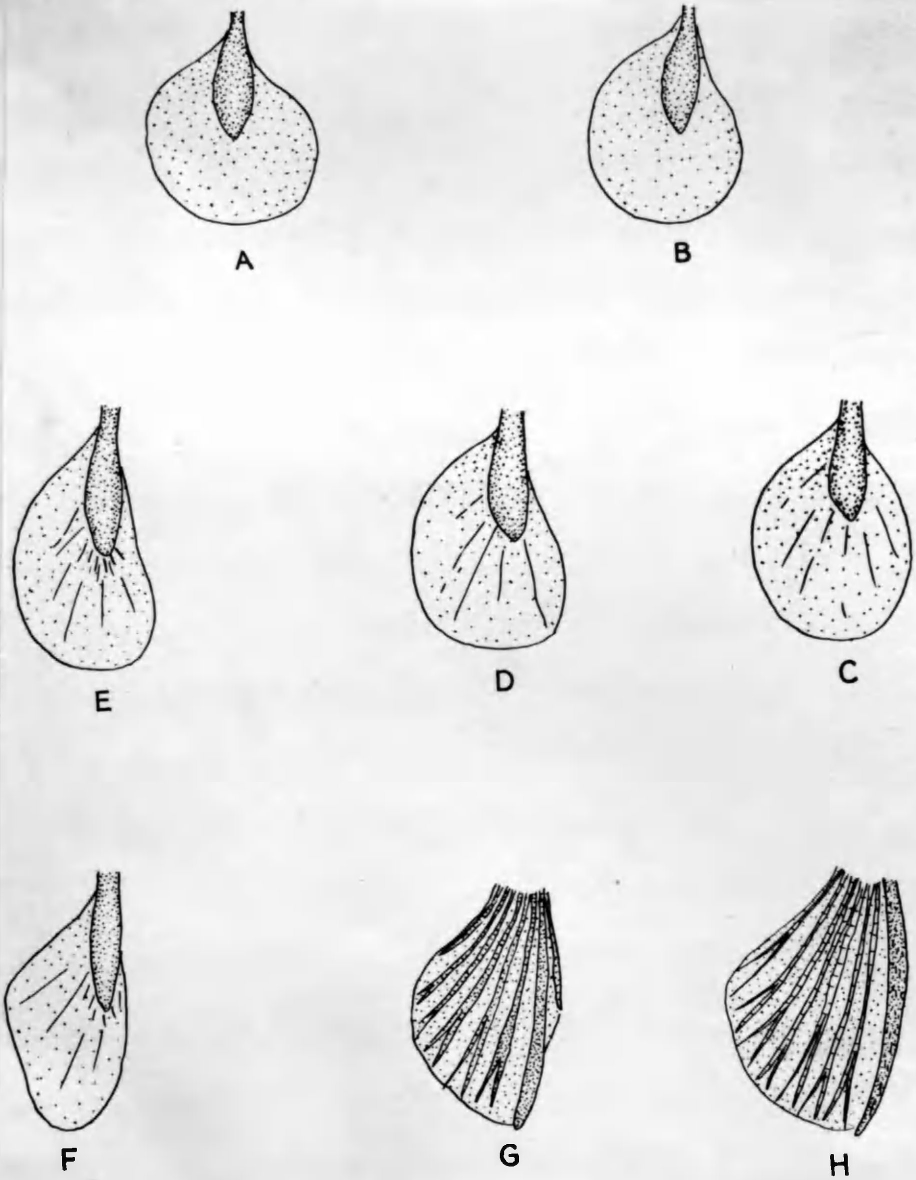

Fig.67. Development of the right pectoral fin. The morphologically pre-axial side is to the left in each case. A.stage 25; B.stage27; C. stage 28; D.stage30; E.stage 31; F.stage 32; G.stage35; H.adult.

girdle. This condition is present as late as stage 35. In the young adult, the cartilaginous skeleton of the fin appears to be continued out into a series of cartilaginous rays more or less fused together distally, and more developed on the now anterior, post-axial side, where the fin has a very stout spine, than on the posterior side of the fin, where it is membranous. To these bars of cartilage the spine and the numerous lepidotrichia are connected. The lepidotrichia, as in the unpaired fins, are paired, with blood vessels running up between them, and bear on the outer side, little denticles which project through the skin on the surface of the fins. The lepidotrichia are becoming segmented in the fins of a larva of stage 35, and the spines are developed by stage 39, but as early as stage 30, there appear to be a number of fibrous strands supporting the membraous fin. The fold of membrane round the solid axis is first developed at stage 25 (Fig.67,A). At this stage it is almost symmetrical on either side of the central axis. By stage 27 the ventral, or primitively post-axial side is becoming slightly smaller than the dorsal, or pre-axial side (Fig.67,B). This process goes on until at stage 32 (Fig.67,F), the membraous fold is confined almost entirely to the dorsal, pre-axial side. At stage 35 (Fig.67,G), the fin has rotated a little further so that the pre-axial side is now posterior. The fin is supported by definite lepidotrichia, the spine being represented by a somewhat enlarged fin-ray along the now anterior, post-axial/

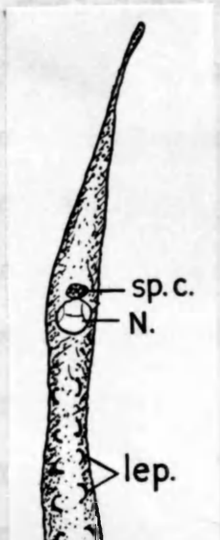

Fig.68.Transverse section of tail of larva of stage 32, showing slight upward bend of spinal cord(sp.c) and notochord(N). lep.lep -idotrichia.

post-axial border of the fin. There appears to be a little short ray beyond the spine, which disappears before the adult condition is reached (Fig.67,H).

Caudal Fin.

The adult Callichthys has a typical, teleostean, homocercal tail, with a symmetrical fin. At the base of the fin are two blocks of bone, each triangular in shape and placed one above the other. Beyond the blocks of bone the fin is supported by fourteen pairs of lepidotrichia which are broken up into little segments and which, towards the tip of the fin, tend to branch dichotomously, all except the outermost at either side branching at least once, and some of them becoming grooved, though not actually split, into two a second time. Between the lepidotrichia, and right out to the tip of the tail there is a rich network of blood-vessels, so that it seems probable that the tail is used for respiratory purposes.

In development the tail passes through all the stages of the true homocercal tail. It is at first quite straight, with the fin as a continuous fold of skin around it, and still, at stage 31, there is hardly any indication of an upward bend, though the uppermost of the two blocks of cartilage has already been formed. At stage 32 (Fig.68) the upward bend has begun, but is still not very far advanced, while in a 12 mm. larva, stage 35, the spinal cord and the notochord are bent up sharply, and extend along the top/

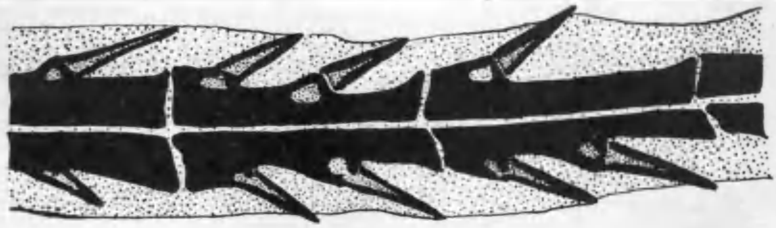

Fig.69. Horizontal section through the tail fin of young adult.

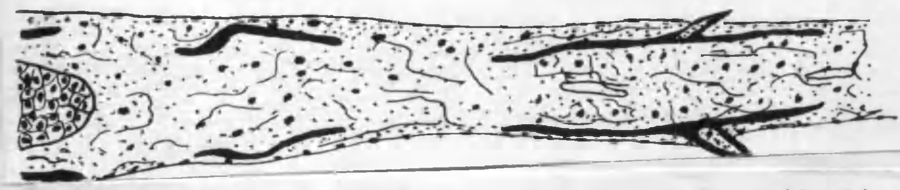

Fig.70. Horizontal section through tail fin of 12mm larva, stage 35.

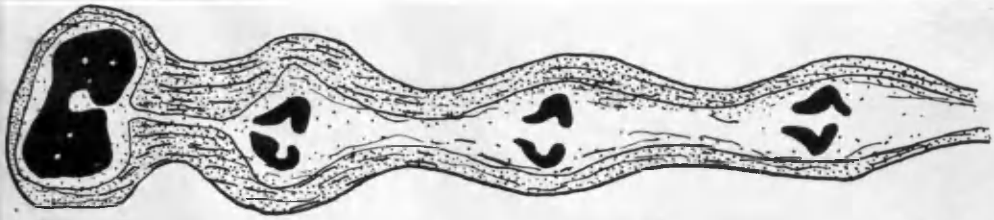

Fig.71. Horizontal section through the dorsal fin of young adult.

top of the upper triangular block of cartilage and beyond it, out into the top of the tail fin, showing how the secondarily symmetrical condition of the tail is attained. In later stages evidently, this narrow strand of spinal cord and notochord degenerates, as in the adult, the vertebral column seems to end abruptly at the point formed by the two triangular pieces of bone or cartilage. These blocks of cartilage are probably modified haemal spines. A similar mode of development is described by GOODRICH for the tail of Pleuronectes in Lankester's Treatise of Zoology.

In the adult the lepidotrichia of the tail fin each bear two spines on the outer side (Fig.69). The lepidotrichia are first visible in the tail fin at stage 30 and at that stage appear to be continuous fibres running nearly the whole length of the tail fin. They are apt to tear when the tail is sectioned. By stage 32 these fibres are becoming replaced by short bony lepidotrichia, especially near the base of the fin, and by stage 35, though still very thin, these bony lepidotrichia are developing spines (Fig. 70).

Dorsal and Anal Fins.

In the Adult, there are two dorsal fins and one anal. All these fins have a very stout, strong spine along the anterior border; behind the spine, the fin is supported by pairs of lepidotrichia (Fig.71) as in the caudal fin. These lepidotrichia are connected with the neural spines of the vertebrae in the dorsal fins, and with the haemal spines in the anal fin. They are segmented/

segmented as in the tail fin and are set with little teeth, each segment of the lepidotrichia bearing two teeth on the outer side. In development the dorsal and anal fins are at first continuous right round the tail, beginning dorsally just behind the head and extending ventrally up to the anus and just beyond it. The first part to differentiate is the tail fin, fin-rays appearing just ventral to the tip of the tail. As these develop, the tail becomes bent upwards and the dorsal fin is separated from the anal, though as late as stage 32, the dorsal fin extends right out on to the tail, and the anal is continuous with the tail fin below. At stage 35, the first dorsal has separated from the rest of the fin, which, though still very long, is separated posteriorly from the caudal fin. The anal fin has also become separated from the tail fin and has receded a little from the anus, though still much longer than in the adult. This process of shortening continues until the adult condition is reached. At stage 32 fibrous lepidotrichia are formed in the anterior part of the dorsal fin and by stage 35, these are becoming segmented and bony, and some of the most anterior of these bony lepidotrichia are developing denticles.

Dermal Skeleton.

The whole body of an adult Callichthys is covered with bony plates. These plates are arranged in two rows down either side of the body with small diamond-shaped plates between them along the/

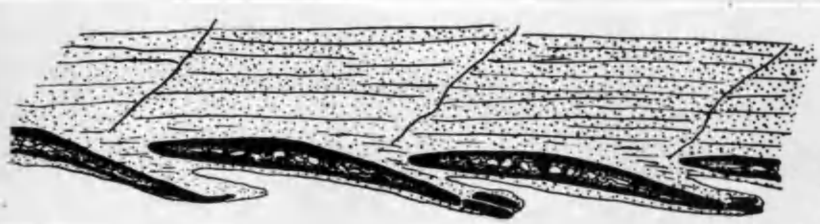

Fig.72. Horizontal section through body-wall of young adult, showing segmental arran-gement of the bony plates.

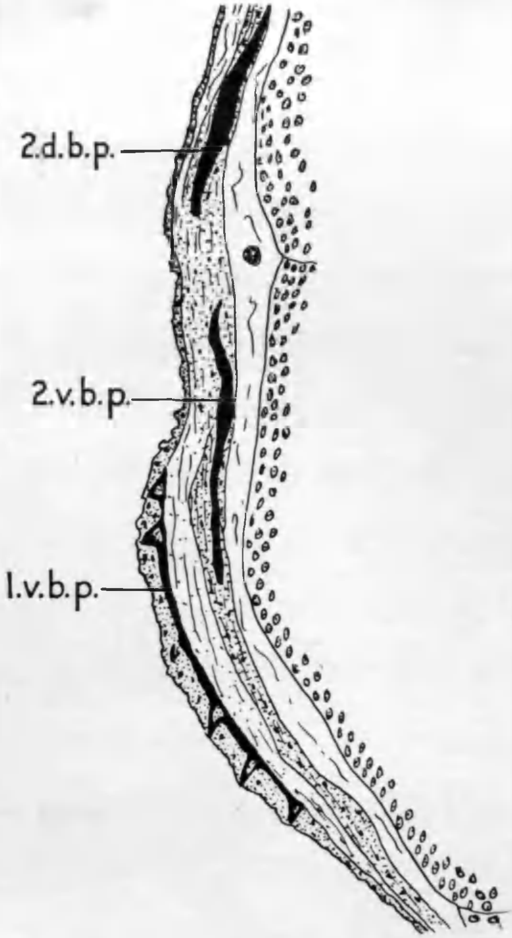

Fig.73. Transverse sect-ion through lateral body-wall of 22mm larva, stage 39. lv.b.p. posterior end of ventral bony plate; 2v.b.p. anterior end of next plate; 2d.b.p. dorsal plate corresponding to 2v.b.p..

the middle of the back. Each of the large lateral plates corresponds to a myotome. The plates are larger anteriorly than they are towards the end of the body. Each is set with little denticles along the posterior border where it overlaps the plate immediately behind it. These denticles are very similar to those on the lepidotrichia of the paired and unpaired fins. Each denticle is hollow at the base and seems to be set in a kind of socket on the edge of the plate. In thick celloidin sections of a young adult, the plates, especially those anteriorly near the skull, are seen to be much thicker near the front where they are embedded in the muscles than they are further back where they taper off towards their posterior border. The thick anterior portion of each plate is not solid, but is formed of a kind of network of bone, with large cavities in it (Fig.72).

The bony plates have not made their appearance in development at stage 35. At stage 39, in a 22 mm. larva, the plates are beginning to form down the sides of the body, but as yet do not reach very far up dorsally, or down ventrally. As in the adult, there is a row of denticles along the posterior border (Fig.73). In the figure, which is a drawing of a transverse section through the middle of the trunk region, the anterior end of one bony plate can be seen lying deeper in the tissue than the posterior end of the plate in front. Along the edge of this plate are numerous little denticles. As will be seen from the figure, the plate lies/

the middle of the body … of the larger dorsal scale-

corresponds to … generally they

they are towards the … little

denticles along the … through the plate

immediately behind it. … very similar to

those on the lepidotrichia … the pectoral fins. Each

denticle is hollow at … set in a kind of

socket on the edge of … sections

of a young adult. The … especially near

the skull, are seen … front where they

are embedded in the … at back where they

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portion of each plate … as a kind of

network of bone, with large cavities in it (Fig.73).

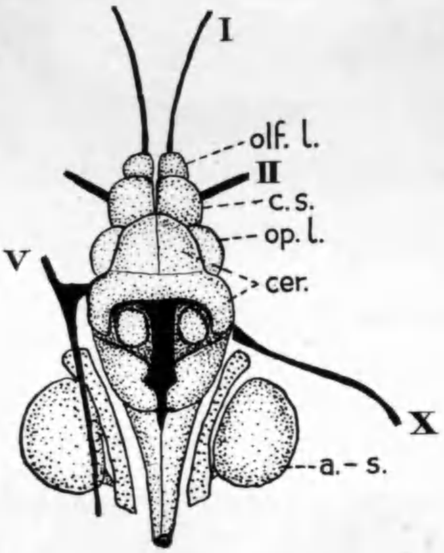

Fig.74. Brain of adult. a.-s.air-sac; cer.cerebellum; c.s.corpora stri-ata; olf.l.olfactory lobes; op.l.optic lobes; I,II,V,X.cranial nerves

The bony plates have not made their appearance in development at stage 35. At stage 39, in a 22 mm. larva, the plates are beginning to form down the sides of the body, but as yet do not reach very far up dorsally, or down ventrally. As in the adult, there is a row of denticles along the posterior border (Fig.75). In the figure, which is a drawing of a transverse section through the middle of the trunk region, the anterior end of one bony plate can be seen lying deeper in the tissue than the posterior end of the plate in front. Along the edge of this plate are numerous little denticles. As will be seen from the figure, the plate lies/

lies in the thick dermal layer just below the epidermis.

As there is a gap in the series of larvae between stages 35 and 39, it is impossible to obtain sections of the earliest stages in the development of the bony plates

NERVOUS SYSTEM.

Brain and Spinal Cord.

In the adult the brain (Fig.74) is comparatively large. It has small, round, olfactory lobes in front, from the anterior end of which the olfactory tracts go off at an angle to the olfactory organ. Behind the olfactory lobes is the cerebrum, which has the usual teleostean form, with a thin roof through which the large and somewhat globular, so-called corpora striata can be seen at either side, giving the undivided cerebrum the appearance of paired hemispheres.

The optic lobes are small and are pressed out to the side by the very large cerebellum which bulges right forward so that in the middle line it overlaps the end of the cerebrum. The cerebellum is curious, for though very large anteriorly, it appears to end abruptly at the front end of the medulla, while the side walls and the lateral part of the floor of the fourth ventricle are very greatly developed. The thickened lateral walls of the medulla bulge inwards posteriorly so that they almost meet, thus, with the cerebellum, enclosing a more or less triangular area of the thin roof of the fourth ventricle. At the base of the triangle/

triangle, near the cerebellum, there is visible on either side through the thin roof, a cushion-like thickening of the floor of the medulla, the "lobus trigemini", (c.f. the figure given by EDINGER, of the brain of a young Cyprinoid). Behind the lateral thickenings, the medulla tapers off rapidly into the spinal cord. On either side of the anterior end of the medulla are the semicircular canals of the otocyst, with immediately behind them, the distended vesicles of the air-bladder. The greater part of the air-sac at each side is enclosed in bone, but just behind the ampulla of the posterior semicircular canal, and again behind the sacculus, the bony capsule seems to give place to a sheet of fibrous membrane. A fuller description, however, is given below in dealing with the air bladder.

In the brain the pineal body appears to be a short stump underlying the "parietal" foramen, but showing no sign of eye structure. It is relatively larger in the larval stages than in the adult. The infundibulum and the pituitary are well marked. From the root of the trigeminal nerve, at either side, a nerve trunk, the ramus recurrens trigemini, runs back close beside the neural spines of the vertebrae, right to the tail fin. This is not the lateralis nerve, for a small branch of the tenth runs back immediately below the lateral line organs, half way down the side of the body.

The spinal cord is rather curious in section, as it appears to/

to have more or less segmentally arranged giant cells at either side in the ventral horns, and also very large neurones near the central canal. The motor nerve roots come away almost ventrally and extend down the sides of the centrum of the vertebra, as very thick strands of nerve. The spinal ganglia seem to lie close beside the motor root at the ventro-lateral border of the spinal cord, the dorsal root running up close to the spinal cord to enter it at the dorso-lateral corner. The dorsal roots are very difficult to trace.

In development the central nervous system is first formed as a solid strand along the mid-dorsal line of the embryo. By stage 16, the fore-brain is distinguishable from the otherwise undifferentiated cord, by the development at either side of the optic rudiments. These rudiments are solid like the rest of the nerve cord, and project a little back from the tip of the brain. By stage 20, a cavity has been formed throughout the spinal cord and the brain and in the optic rudiments. The otocyst and the olfactory organs have also appeared. The brain and spinal cord now rapidly advance. The mesencephalon and the rhombencephalon become marked off by constrictions from one another, and the roof of the hind-brain becomes very thin, so that the cavity of the fourth ventricle can be seen from the outside of the embryo. At this stage the cavity of the brain is very large, but as development goes on much of the cavity is obliterated by the thickening of/

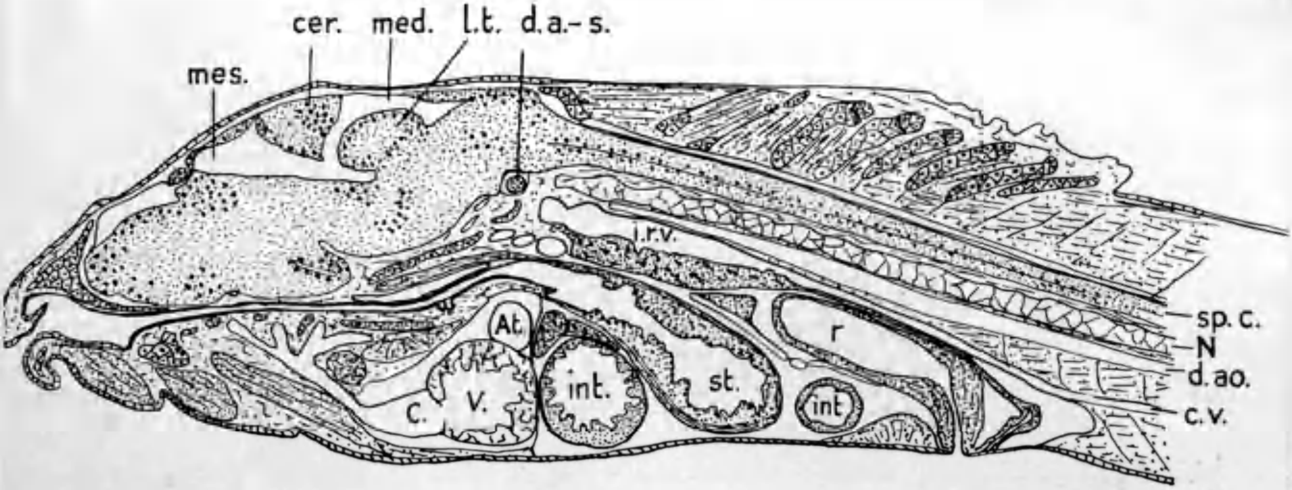

Fig.75. Saggittal section of 12mm larva, stage 35 (slightly diagramatised). At. atrium; C. conus; cer. cerebellum; c.v. caudal vein; d.ao. dorsal aorta; d.a.-s. disc of air-sac; int. intestine; i.r.v. inter-renal vein; l.t. lobus trigemini; med. medulla; mes. mesencephalon; N. notochord; sp.c. spinal cord; st. stomach; V. ventricle; r, rectum.

of the walls. In the mesencephalon, the lateral and dorsal walls become greatly thickened and bulge out to form the optic lobes. By stage 28, when the young larva is just hatched, the brain has attained more or less to its adult form, except that the cerebellum does not extend forward over the surface of the mid-brain. This is true even of the brain of a larva of stage 35 (Fig.75), where the centre of the roof of the mid-brain is still quite thin, the optic lobes being lateral bulgings, as they are in the adult.

In development the brain appears to be quite straight, except that it shares the general flexure of the whole body round the yolk. The spinal cord which is at first solid, has developed a cavity by stage 20. The spinal ganglia are, at first, groups of ganglion cells at the side of, and continuous with, the dorso-lateral borders of the spinal cord. As development proceeds, they gradually pass down the side of the spinal cord, until they become secondarily associated with the ventral nerve roots.

Sense Organs.

Olfactory Organ. In the adult the olfactory organ is a little sac underlying the skin, just behind the mouth and with its two openings dorso-laterally on the surface of the snout. Each opening is double, the anterior aperture being, as in Polypterus, on the tip of a little papilla, the posterior being a wide opening on the surface of the skin. Anteriorly the sac is simple with/

with sensory cells all over the inner surface, but further back, under the second aperture, the floor is folded into a number of little recesses and pockets, all lined with sensory cells. The olfactory tract comes away from the anterior end of the lateral wall of the olfactory lobe of the brain, and runs out at an angle of about 45^{o}, to the posterior surface of the olfactory sac.

In development the olfactory rudiment is first visible in an embryo of about stage 22, as a thickening of the deep layer of the ectoderm on either side of the front of the fore-brain. The rudiment gradually becomes separated from the brain except for a broad strand of nucleated protoplasm which represents the rudimentary olfactory tract. By stage 27, when the embryo is about to hatch, the olfactory rudiment has developed a cup-shaped cavity, and in the young larval stages, the organ rapidly assumes its adult form, except that, even at stage 35, there is only one wide opening at each side. At stage 39 the tubular anterior opening is present, but, as there is a gap in the series of larvae at this point, it is impossible to make out how it is formed. At all the stages of development the organ is in continuity with the brain by a strand of nerve.

<u>Otocyst</u>. In the adult the otocyst has the same structure as has been described for other members of the family Siluridae. From the point in the utriculus where all the semicircular canals meet, a little duct is given off towards the under surface of the brain/

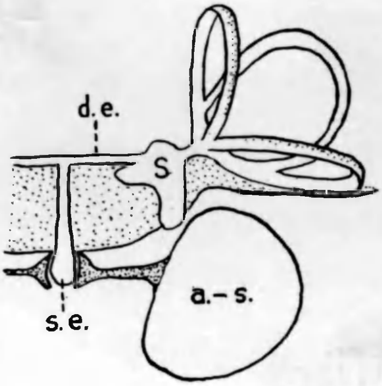

Fig.76. Diagram of otocyst and air-sac. a.-s.air-sac; d.e. ductus endolymphaticus; S.sacculus; s.e.sinus endolymphaticus.

brain, and from this, the sacculus bulges backwards and inwards below the brain and comes into close relationship with the capsule of the air-sac, being pressed up against the wall of the capsule which at this point is thin and fibrous, not bony. (Fig.76).

From the top of the sacculus where it is in communication with the utriculus, a duct is given off horizontally towards the ventral surface of the brain. This is the ductus endolymphaticus, and it unites with its fellow of the other side under the brain, and from their point of junction, a diverticulum is given off posteriorly, the sinus endolymphaticus. The sinus is at first a narrow tube, but posteriorly it bulges up dorsally so as to form a little vesicle which is embraced at either side by the discs of the air-sacs. The sinus ends blindly at the posterior limit of the discs of the air-sacs. The auditory nerve is large and sends branches to the patches of sensory cells in each of the semicircular canals and in the sacculus. The sacculus has a median and a lateral lobe in each of which there are sensory patches. I have not observed any patches of sensory cells in the endolymphatic ducts or in the sinus endolymphaticus. In the adult, opposite each of the sensory patches in the sacculus and also in the lower part of the utriculus, where all the semicircular canals meet, there is a little otolith which seems to be in contact with the hairs of the sensory cells. Thus there are two otoliths in each sacculus and one in the lower part of each utriculus.

In/

In development the otocyst makes its first appearance at stage 18, as a solid thickening of the deep layer of the ectoderm on either side of the anterior end of the medulla, which, however, is not yet marked off from the rest of the brain and from the spinal cord. By stage 20 a cavity has been developed in the rudiment and by stage 25 the sacculus and the semicircular canals are becoming distinguishable. At the time of hatching, stages 27 and 28, the semicircular canals and the sacculus are well developed, though the cavities of the semicircular canals are still very large. At stage 29 the endolymphatic ducts are beginning to form, but do not yet meet each other in the middle line. By stage 30, however, they have fused and the sinus endolymphaticus is formed. Later stages of development show the gradual reduction in size of the cavities of the semicircular canals, and the development of the otoliths and sensory patches in the sacculus and the utriculus.

Lateral Line Organs. The position of the lines of sense organs on the head are shown in the figure of the dorsal surface of the skull (Fig.58). Beginning above the anterior olfactory opening and running in a somewhat wavy line along the dorso-lateral side of the skull, above the eye and then across the outer wall of the auditory and air-sac capsules, there is the main branch of the lateral line system on the head. The sense organs of this branch of the system, are connected by a bony tube just underlying/

underlying the skin, and are arranged at fairly regular intervals, the first one above the anterior olfactory opening, the second above the posterior of the two olfactory apertures, and the third above the centre of the eye. After the third sense organ, there is a fairly long gap where the tube bends down the side of the head to a more lateral position. Just behind the eye, where the bend of the tube begins, a little branch tube runs down vertically at the side of the head and at right angles to the main canal. There are three sense organs in the tube, but only two openings, one opposite the most ventral sense organ, well down the side of the head behind the lower edge of the orbit; the other at the point where the canal leaves the main tube. Behind this vertical canal, the main tube continues back over the lateral wall of the auditory capsule, the fourth sense organ being opposite the lateral semicircular canal of the otocyst. The canal turns even further down the side of the head and the next opening, the fifth, is opposite the anterior, ventro-lateral corner of the air-sac capsule. At this point the bony tube opens out on the inner side, so that the sense organ canal is open to the cavity of the air-sac capsule. Posteriorly where the capsule becomes closed off from the outer skin, the bony tube becomes once more complete, and at this point the sixth sense organ occurs. Three more sense organs with their openings to the exterior, follow in rapid succession and then the canal is continued into the ordinary lateral line canal/

canal with its openings at the posterior border of each bony plate. As the plate corresponds with the myotome, it follows that the sense organs are arranged segmentally. The sense organs have the usual form - a little cup lined with sensory cells, the sensory hairs of which project into the cavity. The arrangements of the sense organs and their canals have been worked out from transverse sections of a larva of stage 39. In addition to these sense organs, there are grooves in the bone of the skull of the adult, running back and towards the middle line on the frontal bone from the position of the third sense organ, above the eye; and also a curved groove which runs forwards and inwards on the pterotic, from the fourth sense organ, on to the postfrontal and then turns inwards and backwards across the supraoccipital, meeting its fellow from the other side in the middle line. This groove has a sense organ on the pterotic, just dorsal to the fourth sense organ of the main canal. The arrangement of the sense organs on the head corresponds fairly closely to that of the sense organs on the head of the fossil fish, *Osteolepis macrolepidotus*, as described by GOODRICH (9), except that there is an additional line running along the side of the head below the eye and across the squamosal bone in *Osteolepis* which is absent in *Callichthys*.

In development the sense organs begin to make their appearance at about stage 35.

Fig. 77. Air-sacs of adult with the remains of the transverse median part embedded in bone.

AIR-BLADDER.

In the adult (Fig.77), the air-bladder consists of two membranous sacs lying one on either side of the end of the medulla and the beginning of the spinal cord. Each of these sacs is almost completely enclosed in a bony capsule which fits round it fairly closely, except towards the sides of the head, where the cavity of the capsule extends right out under a row of sensory tubes just below the skin, in a groove in the bone of the skull. The capsule of the air-sac seems to be open to the outer skin along a narrow slit in the bone just above the groove in which the sense organs are situated. This lateral expansion of the air-sac capsule is packed with a spongy connective tissue. Each air-sac is somewhat egg-shaped with the pointed end directed forwards and the inner side somewhat flattened. To the middle of this flattened, inner surface, a stout and fairly short, solid arm is attached, which ends in a saucer-shaped disc. These arms of the air-sacs are formed of condensed connective tissue, with small ossicles embedded in them, and appear to be quite free from the surrounding structures, except for a band of muscle attaching each one to the posterior wall of the air-sac capsule, so that any movement of the air-sac itself would be communicated to them.

The discs of the two arms are pressed up against either side of the sinus endolymphaticus which, as already described, is a diverticulum extending straight back in the middle line, from the/

Fig.78. Transverse section of alimentary canal of stage 25, showing the first rudiment of the air-bladder(a.b.).

Fig.79. Reconstruction of the air-bladder of stage 28, from horizontal sections.

the point of junction of the two endolymphatic ducts, and which terminates at the hinder limit of the discs of the air-sacs. These discs are seen in section to have small ossicles firmly embedded in them, and I take these to be the ossicles described by BRIDGE and HADDON (4) in a dried skeleton which they had examined. The bones are completely embedded in the tissue of the arm and the disc of each air-sac. There would appear to be at least two ossicles on each side, a disc-shaped one in the outer wall of the disc itself, i.e. in the part pressed against the sinus endolymphaticus; and a straight bone with a forked end in the stalk, with the forked end directed towards the disc-shaped bone. I suppose these represent the "scaphium" and the "tripus" of BRIDGE and HADDON, or the "stapes" and "malleus" of WEBER.

In development the first appearance of the air-bladder is at stage 25 (Fig.78), as a small diverticulum from the right side of the dorsal wall of the alimentary canal, some little distance behind the pharynx. By stage 27, this rudiment has developed into a dorso-ventrally flattened and very small sac lying in the middle line dorsal to the alimentary canal, with which it is connected by a narrow duct. At stage 28 (Fig.79) the same condition is found, except that the walls of the rudiment are very greatly thickened, and in the slightly older larva of stage 29, the air-bladder extends well out to either side, where the cavity swells out slightly, At this stage there is a solid strand/

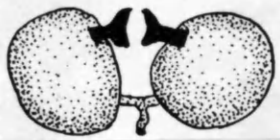

Fig.80. Reconstruction of air-bladder of stage 30, from horizontal sec--tions.

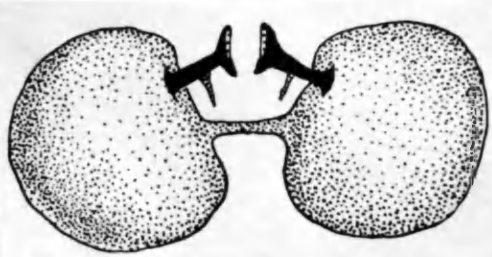

Fig.81. Reconstruction of air-bladder of stage 35, from horizontal sections.

strand of tissue uniting the two sides across the middle line dorsal to the notochord. This solid strand is composed of thickened connective tissue, very similar to that surrounding the notochord, from which it is not very definitely marked off. Reconstructions show that this connecting strand of tissue is close up to the posterior surface of the auditory capsule and within the limits of the chondrocranium. Just anterior to the solid strand uniting the sides of the air-bladder are the developing endolymphatic ducts, which have not yet met in the middle line.

At stage 30 (Fig.80), the lateral swellings have increased in size and the median part of the air-bladder is becoming reduced as is also the pneumatic duct. The, at first, continuous strand of connective tissue uniting the air-sacs dorsal to the notochord, is now interrupted in the middle line by the sinus endolymphaticus, and at each side, where the strand is touching the sinus, it is expanded into a disc. By stage 32, the muscle attaching the arm of connective tissue to the skull is developed and the whole apparatus has assumed its adult structure except that the bones have not been formed and the median part of the air-bladder has still an open cavity communicating by a small, now almost solid, strand with the dorsal wall of the alimentary canal. In late stages of development (Fig.81), the median part of the air-bladder becomes reduced to a solid strand of tissue. It comes away from the posterior inner side of each sac and passes ventral to the backbone/

backbone, dorsal aorta and inter-renal vein, but dorsal to the greater part of the kidney tissue. Even in a larva of 22 mm. length, stage 39, there is still a blind diverticulum from the dorsal wall of the alimentary canal, representing the remains of the once open pneumatic duct.

Sections through the different stages where the otocyst and the air-sacs have assumed more or less their adult form, show that the posterior wall of the sacculus is in close contact with the thin wall of the air-sac capsule, though not with the air-sac itself, and in this region the capsule wall is either fibrous or membranous. The whole capsule is in close relation with the bones of the auditory capsule, and anteriorly the capsule extends up between the sacculus and the posterior semicircular canal. In early stages the air-sac itself is comparatively much larger and almost completely fills the capsule, so that it lies close under the outer skin, where the bone of the capsule is not yet formed. Possibly this difference in comparative size is due to shrinkage in the preserved specimens.

In transverse sections of a 22 mm. larva, below the sinus endolymphaticus, between the discs of the two air-sacs, there is a small cavity bounded below by the basioccipital with the contained notochord, and laterally and dorsally by a sheet of membrane bone which has grown across from the inner wall of the air-sac capsule to form the floor of the cavity in which the sinus endolymphaticus lies./

lies. This little cavity above the basioccipital extends only for a short distance, (about 0.1 mm.) and is split into two lateral cavities posteriorly. Behind it the bone becomes continuous with the cartilage of the basioccipital. This cavity is possibly the "atrium" of BRIDGE and HADDON, and others. It appears to be open in front to the cavity round the sinus endolymphaticus.

RESPIRATORY SYSTEM.

Gills.

The gills are of the normal teleostean type. There are four branchial arches, each supplied with a double row of gill lamellae. The lamellae of the two rows alternate with one another, and each lamella has a little rod of cartilage down the middle, with a blood vessel at either side, and the outer walls folded into a series of secondary respiratory folds. These folds are deeper on that side of the lamella which is towards the outside, i.e. they are deeper on the anterior side of the lamellae of the anterior row on each branchial arch. The lamellae of one row are slightly longer than those of the other row and each lamella has a little process near the tip on the outer border, so that the gill of each arch, as a whole, has a curious three-lobed appearance along its outer border.

In transverse sections it is seen that the cartilaginous arch, ensheathed with bone, lies on the inner side, with the efferent and afferent blood vessels external to it, and the lamellae projecting beyond. From the main afferent vessel, little branches run/

run out alongside the cartilage and send capillaries into the respiratory folds. These capillaries drain into a vessel running up the other side of the cartilage to the main efferent vessel.

In development by stage 27, the first gill cleft is open, and by stage 29 the lamellae are formed and all the clefts are open, though the cartilage of each lamella and its secondary folds are not formed until stage 31.

In sections of a 22 mm. larva, stage 39, there is a curious organ composed of a mass of deeply staining cells, in the dorsal wall of the branchial chamber above the gills, in the angle between the body wall and the operculum, which is probably the thymus.

Intestinal Breathing. In the adult the greater part of the intestine from the stomach to the rectum is very thin walled and greatly distended so that at the points where it is bent upon itself it has a permanent kink and cannot be straightened out, when the gut is unravelled. This part of the alimentary canal has a very rich blood supply, with large veins draining into the interrenal vein in the anterior region of the body cavity. This part of the alimentary canal is used for respiratory purposes as is described by Dr. CARTER (5).

Accessory Breathing Organs.

As has already been stated, the tail fin both in the adult and during development, is very richly supplied with blood, and it, therefore, seems probable that it is used as an accessory breathing/

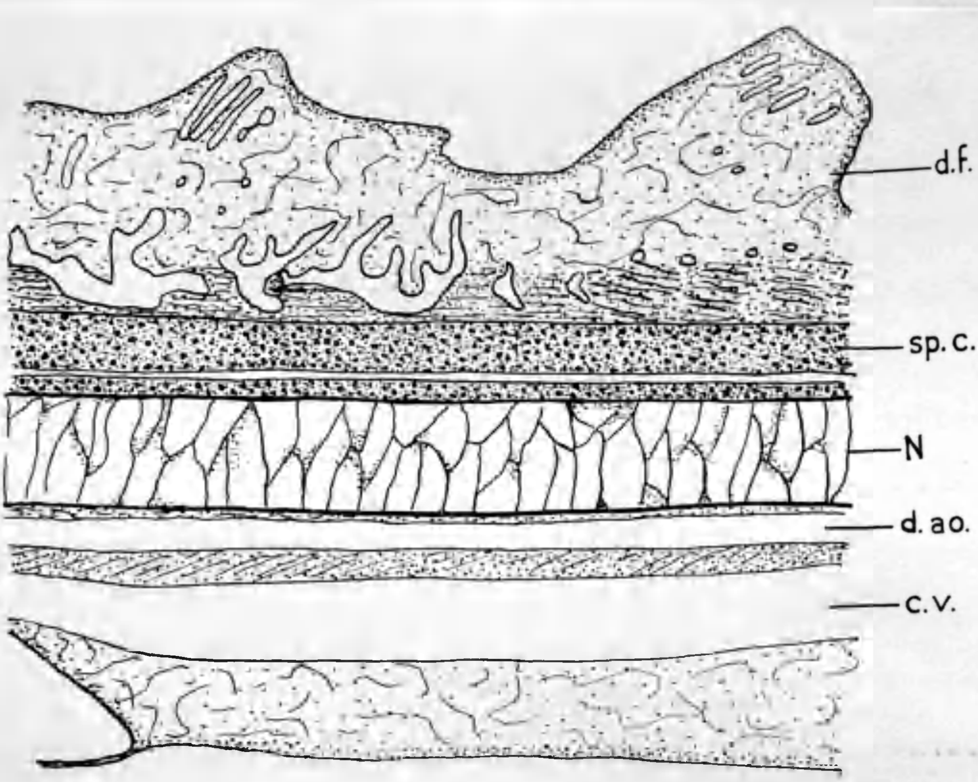

Fig.82. Sagittal section through the anterior part of the tail of a larva of stage 29. c.v.caudal vein; d.ao.dorsal aorta; d.f.dorsal fin; N.notochord; sp.c.spinal cord.

organ. The dorsal fin is also very richly supplied with blood, especially during development (Fig.82), so that probably it too has a respiratory function.

The barbels have a little rod of cartilage up the middle of each of them, and, beside this rod, there is a blood vessel which drains into the inferior jugular vein. In development the barbels first appear as a couple of little projections from a common base on the mandibular arch in embryos of stage 25. As early as this stage, they have a blood vessel running through them, but it is extraordinarily difficult to trace its connection with the rest of the blood supply of the head. However, from the dorsal part of the aortic arch of the first gill arch, a little vessel runs forward into the hyoid arch. Forming a bend round the arch, it runs forward again as a very small vessel into the head and appears to supply the barbels. By stage 27, the veins from the barbels can be traced down into the lower jaw, where they unite into a single vessel in the middle line. At this stage it is very difficult to trace this median vein back towards the heart, but evidently it is the inferior jugular which in the adult passes straight back to open into the right side of the atrium. From their position and their development it seems not improbable that the barbels represent the modified external gills of the mandibular arch and that they are comparable with those of the African Toad _Xenopus_, described by BLES (3).

VASCULAR SYSTEM.

Development.

At stage 18, there is a large longitudinal blood vessel at either side which is first visible just anterior to the position of the otocyst. About the level of the posterior end of the otocyst, these two vitelline veins unite into a wide blood space between the embryo and the yolk, and further back, divide once more and, gradually diminishing in size, disappear altogether. At stage 20, the dorsal aorta is beginning to be differentiated from the mesoderm below the notochord in the "tail" region. The ventral aorta is also being formed and parts of the aortic arches, but these are not joined up to the ventral aorta. The vitelline veins are still very large. At stage 22, the ventral aorta extends forward into the first afferent branchial, and is continued posteriorly by the conus which seems to end blindly. The vitelline veins are very large at either side of the ventral aorta and rudimentary heart. The dorsal aorta is more distinct than formerly.

At stage 23, the heart in the pericardiac cavity is definitely formed and bulges out to the left under the rest of the embryo. The ventral aorta gives off the first afferent branchial, which is continuous round the outside of the arch with the efferent. Dorsally from this aortic arch, a small vessel runs forward into the head, and a large vessel, the aortic root, runs back at either side/

side, the two uniting further back to form the dorsal aorta. At this stage also the anterior and posterior cardinal veins are formed. Posteriorly between the kidneys is a single inter-renal vein which divides anteriorly into the right and left posterior cardinals. Each cardinal is joined by the vitelline vein of its own side, so forming the short duct of Cuvier which enters the heart.

At stage 25, all four afferent branchials are developed and from the dorsal side of the first, a little vessel goes forward and supplies the hyoid arch and then continues forward on either side of the brain sending small branches into the barbels. The chambers of the heart are becoming distinct from one another. Blood vessels are present in the pectoral fin. The posterior cardinals are very wide vessels opening into either side of the atrium by the ducts of Cuvier, the vein on the right being, as in Polypterus, larger than that on the left. As at stage 22, there is a single inter-renal vein, but now it tends to move over to the right anteriorly where it is continuous with the right posterior cardinal, while the left posterior cardinal has already lost its connection with the inter-renal, and only drains the anterior part of the left kidney. The short ducts of Cuvier now receive the anterior cardinal veins as well as the posterior cardinals and the vitelline veins.

At stage 27, the conditions of the vascular system are similar to/

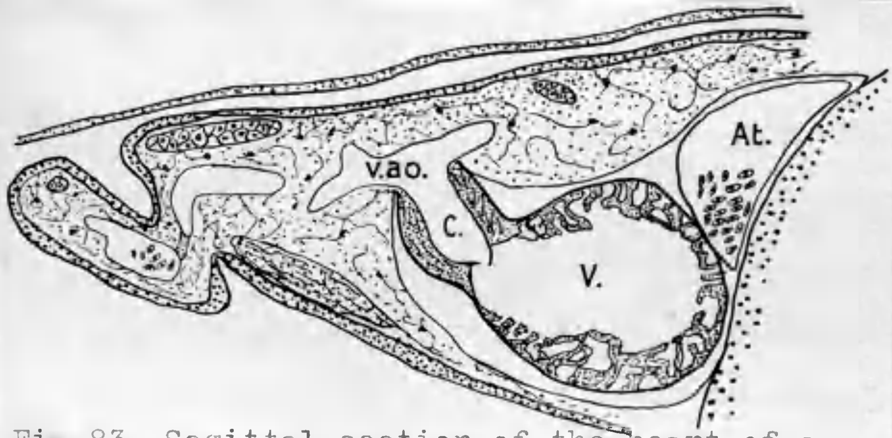

Fig.83. Sagittal section of the heart of a larva of stage 29. At.atrium; C.conus; V.ventricle; v.ao.ventral aorta.

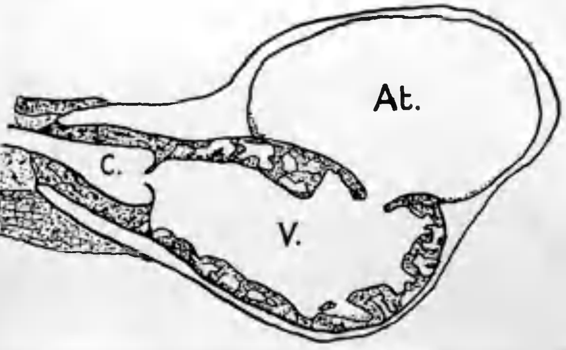

Fig.84. Horizontal section of the heart of a larva of stage 30. At.atrium; C.conus; V. ventricle.

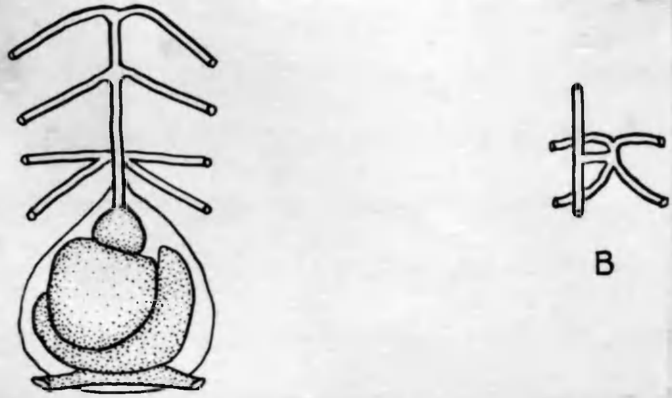

Fig.85. A.Heart from the ventral side. B. Lateral view of the ventral aorta where the two last afferents come off.

to those at stage 25, except that the vitelline veins are becoming smaller and that the left duct of Cuvier is joined by a large vein from the liver, just before it enters the heart. This condition is practically that of the adult, so that by the time of hatching the vascular system is more or less completely formed. In the newly hatched larva, stage 28, the muscular trabeculae in the ventricular wall are well developed. At this and later stages the ventral aorta is very short indeed (Fig.83). A horizontal section (Fig.84) through the heart of a larva of stage 30, shows clearly the respective positions of the chambers of the heart and the valves guarding the openings between them. As development proceeds, the ventricular wall becomes more and more muscular with spaces between the numerous trabeculae.

Heart. (Fig.85).

In the adult the heart is comparatively small and has the typical teleostean form. The sinus venosus extends out laterally into the ducts of Cuvier, which receive on either side an anterior and a posterior cardinal vein. The sinus leads into the atrium which lies dorsal to, and to the left of, the muscular ventricle, into which it opens near to the opening of the ventricle into the conus. The atrio-ventricular opening (Fig.86), which is lateral in position, is guarded by a pair of valves which project from the anterior and posterior walls of the opening and more or less completely close it. The ventricle has very thick walls and the main/

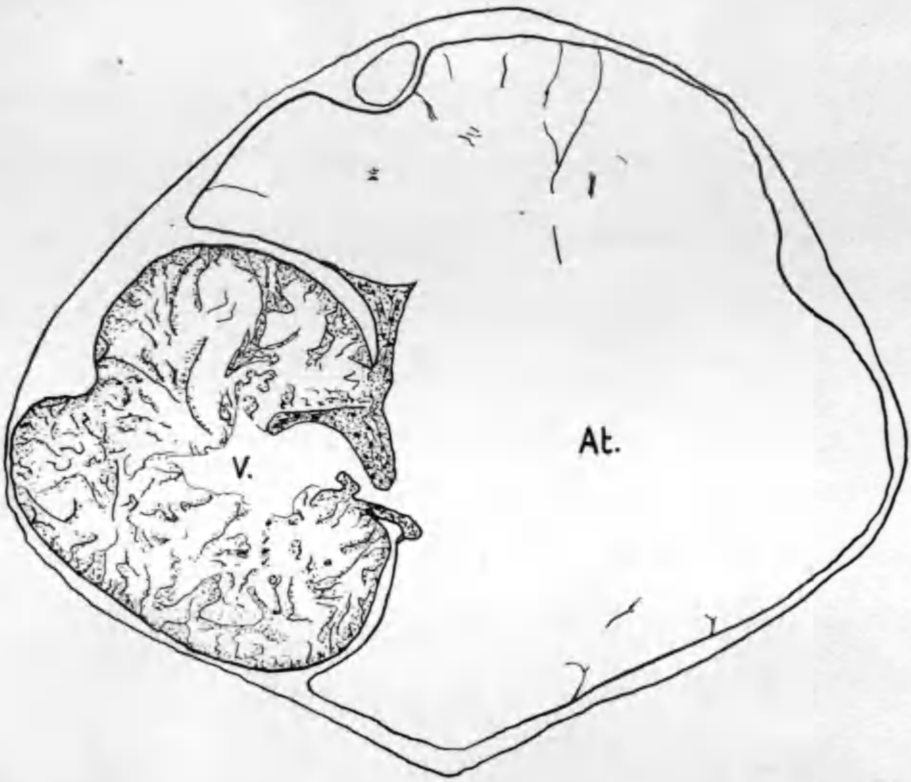

Fig.86. Transverse section of the heart of 22mm larva, stage 39. At.atrium; V.ventricle.

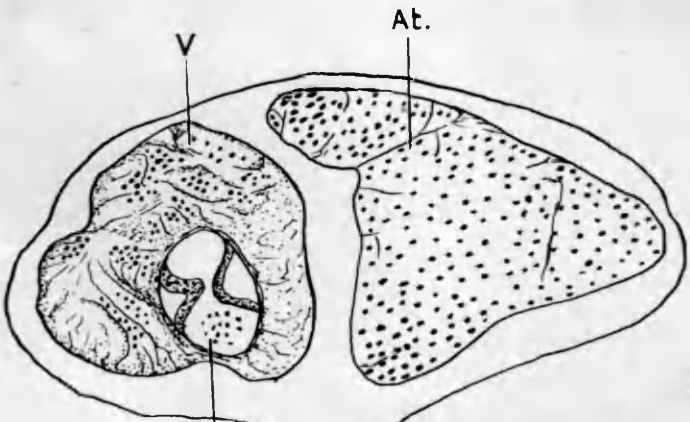

Fig.87. Transverse section of the heart of 22mm larva, stage 39. At.atrium; C.conus; V.ventricle.

main cavity is considerably reduced by the development of muscular trabeculae. The ventricle leads forward into the conus, which is short and very muscular. The opening between the ventricle and the conus (Fig.87), is guarded by two valves projecting from the lateral walls and partially dividing the cavity of the conus into two compartments. Anteriorly the conus leads directly into the very short ventral aorta.

Arteries.

The ventral aorta gives rise to four afferent branchial vessels on either side. The first two pairs come off separately; the last two are at first a single vessel at each side, coming off dorsally as in the Dipnoi, from the ventral aorta, just in front of the pericardiac cavity, and branching almost at once into the two afferents, These last two afferent branchials run back alongside of the pericardiac cavity to reach the last two gills. There are four efferents on each side. The dorsal arterial system, the "circulus cephalicus", has been described by RIDEWOOD (18), and my observations agree with his. The first efferent vessel sends forward an artery to supply the hyoid arch and the head. The main part of the first efferent branchial runs back well out to the side, and is joined by the second. The aortic root so formed, turns obliquely inwards and unites with its fellow of the other side, just where the third and fourth efferent branchials meet their fellows. Thus three arteries from/

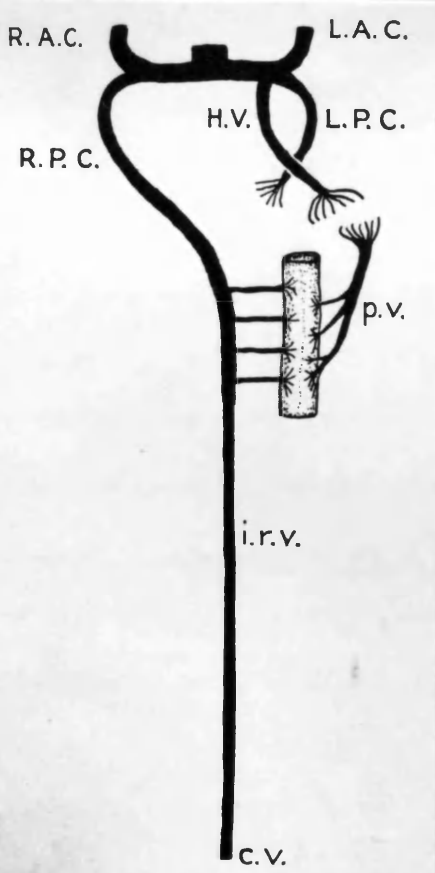

Fig. 88. Diagram of the venous system from the ventral side. c.v. caudal vein; H.V. hepatic vein; i.r.v. inter-renal vein; L.A.C. left anterior cardinal; L.P.C. left posterior cardinal; p.v. portal vein; R.A.C. right anterior cardinal; R.P.C right posterior cardinal.

from either side meet almost at the same place. Just behind this point, the coeliaco-mesenteric artery is given off from the dorsal aorta and, passing to the right of the remains of the pneumatic duct, goes to supply the liver, pancreas, stomach and intestine. The subclavian arteries are given off from the dorsal aorta just posterior to the coeliaco-mesenteric. The only other large artery coming away from the dorsal aorta is one right at the end of the body cavity, supplying the posterior wall of the cavity and the cloaca.

In a 12 mm. larva, stage 35, there is a large blood vessel, the basilar artery, running forward immediately below the brain and sending up numerous branches into the tissue of the brain. Another large vessel lies along the dorsal side of the anterior end of the spinal cord, within the neural arches of the vertebrae, and from it little arteries run up alongside of the neural spines to the dorsal fin. At stage 30, 36 hours after hatching, the blood supply of the dorsal fin, which has as yet no fin rays, is very rich indeed, (Fig.82).

<u>Veins</u>. (Fig.88).

The vitelline veins, as already described, are the first part of the vascular system to appear, being present at stage 18. By stage 23, the anterior and posterior cardinals have appeared, though it is difficult to trace the anterior cardinals forward into the head. As far as can be made out, however, they pass forward/

forward well out to the side of the head, dorsal to the aortic root and then turn in fairly sharply, ventral to the otocyst. At this stage the cranial nerves IX and X are not easily distinguishable in sections, especially owing to the curve of the whole animal round the yolk, but the vein appears to be external to them. Certainly this is so at stage 25, so that it would appear that the "lateral cephalic vein" is incorporated into the anterior cardinal from the first in Callichthys. Both anterior cardinals are present in the adult and open at either side into the duct of Cuvier.

Posteriorly the caudal vein bends down sharply from lying, in the tail, just ventral to the dorsal aorta, to enter the posterior end of the dorsal wall of the body cavity. The vein enters the kidney where the two Wolffian ducts come together and pass round the end of the body cavity as a single duct to open on the urogenital papilla. Having entered the kidney, the caudal vein extends forward as the inter-renal vein, which is continuous with the right posterior cardinal. In the adult, numerous more or less segmentally arranged veins from the wall of the intestine open directly into the anterior part of the inter-renal. The left posterior cardinal in the adult is very short and only drains the blood from the anterior part of the left kidney. As it enters the duct of Cuvier, it is joined by a very large hepatic vein composed of several branches. The hepatic vein appears to be formed/

formed from the left vitelline vein as in Symbranchus, for at stage 28, a short subintestinal vein enters the liver, from which it passes out as the hepatic vein to join the left duct of Cuvier just as it enters the heart. In the adult, there is a very large portal vein coming from the wall of the intestine, through the pancreas to the liver.. By this arrangement of the veins, it comes about that some of the blood from the intestine passes in the usual way viâ the portal vein and the hepatic vein to the heart, while some of the blood, especially from the dorsal side of the intestine, enters the inter-renal and so goes direct to the heart.

In the lower jaw there is a single median vein which receives a little vein from each barbel. This vein runs at first along the right side of the ventral aorta, then as it nears the heart, it becomes dorsal to the aorta and continues along the dorsal wall of the peri-cardiac cavity, finally opening into the dorsal side of the right wall of the atrium. About the same place a little vessel from the muscles of the right body wall opens directly into the atrium. The vein from the lower jaw corresponds to the inferior jugular vein of Polypterus, except that it enters the atrium directly instead of opening into the right duct of Cuvier as it does in Polypterus. The inferior jugular vein is not shown in the diagram.

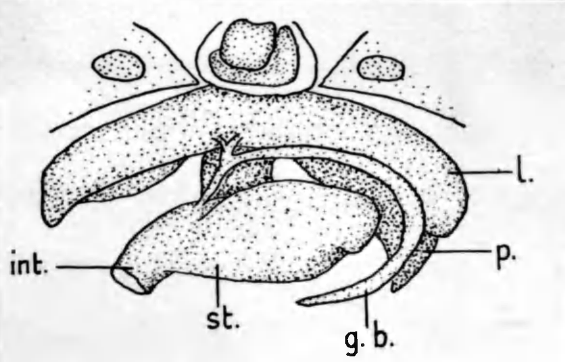

Fig.89. Diagram showing the liver and its duct. g.b. gall-bladder; int.intestine; l.liver; p.pancreas; st. sto -mach.

ALIMENTARY CANAL.

The mouth of the adult is almost terminal and is bounded by thick lips extending out at either side into two long barbels, each supported by a little rod of cartilage. Along the edge of the maxilla there is a row of tiny, conical teeth. The mouth leads back into the wide pharynx with the usual four gill clefts. The branchial arches are armed with small gill-rakers on either side. At the posterior end of the pharynx there is a thick pad on either side of the roof, on which there are conical teeth. Teeth are also present on the floor of this part of the pharynx. A narrow, thick-walled oesophagus leads back to the somewhat kidney-shaped stomach. From the dorsal wall of the oesophagus, in the larva, a pneumatic duct is given off to the air bladder, but in the adult this has degenerated.

The wall of the stomach is thick and muscular. The intestine opens from it fairly near to the opening of the oesophagus. The bile duct from the long, free gall-bladder, opens into the very beginning of the intestine (Fig.89). From sections it appears that this duct is a common hepato-pancreatic duct, the pancreas being a small organ lying between the lobes of the liver and close to the gall-bladder, into the duct of which the pancreatic duct opens. The liver is large, consisting of two main lobes, pressed up against the anterior wall of the body cavity so that the liver partially surrounds the posterior part of the pericardiac cavity and/

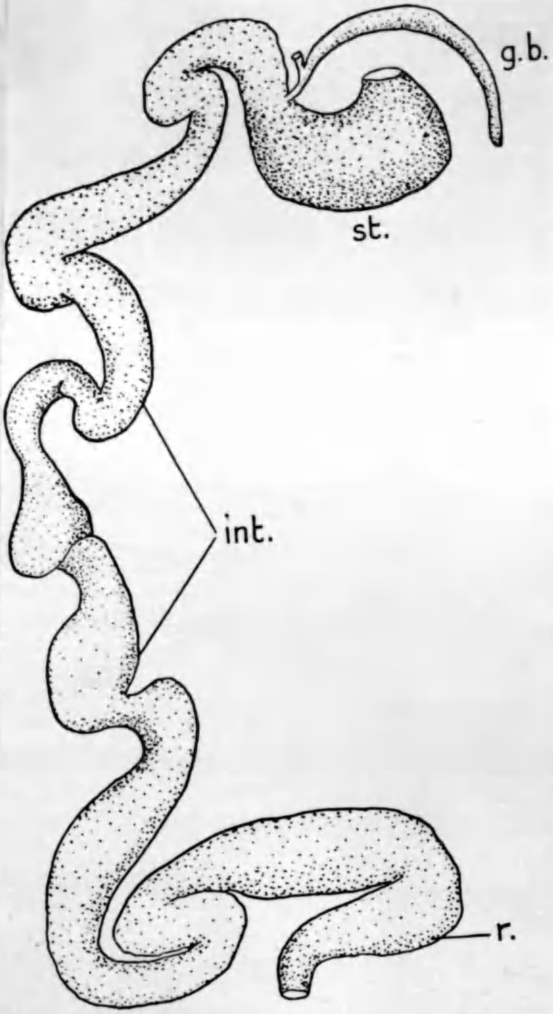

Fig.90. Diagram of alimentary canal. g.b.gall-bladder; int.intestine; r.rectum; st. stomach.

and, in sections, the liver appears along with the posterior part of the heart.

Behind the stomach is the long, coiled, membranous intestine (Fig.90), the walls of which are so distended that the unravelled intestine has a curious kinked and zig-zag appearance. The whole intestine is membranous and greatly distended, leading back into a short and muscular rectum which opens to the exterior between the pelvic fins. The wall of the alimentary canal is richly supplied with blood vessels, the arteries coming from the coeliaco-mesenteric artery and the veins draining into the large inter-renal vein in the anterior part of the body cavity, or by the usual portal vein into the liver.

The alimentary canal is described and figured by Dr. CARTER (5).

In development the foregut has made its appearance at stage 20, as a solid rudiment, very much flattened dorso-ventrally, uniting the rudimentary branchial arches. This seems to be the only part of the alimentary canal recognisable at this stage. By stage 23 a cavity is beginning to be formed in the posterior part of the pharyngeal region, and the solid rudiment is visible some distance behind the branchial region. At stage 25 the whole canal is much more definite, and a small cavity is beginning to be formed behind the pharynx. The rudiment bulges out to the left to form the stomach, and behind the stomach the alimentary canal extends straight back in the middle line to the anus, accompanied/

accompanied by the kidney ducts which are united posteriorly. By stage 27, the mouth is open and in some embryos the cavity is continuous right back to the anus, while in others part of the pharynx is still solid. All the gill clefts are still closed, even where there is a cavity down the centre of the pharynx. By stage 29 all the gill clefts are open. At this stage the yolk is still very large and is quite separate from the alimentary canal which is formed above it, but dips down into it as the canal grows in length. As development proceeds, the canal becomes coiled and membranous, but as late as stage 32, it is still thick-walled. The yolk is still present at stage 31, but has disappeared by stage 32, though the abdominal region is still somewhat distended in larvae of stage 35.

The first rudiment of the liver appears at stage 25, as a little pouch from the lateral wall of the alimentary canal just where it is bulging out to the left into the stomach rudiment. It is a definite organ by stage 27 and is already very vascular. In the adult it has a large portal vein entering it through the pancreas, while the hepatic veins are enormous and enter the left duct of Cuvier.

EXCRETORY SYSTEM.

In the adult the kidneys (opisthonephros) are very large and wide in front, extending almost out to the pectoral girdle in the anterior end of the body cavity, being pressed up against the body/

body wall in front of, and dorsal to the liver. Posteriorly they taper off at first very rapidly and then more gradually, and are represented in the posterior part of the body cavity, only by their ducts which lie just ventral to the inter-renal vein. At the level of the anus, the two Wolffian ducts unite into a common duct which bends round the side of the alimentary canal to open on a little papilla just behind the anus.

Anteriorly the kidneys extend beyond the position of the pneumatic duct and spread out ventral to the air-sac capsule at either side. The anterior cardinals pass through this anterior part of the kidney to meet the posterior cardinals at either side. Just in front of the transverse duct of the air-sacs, the right posterior cardinal turns up alongside the dorsal aorta and passes with it, dorsal to the air-sac duct, though the kidney is ventral to the duct. The left posterior cardinal is only visible in front of the transverse part of the air-sacs. Behind this the two kidneys are closely apposed, with the right posterior cardinal, now the inter-renal vein, dorsal and median to them. For a considerable distance the Wolffian ducts run alongside the ventro-lateral borders of the kidneys, and from a little behind the level of the end of the stomach, there is a very small, solid strand of tissue running along beside and ventral to them. These two strands continue back on either side and finally disappear on the walls of the fused part of the Wolffian ducts near to the opening/

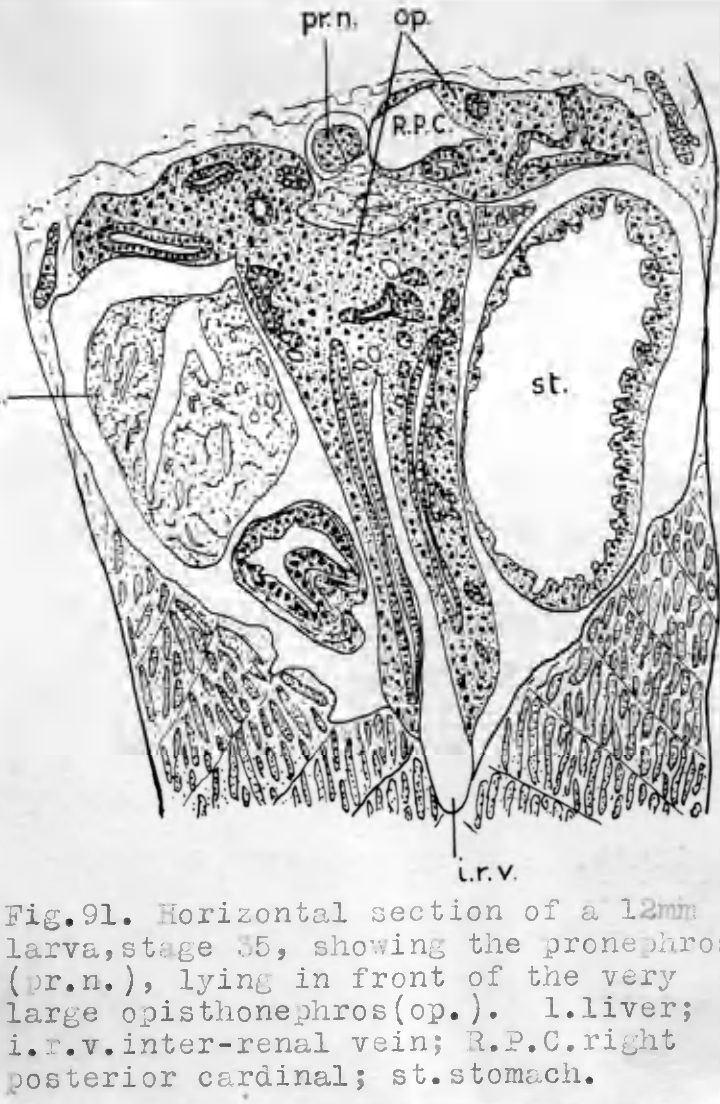

Fig.91. Horizontal section of a 12mm larva, stage 35, showing the pronephros (pr.n.), lying in front of the very large opisthonephros (op.). l.liver; i.r.v.inter-renal vein; R.P.C.right posterior cardinal; st.stomach.

opening on the urinary papilla. I take these to be the persistent vestiges of the Müllerian ducts. Anteriorly just in front of the transverse duct of the air-sacs and ventral to the dorsal aorta, is a little organ (Fig.91) which has the appearance of a very distended glomerulus. This is, I think, the remains of the pronephros, which has developed a single glomerulus such as is described by Schimkewitsch (19) to persist in the adults of a few fish such as Fierasfer, Lepadogaster and Zoarces.

Traces of kidney tubules are visible in an embryo of stage 22, just behind the branchial region, which I take to be the first formation of the pronephros. At stage 23 there are quite definite opisthonephric tubules at the level of the pectoral fins, and by stage 25 these are numerous and the Wolffian ducts run back alongside the alimentary canal. At this stage the pronephros has assumed the form of a single large glomerulus in a much enlarged nephrocoele, in the middle line dorsal to the alimentary canal, just behind the last branchial arch. By this stage the pronephric tubules and their nephrostomes have disappeared. The definitive kidney, the opisthonephros, has assumed its adult form by stage 28 in the newly hatched larva.

SUMMARY.

From the observations chronicled above it will be seen that Callichthys has several primitive features, while being in many ways a typical Teleost. The main points of interest in its development are:-

1. The process of segmentation is similar to that of the egg of Salmo.
2. Development is very rapid and the embryo has many adult features by the time of hatching.
3. The chondrocranium is of the primitive platybasic type, and the trabeculae and parachordals are continuous.
4. The cartilaginous pectoral girdle closely resembles the visceral arches.
5. The anterior part of the vertebral column, with the air-sac capsule connected with it, is similar to that of other Siluridae, except that the first normal, probably the fifth, vertebra carries a large rib, comparable with the cranial rib of the Dipnoi.
6. The bony plates of the body, and probably some of the superficial bones of the skull, are of dermal origin. The bony plates have a row of denticles along their posterior border, similar to the denticles on the lepidotrichia of the paired and unpaired fins.

7. In the otocyst the two endolymphatic ducts meet in the middle line ventral to the brain, and send back a little diverticulum, the sinus endolymphaticus, which is embraced at the sides by the discs of the air-sacs.

8. The air-bladder is represented in the adult by a pair of air-sacs lying at either side in a bony capsule just behind the ear, with which they are connected by means of the Weberian apparatus.

9. The arrangement of the lateral line sense organs on the head is similar to that of the sense organs on the head of *Osteolepis*, one of the fossil Ganoids.

10. The gills are normal, but the fish also breathes by its membranous and vascular intestine. Probably the caudal and dorsal fins have a respiratory function as they are very vascular, especially during development.

11. The heart has a muscular conus. The last two afferents of each side come from the dorsal side of the ventral aorta as a single vessel, as they do in the Dipnoi.

12. The caudal vein continues forward as the inter-renal vein, which is again continuous with the right posterior cardinal. The left posterior cardinal is very short and in the adult has lost its connection with the inter-renal. There is a single inferior jugular vein, comparable/

comparable with that of Polypterus, which opens into the right side of the atrium.

13. The development of the pectoral fins is somewhat similar to that of the pectoral fins of Polypterus, the fin having at first a solid axis with a fold of membrane around it.

14. There appears to be a persistent pronephros with a single very much enlarged glomerulus, right at the front of the opisthonephros in the middle line.

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