



<https://theses.gla.ac.uk/>

Theses Digitisation:

<https://www.gla.ac.uk/myglasgow/research/enlighten/theses/digitisation/>

This is a digitised version of the original print thesis.

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study,  
without prior permission or charge

This work cannot be reproduced or quoted extensively from without first  
obtaining permission in writing from the author

The content must not be changed in any way or sold commercially in any  
format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author,  
title, awarding institution and date of the thesis must be given

Enlighten: Theses

<https://theses.gla.ac.uk/>  
[research-enlighten@glasgow.ac.uk](mailto:research-enlighten@glasgow.ac.uk)

STUDIES IN DIPNOAN STRUCTURE

T H E S I S

for the

Degree of Doctor of Philosophy

in the

University of Glasgow

by

Agnes E. Miller, M.A.

February, 1962

ProQuest Number: 10656289

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10656289

Published by ProQuest LLC (2017). Copyright of the Dissertation is held by the Author.

All rights reserved.

This work is protected against unauthorized copying under Title 17, United States Code  
Microform Edition © ProQuest LLC.

ProQuest LLC.  
789 East Eisenhower Parkway  
P.O. Box 1346  
Ann Arbor, MI 48106 – 1346

# C O N T E N T S

	<u>Page</u>
ACKNOWLEDGEMENTS	i
PREFACE	1
A BRIEF GENERAL ACCOUNT OF WORK ON THE SHEATHS OF THE NOTOCHORD	11
A SUMMARY OF PROFESSOR HELD'S CONCLUSIONS	19
DESCRIPTION OF THE DEVELOPMENT OF NOTOCHORDAL SHEATHS IN <u>LEPIDOSIREN</u> EMBRYOS FROM STAGE 12 TO STAGE 36	29
ARGUMENT	55
CONCLUSION	64
PARTICULARS OF FIXATION, STAINING AND EMBEDDING MEDIA OF THE <u>LEPIDOSIREN</u> EMBRYOS FIGURED	67
BIBLIOGRAPHY	69
ADDITIONAL PAPERS	

## ACKNOWLEDGEMENTS

I would pay grateful tribute to the training and inspiration of my former teacher and colleague, the late Professor Sir John Graham Kerr. To Professor C.M. Yonge, F.R.S., very special thanks for much helpful advice and useful criticism.

# STUDIES IN DIPNOAN STRUCTURE

## PREFACE

An examination of certain structures at an early stage in the development of the living Dipnoi is of interest and importance morphologically since this group is one of great antiquity and may on that account be expected to contain certain primitive features.

In one genus particularly, Lepidosiren paradoxa, the tissue cells are of very large size and this fact makes for greater clarity in histological investigation than is the case in several other groups which do not possess this advantage.

The following thesis deals with work, first of all, on the appendicular skeleton and considers the structure of the pectoral fin skeleton of Ceratodus forsteri and its bearing on the condition met with in certain other primitive vertebrates.

The second part of the work concerns the axial skeleton and of this there are two aspects. One of these deals with the arrangement met with in the hinder part of the body of Lepidosiren and Protopterus, the other is an investigation into the origin of the sheaths surrounding the notochord in early stages.

## APPENDICULAR SKELETON

The Ceratodus fin is generally acknowledged to be of an archaic type and indeed it is probably the most primitive type of paired fin known to exist in vertebrates.

This claim rests on two main bits of evidence, first of all, when considered physiologically this archipterygial fin is a clumsy and inefficient locomotory organ when compared with that of modern fishes. Then, secondly, according to the evidence of palaeontology this type of fin is found occurring frequently among the more archaic fossil fishes, being typical not only of lungfish but also of fossil sharks and ganoids.

Some light was cast on the probable course of evolution of the pectoral fin of sharks when Braus (1906) found in one specimen of an embryo Centrophorus that there were present a number of radial elements along the post-axial (= inner) side of the axis of the fin. In order to decide whether this Centrophorus embryo condition of the pectoral fin was a regular or a chance variation a large number of embryo fins of Acanthias vulgaris were examined and it was confirmed that these post-axial radial elements are a regular feature of the fin skeleton in Acanthias and in all likelihood for the shark-like elasmobranchs in general. When compared with the fin in

Ceratodus the axis of the fin in Acanthias shows an enormous increase in thickness particularly at the basal end. This tendency towards thickening of the axis in that region may be seen in the fin skeleton of Pleuracanthus (Miller, 1952; Text-Fig. 1 B). The axis in Pleuracanthus, however, differs from that of Acanthias in that it is more fully segmented, while in Acanthias segmentation has largely been lost and the number of elements reduced to five recognisable sections (Idem; Text-Fig. 3, No. 5).

It might be remarked that there is little resemblance between the pectoral fin of the embryo Polypterus (Idem; Text-Fig. 1 F) and that of Acanthias. In the examination of so large a number of specimens there recurred in the majority of fins an expansion of the outer ends of the pre-axial radial elements (Idem; Text-Fig. 6, No. 1, e.r.). In many cases this expansion amounts almost to fusion and this, had it been complete, would have resulted in a continuous outer rim of cartilage with, on the inside the gaps or slits between the radials, the whole very reminiscent of the condition in the embryo fin of Polypterus. In the case of the adult Polypterus (Idem; Text-Fig. 1 G) there are present a number of small irregular cartilage elements fringing the outer border of the fin. Irregularities of a similar nature, and of no functional

value, in some Acanthias fins (Idem; Text-Fig. 6, Nos 2, 3, 4 and 6, cl.r. and \*\*) are to be seen at the tips of the pre-axial radials in the region of the propterygium (Fig. 6, No. 1, prop.).

As the result of this investigation of the fins of Acanthias embryos evidence has been brought forward which makes it difficult to escape the conclusion that the fin of the actively swimming sharks has passed through a phase of skeletal evolution from the primitive archipterygium which today still persists in the pectoral fin of Ceratodus.

---

According to Moy-Thomas, in his paper, " Early Evolution & Relationships of the Elasmobranchii " ( Biol. Rev. Cambridge 1938 ) many palaeontologists dispute the primitiveness of the archipterygium since it does not appear to fit in with the theory of the fin-fold origin of the paired limbs. On the other hand, the more recent theory, the external-gill hypothesis, offers fewer difficulties in accepting the archipterygium as probably the earliest type of paired fin.

REPRINT FROM THE  
PROCEEDINGS

OF THE

ROYAL SOCIETY OF EDINBURGH

Section B (Biology)

VOL. LXV—Part I (No. 2)

Note on the Evolutionary History of the Paired Fins  
of Elasmobranchs

By Miss A. E. Miller, M.A., University of Glasgow

PUBLISHED BY  
OLIVER & BOYD  
EDINBURGH: TWEEDDALE COURT  
LONDON: 98 GREAT RUSSELL STREET, W.C.1

1952

Price 1s. 6d.

II.—Note on the Evolutionary History of the Paired Fins of Elasmobranchs.\* By Miss A. E. Miller, M.A., University of Glasgow. Communicated by Sir JOHN GRAHAM KERR, F.R.S. (With Six Text-figures.)

(MS. received October 24, 1951. Revised MS. received May 1, 1952.  
Read June 2, 1952)

SYNOPSIS

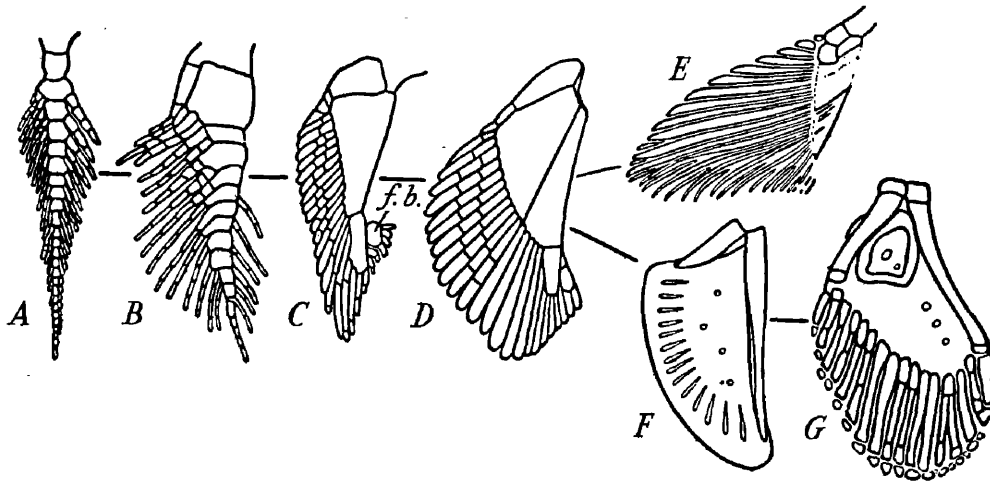
The subjoined Note summarises an investigation into the skeleton of the pectoral fin of a common Selachian—*Acanthias vulgaris*—with the object of determining whether, and if so, to what extent it shows any vestige of the archipterygial type of structure. To this end careful examination was made of five hundred random embryos, and the result demonstrated the general occurrence of vestigial post-axial rays at the tip of the fin. The suggestion that such rays—stated by Braus to occur in a specimen of the embryo of *Centrophorus*—are to be regarded as a mere casual variation without evolutionary significance is thus shown to be devoid of weight, and the pectoral fin skeleton of Selachians established definitely in line with that of *Pleuracanthus* and *Ceratodus*.

ONE of the most important chapters in the morphology of vertebrates is the evolutionary history of the two pairs of *limbs*—pectoral and pelvic—and of this chapter there are two sections: *one*, the evolutionary origin of the limbs, and *two*, the divergence of the original type of limb into (*a*) the swimming type of paired fin as seen in the more highly developed fishes and, on the other hand, (*b*) the pentadactyl limb of land animals. As regards *one* there exist two quite separate hypotheses—the older and generally accepted one, according to which the pectoral and pelvic limbs are surviving and differentiated portions of a once continuous lateral fold of the body-wall, and the more modern one, that each limb has arisen quite separately from an outgrowth of the body-wall which was originally respiratory in function—the external gill. A fuller discussion of these theories than is possible in a short paper like this will be found in Graham Kerr, *Phil. Trans. Roy. Soc.*, 1900, or *Textbook of Embryology*, 1919.

The first hypothesis—lateral fold—was originally based upon Balfour's observation of a continuous lateral ridge in certain Elasmobranch embryos connecting the embryonic rudiments of pectoral and pelvic fin. This ridge, however, turned out to occur only in skates and rays and not in

\* This paper was assisted in publication by a grant from the Carnegie Trust for the Universities of Scotland.

the more primitive sharks and dogfish. Later investigations showed further that where it did occur it was a secondary development—the limb rudiments being at first discontinuous. The fact that the lateral fold theory has not been discarded is due mainly to the influence of palæontology, which has adduced facts in the skeleton of extinct sharks (*Cladoselache*, text-fig. 1, E) most easily interpretable on this hypothesis. The external gill hypothesis rests upon studies of the young developmental stages of a number of vertebrates which are acknowledged to be relatively archaic or “primitive” in their general features—Lung fish, *Polypterus* and the Urodele Amphibians. According to this hypothesis, the highly



TEXT-FIG. 1.—Pectoral fin skeletons of: A, *Ceratodus* (Semon); B, *Pleuracanthus* (Fritsch); C, *Centrophorus* embryo (Braus); D, *Acanthias* (Gegenbaur); E, *Cladoselache* (Bashford Dean); F, young *Polypterus* (Budgett); G, adult *Polypterus* (Wiedersheim).

f.b., fused base of post-axial rays.

developed paired fin as seen in the more actively swimming fishes such as sharks and many teleosts has passed through the ancestral phase of a clumsy paddle such as is still seen persisting to-day in the dipnoan fish *Ceratodus* (text-fig. 1, A), the supporting skeleton of which was long ago given the name archipterygium by Gegenbaur. It has to be borne in mind that Gegenbaur assumed the origin of the limb not from an external gill—since our knowledge of this type of organ has been built up subsequently to his time—but from a gill septum.

Much importance as evidence bearing upon the relationship between the modern paired fin and the archipterygial fin has been attached to Braus's account of the structure of the young pectoral fin of *Centrophorus* (text-fig. 1, C), in which the fin rays are not confined to the outer side of the fin skeleton but extend round its tip to its inner side—a condition clearly explicable by their interpretation as the last remnants of the inner

row of rays of the archipterygium. This at once raises the question, Are we justified in accepting the presence of these inner rays seen in the young *Centrophorus* as being a normal occurrence in a young shark or dogfish rather than a mere odd and inexplicable variation?

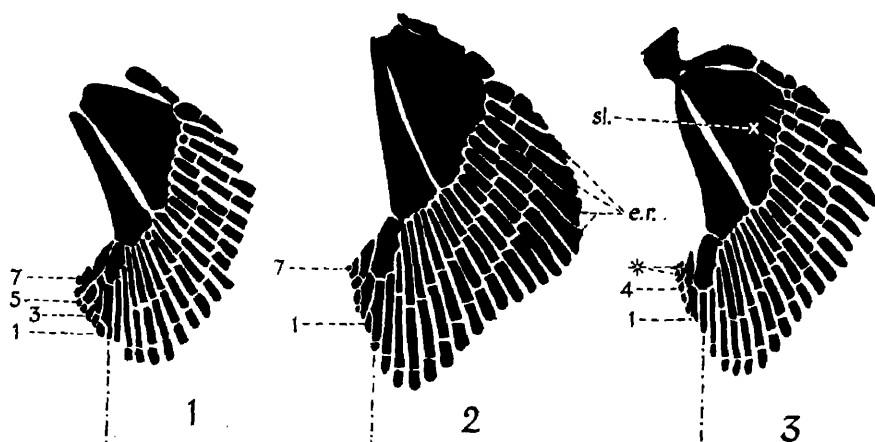
The work here recorded had for its object the settlement of this question, and, having subjected to examination the fins of five hundred embryos of *Acanthias*, I may say at once that the evidence is convincing that the condition figured by Braus for *Centrophorus* is no mere chance variation but represents a normal phase of development in the case of *Acanthias*, and therefore in all probability of shark-like Elasmobranchs in general.

#### TECHNIQUE

The pectoral fins of five hundred embryos of *Acanthias vulgaris* (*Squalus acanthias*), varying from 5 to 10 inches in length, were fixed in formalin (10 per cent.), stained with Victoria blue, the muscles dissected off and the specimen mounted in Canada Balsam. The fins were then critically and repeatedly worked through and damaged specimens eliminated. In a number of cases the delicate post-axial (inner) rays did not stain deeply, so that special care was needed to ascertain the precise arrangement of the cartilage elements in that area. The fin skeletons were drawn with the Edinger apparatus under a low power.

#### DESCRIPTION

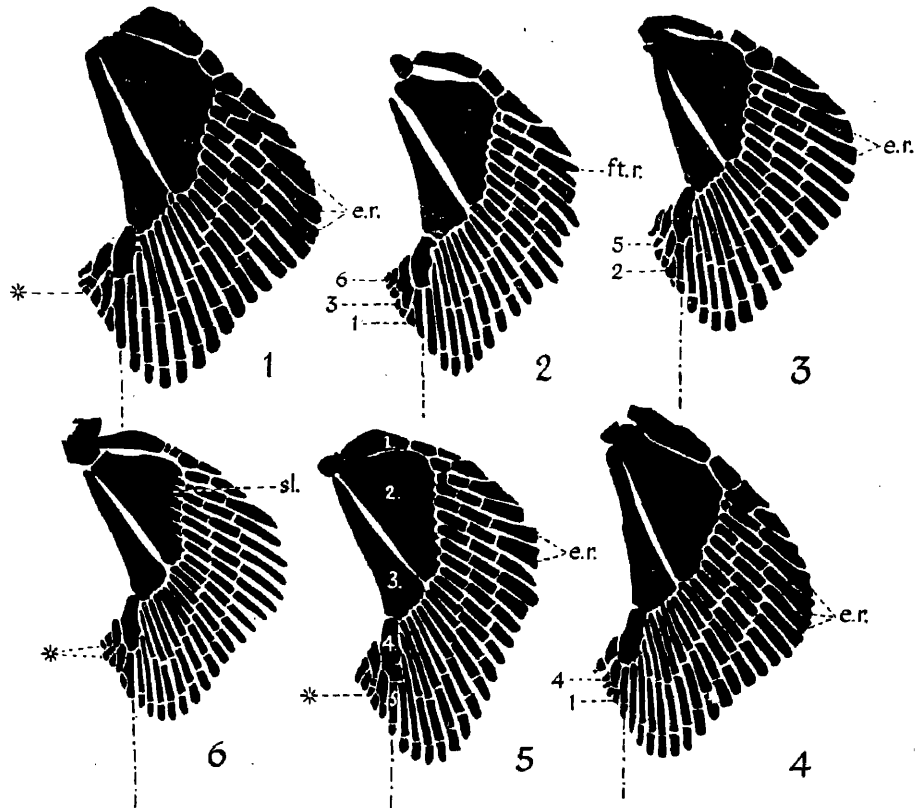
Three fins (text-fig. 2, figs. 1, 2 and 3) show seven inner or post-axial rays (numbered 1-7). In contrast to Braus's *Centrophorus* fin (text-fig. 1,



TEXT-FIG. 2.

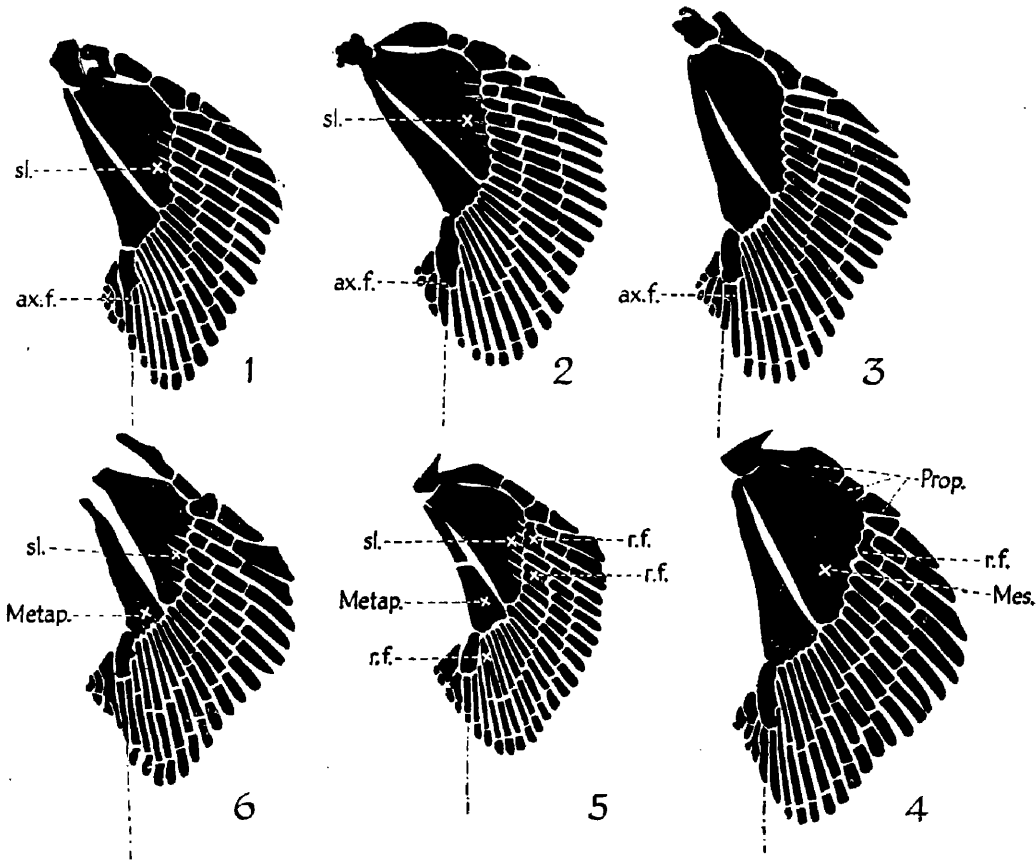
C) it will be remarked that these elements in *Acanthias* are smaller, tend to fuse up together and exhibit a jointed arrangement (text-fig. 2, fig. 3, \*).

Three fins (text-fig. 3, figs. 1-3) with *six* post-axial rays and three with *five* (text-fig. 3, figs. 4-6) post-axial rays illustrate the variability of the arrangement of these elements. The jointed (\*) appearance already mentioned may be seen again (text-fig. 3, figs. 1, 5 and 6). The fused base bearing elements 4 to 7 in *Centrophorus* (text-fig. 1, C, *f.b.*) occurs also in *Acanthias* but it is not a constant feature.

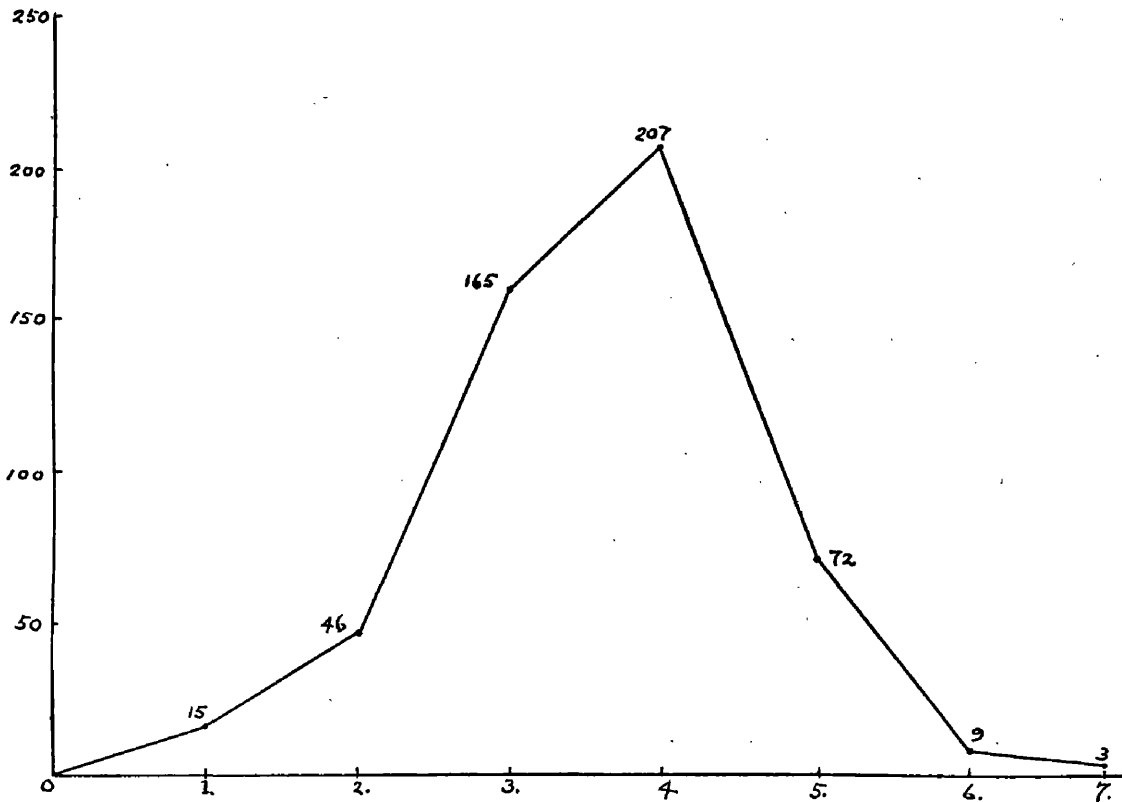


TEXT-FIG. 3.

The limit of the series of post-axial rays is determined by the ray-like terminal portion of the main axis of the fin which separates them from the series of pre-axial rays. A dotted line is drawn from the tip of that terminal portion to assist the eye in picking out the division between the two sets of rays. A glance over the illustrations will reveal that this terminal portion of the axis is as a rule an independent structure, but in two cases (text-fig. 4, figs. 1 and 2, *ax.f.*) the line of axis is emphasised by its continuity with the larger cartilage at its base. Even when fusion with a neighbouring ray takes place (text-fig. 4, fig. 3, *ax.f.*) the line of axis is easily recognisable.



TEXT-FIG. 4.



TEXT-FIG. 5.—Graph to show the relation between the numbers of fins and post-axial rays. The *abscissa* shows the number of post-axial rays, the *ordinate* the number of fins.

## NUMBER OF POST-AXIAL RAYS

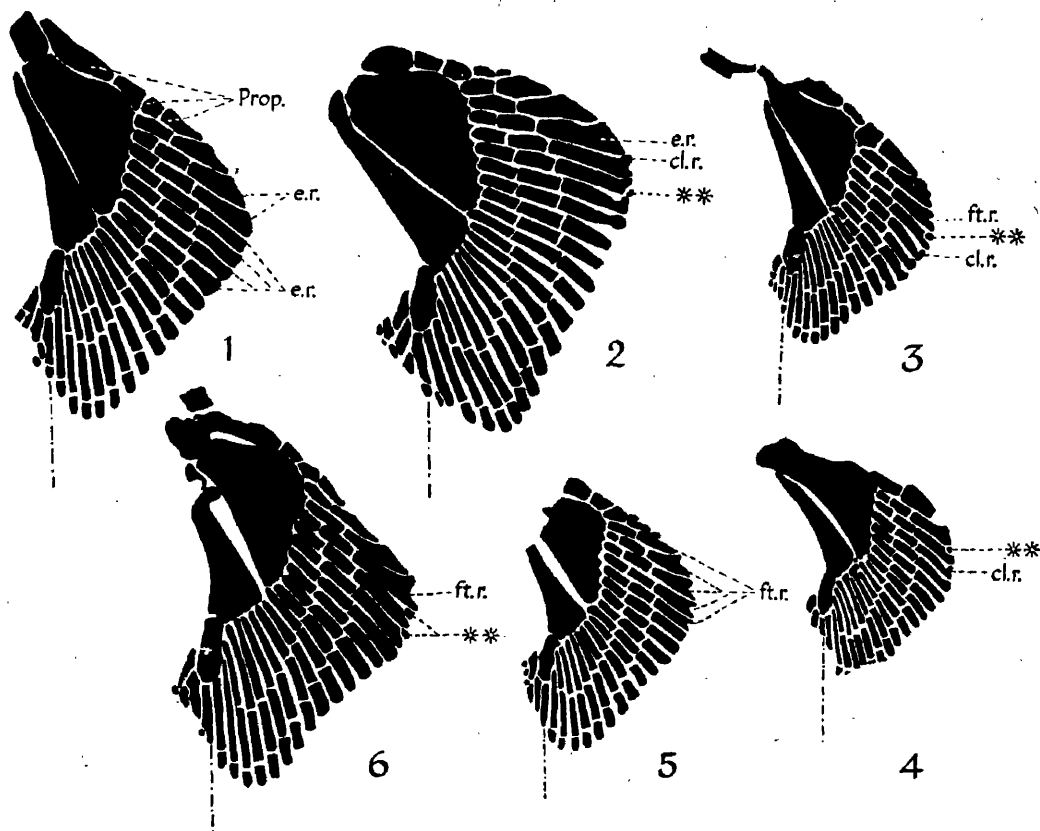
As has already been stated only three fins from the whole collection show post-axial rays of the same number as those of Braus's *Centrophorus* embryo. It was a matter of interest to determine how the number of post-axial rays varies. From a graph (text-fig. 5), it will be seen that the largest number of fins show four post-axial rays, and this may be regarded as the number typical for *Acanthias*. So far as my investigations go, no fins entirely lacking post-axial elements were observed. The bulge in the curve of the graph towards the smaller number, however, would seem to indicate that the post-axial rays are to-day still undergoing reduction in number.

## FUSION OF RADIAL ELEMENTS

In those fins where fusion occurs this affects mostly rays of the mesopterygial region (text-fig. 4, fig. 4, *Mes.*) at that part lying close to the propterygium (*Prop.*). Two specimens are figured, the first (text-fig. 4, fig. 4, *r.f.*)—an example of minimum fusion—showing two rays fused; the second—illustrating maximum fusion—showing several mesopterygial rays and, in addition, two rays of the metapterygial region (text-fig. 4, fig. 5, *Metap. r.f.*) fused together. Between these two examples of radial fusion there occur others intermediate in extent. Of the total collection only about four per cent. show any fusion. It is accordingly not justifiable to conclude that the cartilaginous elements of *Acanthias* are in a state of active fusion.

In a large number of fins the mesopterygium along its outer border shows the cartilage becoming markedly thinner at regular intervals. These thin parts have been depicted as white slits (*sl.*) in those fins in which they occur (text-fig. 2, fig. 3; text-fig. 3, fig. 6; text-fig. 4, figs. 1, 2, 5 and 6, *sl.*). Though this appearance is in most cases confined to the edge of the mesopterygium, the metapterygium in one fin (text-fig. 4, fig. 6, *Metap.*) shows a like condition. In no case was there present a definite perforation of the edge of the mesopterygium, and in many fins the whole mesopterygium stains uniformly. In order to ascertain whether the furrows are caused by pressure from the muscles, transverse sections were cut through the fin, and the evidence points to the complete independence of muscles from skeleton at that part. This agrees with the finding of Braus for early stages in shark embryos (*Entwickl. d. Wirbelt.*, 1906; "Entw. d. Extremit.", p. 221). It might be argued that this phenomenon represents the last traces of a row of once separate contiguous rays resembling in form those now fringing the border of the mesopterygium.

A similar appearance would result, however, from those portions of the mesopterygium in close proximity to the adjacent rays having to carry greater strain and reacting thereto by becoming thicker.



TEXT-FIG. 6.

#### IRREGULARITIES IN THE SHAPE OF RADIAL ELEMENTS

A noticeable feature in many fins is the expanded (*e.r.*) and sometimes "fish-tail" (*ft.r.*) appearance of the pre-axial rays. This expansion occurs in those rays situated next the propterygium, and may be seen to affect one element (text-fig. 3, fig. 2, *ft.r.*; text-fig. 6, fig. 2, *e.r.*, figs. 3 and 6, *ft.r.*), two elements (text-fig. 3, figs. 3 and 5, *e.r.*), three elements (text-fig. 3, figs. 1 and 4, *e.r.*), four elements (text-fig. 2, fig. 2, *e.r.*), five elements (text-fig. 6, fig. 5, *ft.r.*), and as many as six elements (text-fig. 6, fig. 1, *e.r.*). In the last-mentioned fin (text-fig. 6, fig. 1) the rays have become so broad at their outer edge that they appeared to be fused together. Examination with a high power, however, revealed a fine tract of unmodified connective tissue separating the elements.

Further irregularities occur: the edges of the rays instead of merely broadening out have an outgrowth (*cl.r.*) directed towards the prop-

terygium (text-fig. 6, figs. 2, 3 and 4, *cl.r.*). Others exhibit small isolated segments of cartilage (\*\*) some of which (text-fig. 6, figs. 2, 3, 4 and 6, \*\*) have the appearance of fitting into a socket of the element.

Consideration of those fins with "enlarged" radials (*er.*) obviously suggests that actual fusion of their extremities would bring about the condition seen in the young *Polypterus* (text-fig. 1, F), where a continuous border of cartilage forms the outer rim of the fin skeleton while proximally a series of slits indicates the former spaces between the radial elements. Those fins with club-shaped projections (*cl.r.* and \*\*) recall the condition of the adult *Polypterus* (text-fig. 1, G) with its series of small irregular cartilage elements fringing the outer ends of the rays.

A further point noticeable in a survey of the *Acanthias* fin skeletons concerns the propterygium. This has the form of a styliiform rod longer in some cases (text-fig. 6, fig. 1, *Prop.*), shorter in others (text-fig. 4, fig. 4, *Prop.*), but in almost all fins showing the same depth of stain as the other two more bulky elements, mesopterygium and metapterygium.

*Note.*—The axial elements in the fin skeleton of *Acanthias* appear to be five in number (*e.g.* text-fig. 3, fig. 5, numbers indicated in white 1–5), propterygium, 1, mesopterygium, 2, metapterygium, 3, two terminal elements, 4 and 5; from this latter one of which a dotted line is drawn in the figure. As compared with those of *Ceratodus* they have greatly increased in bulk and have to a large extent lost their segmentation.

#### SUMMARY

The foregoing paper summarises an investigation of the skeleton of the pectoral fin in five hundred embryos of *Acanthias*, all of which show post-axial rays varying in number from one to seven, the majority of specimens having three or four. Such an investigation would supply evidence of the extent it is justifiable to regard the skeleton of Selachians as having evolved from the archipterygial type of fin skeleton as exemplified by *Ceratodus*. It is held that the acceptance of this view is justified by the facts disclosed.

In conclusion I should like to acknowledge with much gratitude the help and criticism of Professor Sir John Graham Kerr, F.R.S., in the course of the work.

My grateful thanks are also due to Mr A. Mackinnon of this department for the care and skill he has taken in preparing the specimens which have made these investigations possible.

REFERENCES TO LITERATURE

- BALFOUR, F. M., 1878. *Monographs on the Development of Elasmobranch Fishes*, London.
- BRAUS, H., 1906. Hertwig's *Handbuch der Entwicklungslehre*.
- GEGENBAUR, C., 1872. *Untersuchungen zur vergleich. Anat. der Wirbelthiere*, Leipzig.
- KERR, J. GRAHAM, 1900. "The External Features in the Development of *Lepidosiren paradoxa*", Fitz., *Phil. Trans. Roy. Soc. London*, CXII, B, 299-330.
- , 1907. *The Work of J. S. Budgett*, Cambridge.
- , 1919. *Textbook of Embryology*.
- MOLLIER, S., 1892. "Zur Entwicklung der Selachierextremitäten", *Anat. Anz.*, VII, 351-365.
- RABL, C., 1901. "Gedanken und Studien über den Ursprung der Extremitäten", *Zeits. Wiss. Zool.*, LXX, 474-558.

(Issued separately December 31, 1952)

## AXIAL SKELETON

The first of the studies on the axial skeleton deals with the appearance in the tail region of two of the Dipnoi, Lepidosiren and Protopterus, of blocks of cartilage.

The Dipnoi are recognised as possessing a cylindrical notochord throughout life, but this regular feature occurring, not merely in the early stages of development but persisting in the adult, suggested that here was a condition of considerable evolutionary importance.

These cartilage blocks show a remarkable regularity and taper off gradually towards the tip of the tail (Miller, 1930; Text-Fig. 1) in the young specimen. In the adult they are present (Idem; Pl. 1), are less numerous and much less regular in form. In a verbal communication Dr G.S. Carter declared, as a result of his experience in the mudfish swamps of Central South America, that every adult Lepidosiren brought in had the tail damaged, sometimes severely so, due to having been bitten off and no doubt this may account for the irregular appearance in Plate 1 due to regeneration.

These structures, then, have every justification for being regarded as vertebral centra. If this is granted, the claim that the Dipnoi possess a cylindrical notochord

must be amended, for these elements now appear as the last vestiges of a once-segmented vertebral column.

The fossil dipnoans have up till now not been shown to offer any corroborative evidence and this may be due to the fact that cartilaginous centra are seldom sufficiently calcified to endure and also to the further fact that in the fossil dipnoans the vertebral column is generally hidden under a thick coating of calcified scales. If, however, a completely segmented vertebral column were the condition in ancient dipnoans this would have interesting repercussions particularly on the argument raised regarding the position of the fossil Palaeospondylus gunni. The main objection to considering this enigmatical creature as a member of the Dipnoi was the fact that it possesses a completely segmented vertebral column.

The various resemblances of skeleton between the skull of the larval Lepidosiren and the two specimens of Palaeospondylus concern the olfactory region, the area of the tooth-plates, the strongly marked halves of the hyoid arch and particularly a pair of structures very conspicuous in the fossil (Idem; Text-Fig. 5 B and C, o.r.) and which closely resemble the occipital ribs of the Lepidosiren figured (Idem; Text-Fig. 5 A, o.r.). These latter structures are unique and found only in the Dipnoi

among vertebrates.

The remaining objection to regarding Palaeospondylus as dipnoan, probably a young dipnoan on account of its small size, would be removed if these cartilage blocks in the tail region of Lepidosiren and Protopterus were recognised as the remains of an earlier segmented condition of the entire vertebral column.

---

Since the publication of this paper in 1930, extensive researches have been carried out on a large number of specimens of Palaeospondylus gunni by Moy-Thomas (Palaeospondylus gunni, Traquair. Phil Trans. Roy.Soc. London 1940).

He considers Traquair's figures misleading & has found that the specimen designated "C" (Miller: Tx. fig.5) is not the ventral but the dorsal aspect of the cranium of the fossil. He also quotes the findings of Graham Kerr (1900) regarding similarities of features in the skulls of Lepidosiren larvae and Palaeospondylus, but he does not accept the suggestions of dipnoan affinity therein offered.

While recognising the enormous labour involved in working over so great a collection of these fossils, to enter into a discussion of Dr. Moy-Thomas's interpretations of these elements is outwith the scope of this study which is concerned with the segmented vertebral column of Palaeospondylus and that is not in dispute.

In 1952 there appeared what would seem to be an important piece of evidence supporting the suggestion of this paper (1930) when Jarvik ("On the Fish-like Tail in the Ichthyostegid Stegocephalians." Meddel. om Gronland, Bd. 114, No. 12 1952) made known the existence of certain Dipnoans of the Middle Devonian which possessed not only clearly segmented vertebral columns but also well ossified vertebral centra.

NOTES ON THE TAIL SKELETON OF LEPIDOSIREN  
PARADOXA, WITH REMARKS ON THE AFFINITIES OF  
PALÆOSPONDYLUS. BY AGNES E. MILLER, M.A., Uni-  
versity of Glasgow.

---

[From the PROCEEDINGS OF THE ZOOLOGICAL SOCIETY OF LONDON, 1930.]  
[Published October 22nd, 1930.]

---

Note on the Tail Skeleton of *Lepidosiren paradoxa*, with Remarks on the Affinities of *Palaeospondylus*. By AGNES E. MILLER, M.A.\*, University of Glasgow.

(Plate I.†; Text-figures 1-5.)

A well-known and interesting characteristic of modern Dipnoan fishes is the presence in the tail region of a number of blocks of cartilage presenting a superficial resemblance to vertebral centra, which are otherwise conspicuous by their absence in that group. The purpose of this note is to make known the main results of a detailed examination of the structures in question as they occur in the South American Lungfish, *Lepidosiren paradoxa*.

The typical adult condition is shown in Plate I., where the notochord enclosed in its cartilaginous secondary sheath is seen to be continued towards the tip of the tail by a series of thirty-six cartilaginous blocks closely resembling typical vertebral centra. The number of such blocks shows great differences in different individuals, and it is impossible to decide upon any particular number as being "normal," owing to the fact that the tip of the tail, and indeed the tail region generally, is exceedingly liable to damage, and has in the great majority of specimens been secondarily shortened by disease or other injury.

The question now presents itself—Is the resemblance of the blocks of cartilage to vertebral centra merely superficial, or is it fundamental? The importance of this question will be recognized when it is recalled that every zoological text-book gives us one of the main Dipnoan characteristics that their axial skeleton is devoid of centra.

The obvious course is to turn to the evidence of embryology for the answer. In text-fig. 1, a reconstruction drawing from a young *Lepidosiren* of stage 37, there are present in all twenty-four blocks of cartilage, extending from the end of the notochord to the tip of the tail.

From the next illustration (text-fig. 2), which shows the hind end of the notochord and the first of these cartilage blocks, it is obvious that the entire series of cartilage blocks lies quite outside the notochordal sheath. The notochord itself comes to an abrupt end, and the primary sheath is to be observed terminating in the substance of the first cartilage block of the series.

In this particular section the cells of the notochord have shrunk away somewhat from the primary sheath, which nevertheless is obviously vanishing off in the substance of the first of these cartilage blocks.

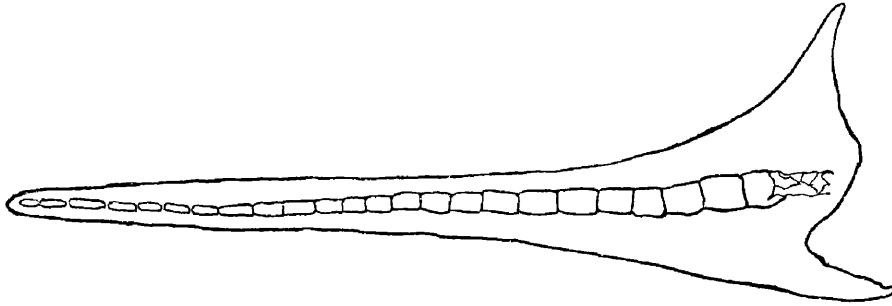
Text-fig. 3, a section through the tail of a young *Protopterus* of 96 mm., makes quite clear the fact that the cartilage of the secondary sheath is continuous with that of the first cartilage block of the series.

The possibility that these cartilaginous centra are not normal, but due to regeneration, is naturally suggested by the appearance of such sections (*cf.* also Plate I.), and it is not lightly to be dismissed. It should be said, however, that although the tip of the tail is exceedingly liable to injury during the life of the *Lepidosiren*, examination of large numbers of individuals of early stages from about stage 32 onwards has failed to disclose a single one in which the segmental

\* Communicated by Prof. J. GRAHAM KERR, F.R.S., F.Z.S.

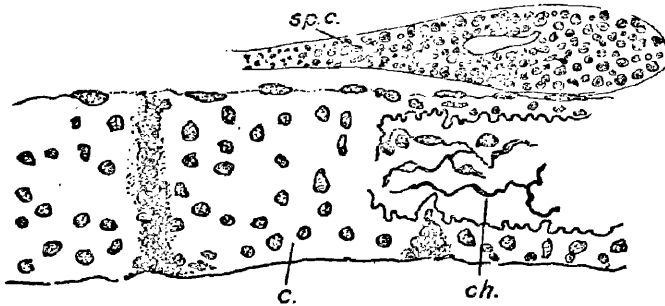
† For explanation of the Plate, see p. 789.

Text-figure 1.



Reconstruction of tail of *Lepidosiren* larva of stage 37, with twenty-four cartilage blocks.

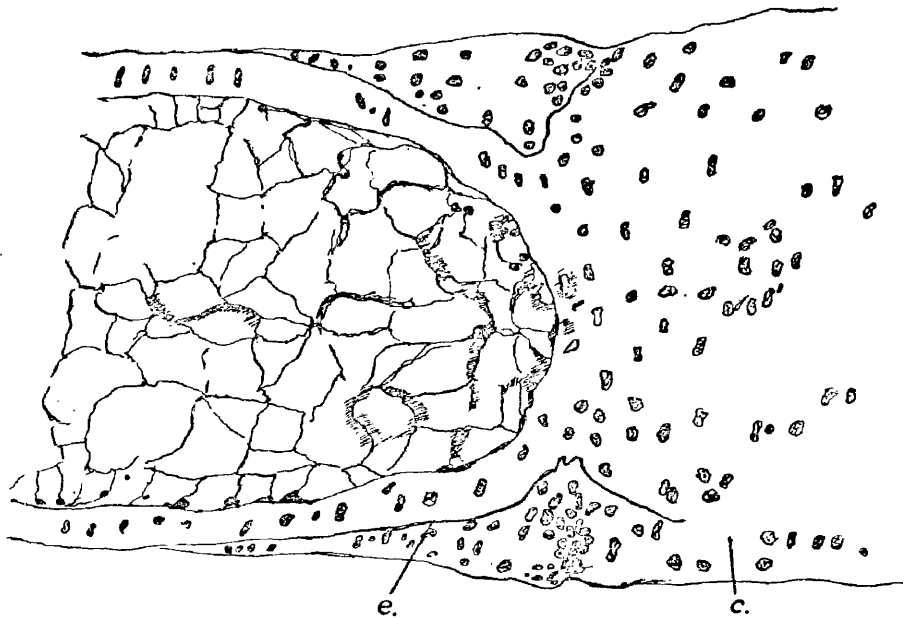
Text-figure 2.



*Lepidosiren* larva of stage 37. End of notochord as seen in longitudinal section under high power (oc. 2, Zeiss D).

c, first cartilage block of series; ch., notochord; sp.c., spinal cord.

Text-figure 3.



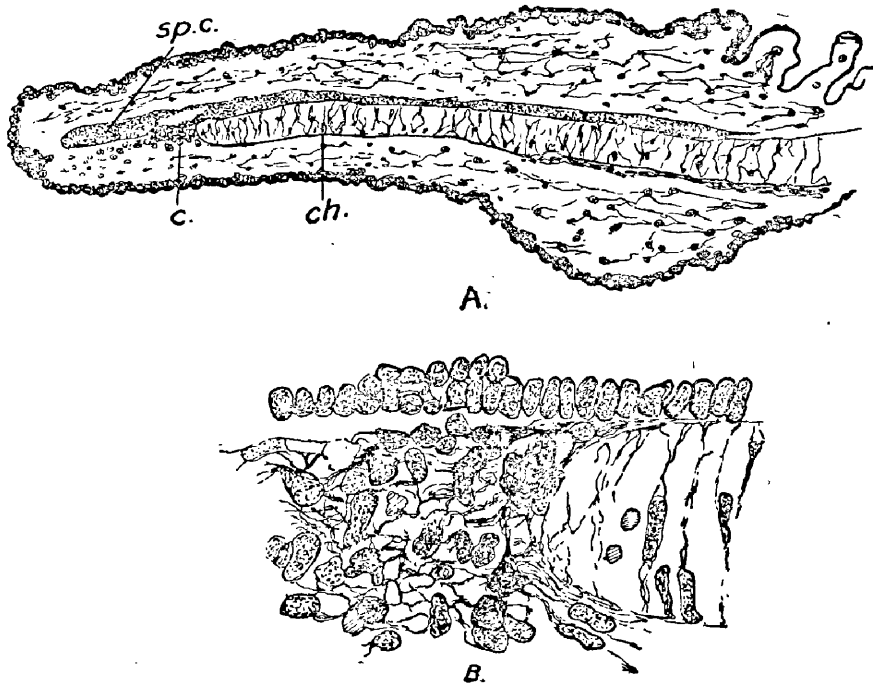
Tail of young *Protopterus* of 96 mm., as seen in longitudinal section.

c., first cartilage block of the series; e., primary sheath of the notochord.

blocks of cartilage were absent. The really important point is that here in the tail region of those Lungfish we have present an innate tendency towards the production of segmentally arranged blocks of cartilage; the question whether injury followed by regeneration is normally required for this tendency to find full expression would, even if answered in the affirmative, be of minor though still considerable interest. In this connection it is worth recalling that the young *Lepidosiren* of stage 32 have not yet reached a condition of development when they are capable of biting, so that the risk of such injury between stage 32, when cartilage blocks are first quite definitely to be observed, and stage 38, when the jaws are sufficiently developed to enable the creature to feed, is considerably lessened.

So far as my investigations go, there are no definite cartilage blocks present

Text-figure 4.



A. Longitudinal section through tail of *Lepidosiren* larva of about stage 31. B. Part of A more highly magnified, showing connective tissue becoming converted into cartilage.

(Letters as on previous text-figures.)

before stage 31, although at this stage it is becoming obvious that the connective-tissue cells at the end of the notochord are becoming converted into cartilage (text-fig. 4).

It has been stated by Gadow that the notochord of the Dipnoi is potentially chordo-centrous on account of the fact that the chordal sheath is partly cartilaginous, and in spite of the fact that it shows no trace of division into vertebrae. It seems, however, that the conception of a centrum derived entirely from the chordal sheath is somewhat hypothetical, since not only is the chondrification of the secondary sheath affected by the immigrant cells from the cartilaginous arch elements which have broken through the primary sheath, but in the case of Elasmobranchs the spreading bases of the arch elements come to envelop the whole notochord with its sheaths in a layer of external cartilage. While in early

stages the primary sheath forms a distinct boundary between the two layers of cartilage—of sheath and arch origin respectively—this tends to disappear later, and it becomes quite impossible to say where the one ends and the other begins. Further, the general facts of comparative embryology impress upon us the very much greater evolutionary importance of the arch centrum as compared with the sheath centrum.

The general impression borne in upon me by my study of these blocks of cartilage in the Dipnoan tail is that we are not justified in refusing to them the term "vertebral centra," and that in consequence we should define Dipnoi not as fish without vertebral centra, but rather as fish in which there are present vertebral centra only towards the tip of the caudal region.

If we consider the presence of these centra from the point of view of evolution, we cannot but remember that the normal condition is for the vertebral column to be homogeneous from end to end. This at once raises the question as to whether the segmentation of the vertebral column towards the posterior end of the Dipnoan body is an incipient stage in the segmentation of the axial skeleton, or whether it is the last remnant of a segmentation which once extended throughout the body. This morphological problem must be kept in relation to physiological considerations. In the living Dipnoan the creature still retains the primitive vertebrate method of progression by passing waves of lateral flexure back along the length of the body, so that there is in these animals, so far as surviving members of the group are concerned, none of that concentration of propulsive force at the hinder tip of the body which finds its expression in a spread-out caudal fin.

This consideration renders it improbable that the tail region of the Dipnoi is in any active condition of forward evolution; and indeed the probability lies in quite the opposite direction, namely that the terminal region of the body is of no functional significance, and therefore degenerative. One may recall the apparently meaningless growth in length which often takes place in the young Dipnoan, as for example in the young *Protopterus*, where the tail region may grow out into a filament as long as the body. The tendency towards segmentation, which becomes visible in the Dipnoan in this terminal part of the tail free from adaptive necessity, seems to fit in most easily with the hypothesis that here we have the last vestige of a once segmented condition of the vertebral column as a whole. The paucity of corroborative evidence from the side of palæontology ceases to be a formidable difficulty when we bear in mind, firstly, that uncalcified cartilaginous centra are in the highest degree perishable, and, secondly, that the vertebral column of ancient Dipnoans is so generally concealed in a body the outer layer of which consists of a thick coating of calcified scales.

The possibility that a completely segmented vertebral column may have been the condition in the ancestral forms of the modern Dipnoi would have an interesting bearing upon the problem of the affinities to this group of that much discussed fossil enigma, *Palæospondylus gunni*.

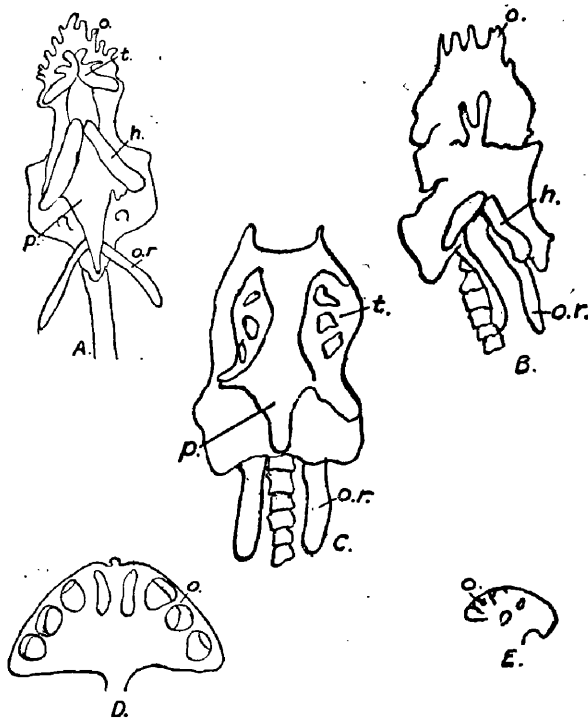
To the opinion still held by various palæontologists, following Traquair, its original describer, that this creature is to be classed as a Devonian representative of the Cyclostomata, there are several objections, one of the most serious being that *Palæospondylus* shows a completely segmented and apparently chordo-centrous vertebral column.

Professor Graham Kerr, in pointing out many years ago that the affinities of this creature were strongly Dipnoan, found the most important argument against this view in the fact that *Palæospondylus* possessed definite vertebral centra.

It may be recalled that *Palæospondylus* was first described by Traquair in the 'Annals and Magazine of Natural History,' ser. 6, vol. vi., having been got from the Achanarras quarry, near Thurso. This great authority on the palæontology of fishes concluded that it was a "primitive lamprey." Professor Kerr, as a member

of the Royal Physical Society and a personal friend of Traquair, was particularly interested in *Palæospondylus*, and had the main facts of its structure clearly in mind during his second *Lepidosiren* expedition, 1896-97. While on that expedition it happened that a particular batch of small *Lepidosiren* larvæ were dead when brought in and were just put on one side; by next morning the specimens were completely macerated and, on pouring away the water, there were found merely skeletal remains. In such specimens the limbs and limb-girdles being free from the axial skeleton had naturally disappeared, and the same applied to the massive lower jaw, which drops off, along with, as a rule, the branchial arches, at an early stage of maceration; in addition, the bars of the olfactory capsules were commonly frayed out into little tags projecting from the front end

Text-figure 5.



A. Macerated skull of *Lepidosiren* larva, ventral view. B and C. Specimens of *Palæospondylus*, after Traquair. D. Dorsal view of olfactory capsule of *Lepidosiren* larva. E. Front end of skull of *Palæospondylus*, after Bashford Dean.

h., hyoid; o., olfactory capsule; o.r., occipital rib; p., parasphenoid; t., tooth-plates.

of the skull (text-fig. 5, A, o). The appearance of *Lepidosiren* skeletons in this condition suggested irresistibly those of so many specimens of *Palæospondylus*. Professor Kerr, after his many years of research upon the morphology of the Dipnoi and other lower vertebrates, adheres to the opinion that it is quite unjustifiable to hold that *Palæospondylus* is anything else than a member of that group of primitive fishes to which we apply the name Dipnoi.

In estimating what weight is to be attached to this opinion, we cannot ignore the fact that its protagonist is probably the only living morphologist who has had the opportunity of examining large numbers of small Dipnoan skeletons in that macerated condition, which provides the usual material for fossil remains.

On account of the great interest taken by palæontologists in *Palæospondylus*, I will summarise shortly the interpretation of its representations by Traquair that would be given by one familiar with Dipnoan embryology. I confine myself naturally to the representations of actual individual specimens and ignore figures expressing theoretical interpretations.

In text-fig. 5 there are reproduced in outline (B and C) Traquair's figures of two of his most perfect specimens, and alongside (A) I give an outline of what appear to be the corresponding structures in *Lepidosiren* as shown by the macerated skeleton of a young specimen. To facilitate comparison I have made the text-figures of approximately the same size, although in actual fact *Palæospondylus* is much smaller.

Two bones (text-fig. 5, B; *h.*) correspond in position, shape, and relative size to the hyoid arch of *Lepidosiren*; of this one feels more particularly assured by Dr. Traquair's statement that we are viewing the ventral surface of the skull of *Palæospondylus*. In the second specimen (C) there is again a structure showing strong resemblance in shape, position, and relative size to the parasphenoid bone (*p.*) of *Lepidosiren*; while marks further forward recall the characteristic tooth-plates (*t.*) of the Dipnoi.

The tags ("cirri" of Traquair) seen projecting from the front end of many specimens of *Palæospondylus* (*o.*) are represented in the macerated *Lepidosiren* by the remains of the cartilaginous trabeculæ of the olfactory capsule; and in this connection it is of interest to observe that Bashford Dean figures a specimen of *Palæospondylus* (text-fig. 5, E) in which the front end of the head skeleton is limited by a curved bar. The figure of the front end of a *Lepidosiren* larva head (text-fig. 5, D) shows how closely this agrees with the cartilaginous support of the olfactory capsule before it becomes frayed out in the process of maceration. Perhaps the most characteristic single feature of *Palæospondylus* is the pair of elongated structures (*o.r.*) attached to the skull one on each side posteriorly. The Dipnoi alone amongst vertebrates are characterised by a pair of elongated bones (*o.r.*) attached to the skull, one on each side posteriorly—the enlarged ribs of the occipital vertebra, which has become fused with the cranium. Graham Kerr homologises these *Palæospondylus* structures with those occipital ribs—a possibility which is also contemplated by Bashford Dean.

W. J. and I. Sollas believe the structures in question to be attached to the "last branchial arch." One is justified, however, in being somewhat sceptical as to this, owing to the liability to error inherent in the wax-plate method of reconstruction, more especially when applied to the macerated and crushed remains of fossil organisms. Even if the Sollases were correct as to the attachment of these rods to a branchial arch, they might quite possibly represent the skeletal support of external gills, such as are to be seen in an incipient or a vestigial form in the external gill of a young *Polypterus*, for external gills are known to exist in the larvæ of two of the three living Dipnoi; and now that we know more regarding the morphology and development of external gills, there seems every reason for regarding them as archaic organs and by no means modern adaptations.

Enough has, I think, been said to bring out the strong resemblances between the skeleton of *Palæospondylus* and that of the modern Lungfish, and to justify of us in the belief that *Palæospondylus* was simply a small-sized Dipnoan.

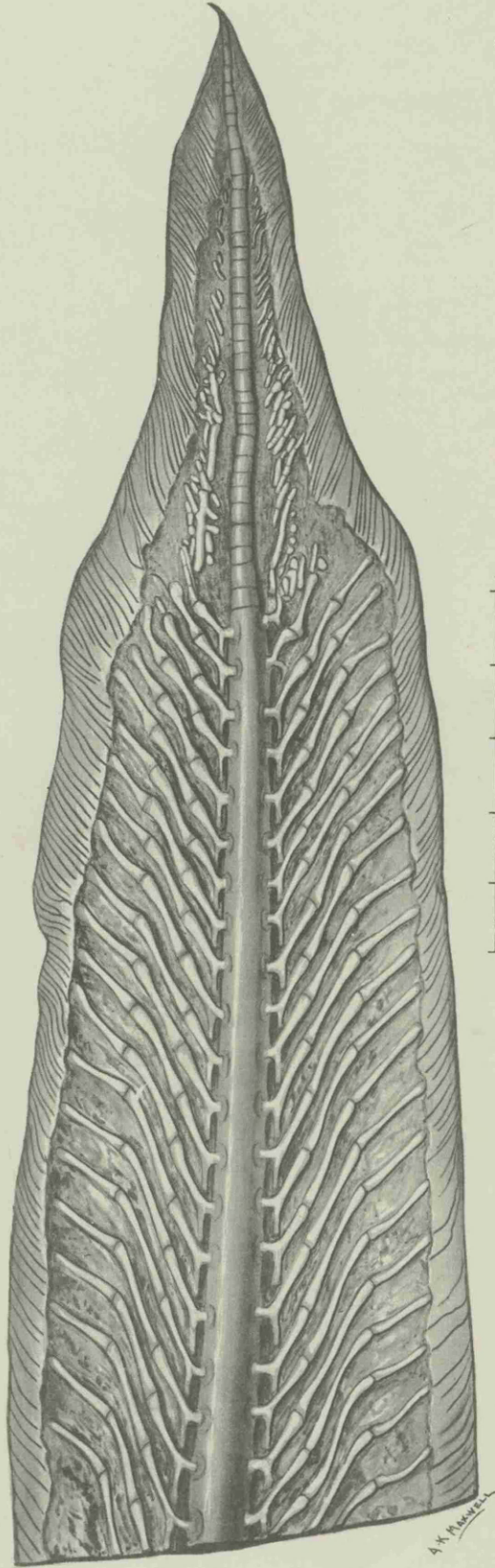
The most conspicuous difficulty in the way to this conclusion is, as already indicated, the completely segmented vertebral column of *Palæospondylus*; but even that difficulty, such as it is, would be removed if we accept the view supported in this note that the blocks of cartilage in the tail of the modern Lungfish are to be regarded as a vestige or a reminiscence of an earlier segmented condition of the whole vertebral column.

## LITERATURE.

- AGAR, W. E. Development of Skull and Visceral Arches in *Lepidosiren* and *Protopterus*. Trans. Roy. Soc. Edin. vol. xlv. pt. 1, no. 3, 1905.
- BRINDLEY, H. H. Note on some Abnormalities of the Limbs and Tail of Dipnoan Fishes. Pub. Phil. Soc. Camb. vol. x. 1900.
- DEAN, BASHFORD. Affinities of *Palæospondylus*. Proc. Zool. Soc. London, 1898.
- Devonian Lamprey. New York Ac. of Sc. vol. ii. pt. 1, 1900.
- Further Memoir on *Palæospondylus*. Science, n. s. vol. xix. no. 480, 1904.
- GADOW, H. Evolution of Vertebral Column of Fishes. Phil. Trans. Roy. Soc. vol. clxxxvi. 1894.
- GÜNTHER, A. *Ceratodus*. Phil. Trans. Roy. Soc. 1871.
- HOLMGREN, N. Some Observations about the Growth of the Tail in *Lepidosiren*. Acta Zool. vol. ix. 1928.
- KERR, J. GRAHAM. Keibels Normentafeln Entw. Wirb., Heft x. Jena, 1909.
- The Zoological Position of *Palæospondylus gunni*. Pub. Phil. Soc. Camb. vol. x. pt. 5, 1900.
- Text-book of Embryology.—Vol. II. Vertebrata. London, 1919.
- KISSLEWA, S. Skelett von *Lepidosiren*. Bull. Soc. Natur. Moscow, 1928.
- SOLLAS, W. J. & I. An Account of the Devonian Fish *Palæospondylus gunni*. Phil. Trans. Roy. Soc. vol. cxevi. 1903.
- TRAQUAIR, R. H. On some Fossil Fishes. Ann. & Mag. Nat. Hist. ser. 6, vol. vi. 1890.
- Further Description of *Palæospondylus gunni*. Proc. Roy. Phys. Soc. Edin. vol. xii. 1893.
- Still Further Contributions to our Knowledge of *Palæospondylus gunni*. Proc. Roy. Phys. Soc. Edin. vol. xii. 1893.

## EXPLANATION OF THE PLATE.

Tail of adult *Lepidosiren* dissected to show vertebral column and cartilage blocks.



0 1 2 3 4 5 cms.

John Bull, Sons & Danielsson, Ltd., London.

TAIL OF ADULT LEPIDOSIREN PARADOXA DISSECTED TO SHOW  
VERTEBRAL COLUMN AND CARTILAGE BLOCKS.

The second study on the axial skeleton of the Dipnoi is concerned with the origin and development of the sheaths of the notochord in Lepidosiren. It is generally assumed that the sheaths are formed by the activity of the actual cells which constitute the structure. The fact, however, that these sheaths are composed of two apparently different substances received closer attention and investigation was carried out on many types of vertebrates, particularly primitive vertebrates as e.g. elasmobranchs, ganoids and amphibians, by numerous workers. The conclusions reached were in many cases conflicting and after reviewing most of the work done it seemed that there were two factors which contributed to such a state. First, the difficulty of obtaining a sufficient number of early stages of developing embryos of primitive vertebrates; and, second, though to a lesser extent, the differing histological techniques employed which made comparisons less certain.

The present investigation concerns a series of Lepidosiren embryos commencing at a stage in development when the rudiment of the notochord and the mesoderm are not yet separated and continuing through each stage up to that one in which the two sheaths attain their maximum growth before the cartilage cells from the arch elements have penetrated the primary sheath and have begun to colonise the secondary sheath converting it into the

characteristic cylinder of cartilage of the adult condition.

The material used in this study consisted of Lepidosiren embryos which had been sectioned serially and slides prepared under direction of the late Professor J. Graham Kerr and which now form part of the unique collection of slides of these primitive vertebrates in the Zoology Department of Glasgow University.

In some cases details of the fixative, stain and embedding media used are noted and this information is given on a separate page at the end of this thesis. In other cases some of these particulars have not been recorded.

It will be obvious that since part of the material was despatched by Mr Pride and others from South America it had already been fixed in formalin 10%; sublimate acetic was another fixative commonly used. With regard to the embedding medium that most employed in very early stages where a quantity of yolk was present was celloidin and in some cases the celloidin block was immersed in soft paraffin before cutting - apparently with most satisfactory results. Later when the quantity of yolk was less the embryo was embedded and cut in paraffin (52°C melting point).

The stains used are also recorded where these are known; the later stages of development were usually

treated with Heidenhein's haematoxylin and counterstained with eosin; borax carmine with orange G, however, was also used. The stain has endured with very little fading and the large size of the cells - already referred to - was one of the most valuable assets in reaching the definite conclusions which the work of investigation required.

A BRIEF GENERAL ACCOUNT OF WORK ON THE ORIGIN  
OF THE SHEATHS OF THE NOTOCHORD

Johannes Müller (1834) differentiated the "inner fibrous and proper sheath of the chorda from an outer cutaneous in cyclostomes". Leydig (1851-53) found in the sturgeon that the inner fibrous sheath seemed to have two parts, the inner portion being composed of connective tissue capable of swelling and an outer membrane of elastic property. Kölliker (1860) followed with investigations on a series of fishes - elasmobranchs, Chimera, and sturgeons. His conclusion was that they commonly possess notochords with two sheaths - an inner fibrous, which he termed "elastic" and an outer or external elastica. His theory was that these were produced as a secretion of the chorda cells, and corresponded to the structureless sheaths of all higher vertebrates. Gegenbaur (1867) agreed with Kölliker but pointed out that all the cells of the notochord were not capable of secreting, only those situated round the periphery - this was the theory advanced though no direct observations of such a process seem to have been made. W. Müller (1871) found in a four-day-old chick a fine homogeneous membrane round the chorda which he named the "cuticula chordae". He put forward the suggestion that this sheath was derived from certain connective tissue cells which had arisen from the

outer wall of the primitive aorta.

Goette (1878) after working on elasmobranchs and teleosts objected to the definition of the sheath as "cuticular", considering that the chordal cells are not an epithelium but a mass of protoplasm and nuclei - a plasmozone - the outer portion of which becomes modified locally as sheath. He, however, agreed with Kölliker in deriving the sheaths from chordal cells.

Hasse working on elasmobranchs (1892) found a cuticula chordae, a thin membrane enclosing a distinct notochordal epithelium, on the outside of which was another bounding membrane, the cuticula skeleti or elastica externa and between these two, a sheath in which cells have appeared - the inner cell sheath. In common with other workers he considered that there could be no doubt of the relationship of these sheaths to the chorda. v. Ebner (1896) dealing with the history of the chorda sheaths in vertebrate embryos at an advanced stage of development found that the sheaths consisted of connective tissue. He argued that the fibrous sheath is formed from the chordal epithelium and suggested that it had long before lost all direct contact with the connective tissue cells.

Claus (1894) found in a 5 mm. Acanthias embryo the smooth chorda sheath as a "strong brilliant peripheral

bounding edge which at an older stage appeared strengthened to a double-contoured brilliant sheath with all the characteristics of the elastica", (presumably the elastica externa). This he declared to be a product of the chorda for the reason that at this early stage no skeletogenous cell-sheath was obvious. Somewhat later and simultaneously with the appearance of the chorda epithelium the fibrous sheath arises below the elastica as a "layer of secretion with a very pronounced perpendicular stiffening". Up till then Klaatsch (1895) had called the elastica externa the "skeletoblastic sheath", but after investigating certain embryos of Torpedo he regarded both sheaths of the chorda, cuticular and elastic, as the primary sheath; the fibrillar lying below as the secondary sheath; this nomenclature has been increasingly adopted.

Bergfeldt (1896) observed in a young Alytes obstetricans of 4-6 vertebrae the sheath of the chorda as a "fine but definite double-contoured covering which lies closely to the chorda cells", much the same observation as that of Claus and from whose general conclusions he does not differ. Bergfeldt does add, however, that in the absence of any further cells in the vicinity of the chorda there remains only the assumption that the cells which form the membrane of the spinal cord, intestine, myotome, etc., have formed also the sheath of the chorda and this must therefore be

the common product of all three cell-layers.

Schauinsland (1903) working on Callorhynchus offers as a simple hypothesis that the elastica (externa) and the fibrous sheath immediately after, are separated from the still unvacuolated cells of the chorda. He did not bring forward any direct observations as to the manner of their origin. O. Schneider (1913) working on the histology of the chorda sheaths of cyclostomes and other fishes still derived the sheaths from the cells of the notochord. Tretiakov (1921) working on Acanthias comes to the conclusion that part of the structure of the sheaths is formed from the mesoderm. The specimen of Acanthias embryo investigated was at an advanced stage of development - 60 cms - when cartilage had already invaded the notochord to the point where the vertebral centrum was obvious. The notochord sheaths at this late stage tend to be much distorted and to render the normal course of development obscure. Conclusions, therefore, drawn from a study of such material are apt to be misleading.

From the time of appearance of Held's work (1921) subsequent work seems to have been concentrated on the later phases of the vertebral column, and interest in the earlier stages of notochord development appears to have waned; though Wenig (1929) discusses the condition of

the secondary sheath in an Ammocetes - 12 cms - he offers no observations on the probable origin since he deals only with the sheath at its maximum stage of development.

#### THE ELASTICA INTERNA

This term has been applied to a structure which is variable in extent and nature and which in some cases is apparently wanting altogether. Some confusion has arisen since Kölliker first used the name. To begin with he described it as a sheath round the notochord, in selachians and ganoids (1860) as a membrane about  $2\mu$  thick which "always" consists of "a thick network of fibres which chemically and in part also microscopically correspond to elastic fibres". In a later work (1872) he denoted this same layer as the real or inner chorda sheath and he applied the term to the actual fibrous sheath which he found to vary in different groups of animals, "thickest in teleosts, cyclostomes and ganoids where an inner (Limitans interna) and an outer (Limitans externa) specific elastic layer can be found and the middle layer assumed a fibrillar structure". In two short following works Kölliker labels this layer with two different names and in attempting to homologise the parts has caused a certain confusion.

Gegenbaur (1867) came to the conclusion that in cyclostomes, ganoids and teleosts which he investigated

no elastica interna was formed. W. Müller (1871) noted a "covering", cell-less, in the vicinity of the chorda but he does not identify it with a particular chorda sheath and it was Balfour (1878) who suggested that Müller's cuticula chordae (p. 11) may be identified with Kölliker's elastica interna. Goette, working on elasmobranchs (1878) and teleosts (1879) could not discover an elastica interna. He therefore identified Kölliker's elastica interna with what he called the cuticular sheath, but he could not recognise it as elastic in nature.

Hasse in his first work on ganoids (1883) found an elastica interna lying up against the chordal epithelium and described it as an elastic perforate membrane which he named "cuticula chordae". In a later work (1894) he denied the appearance of such a membrane and described it as "a lightly staining shadow of the inner surface of the fibrous sheath". Lwoff (1887) investigating a variety of fishes - cyclostomes, ganoids and some teleosts and also some amphibians, defined the elastica interna as a product of metamorphosis of the outer part of the chorda epithelium, "since it can in no wise be seen to be part of the chorda sheath". He, however, allowed that since it was a fine structureless cuticle Hasse's name "cuticula chordae" might be retained.

Agreeing with the conclusion that the *elastica interna* is not a separate structure and suggesting that it might therefore be disregarded is Grassi (1883) working on teleosts and likewise also Scheel (1893) working on Rhodeus. Rabl (1892) investigating Pristiurus and Scyllium mentions a "tunica propia" but does not go into any detail.

v. Ebner (1895) from his work on cyclostomes, ganoids, teleosts and amphibians, sums up his findings as follows:- "the *elastica interna* is wanting in amphibians, is not clear in cyclostomes and Acipenseridae but shows a varied development in elasmobranchs and teleosts". He concludes that this perforate elastic membrane might be a late and variable development of the chorda.

Gadow and Abbott (1895) label the fibrous sheath in cyclostomes and ganoids the "chorda" sheath; they mark the cell-less part of the fibrous sheath of elasmobranchs and also the fibrous sheath of teleosts with the expression *elastica interna*. Albrecht (1902) working on Esox and Salmo fario denies any appearance of an *elastica interna* and declares that what earlier workers have regarded as such is the inner structureless layer of the fibrous sheath. Schauinsland (1906) also agrees after gathering together all his findings that the *elastica interna* is no independent formation but a later modification of the inner layer of the fibrous sheath, in spite of the fact that there are

present in it elastic fibres. It is interesting to find that Henneguy (1907) finds an elastica interna in Acanthias and described it as "a layer of fibres crossing in all directions, staining with resorcin just like elastic fibres" but since he only finds it by means of staining he does not attach much morphological importance to it! Roth (1911) finds such a membrane in different elasmobranchs and without going into details of structure described it as from 0.5-3 $\mu$  thick and says it may be stripped off with forceps. O. Schneider (1913) investigating the histology of the chorda sheaths in cyclostomes and other fishes, finds an elastica interna existing, though developed in a variable manner, and which even in certain teleosts inserts itself into the epithelium of the chorda. Wurmbach (1932) in dealing with the cartilaginous phase of development of the vertebral column states as an accepted fact that the chorda consists of an outer elastica externa, a collaginous fibrous sheath and an elastica interna. It is clear from the illustrations that his Acanthias material is at a stage of development noticeably more advanced than the last figured stage of Lepidosiren (Fig. 33, p.53).

A SUMMARY OF PROFESSOR HELD'S CONCLUSIONS BASED ON INVESTIGATION OF CERTAIN VERTEBRATES IN HIS PAPER "UBER DIE ENTWICKLUNG DES AXENSKELETTS DER WIRBELTIERE"

In putting forward his point of view regarding the origin of the sheaths of the notochord Held (1921) gives it as the result of his research that the cell-free sheaths of the chorda in all the chief classes of vertebrates are in a similar fashion derived from the mesoderm. From an accumulation of connective tissue-like substance round the notochord and with help from the extensive outgrowths of the cells of the myotome, the endodermal axial rod is supplied with firm sheaths. The fibrillar collagenous sheath is not only the innermost but also the first appearing of all sheaths. Very soon after its appearance and within its substance the elastica externa is visible, surrounding these an outer celled sheath appears and this though spongy in texture at first, later becomes firmer due to strengthening by the cells of the mesoderm.

Held figures first a section through the tail-end of a Petromyzon embryo 16 days after fertilization and there he identifies the cells of the notochord surrounded by a dark membrane enclosing their basal parts to which he gives the name "limitans chordae propria". He finds surrounding the "limitans" a thin and not entirely homogeneous membrane

**Petromyzon Plan.**  
16 Tage.

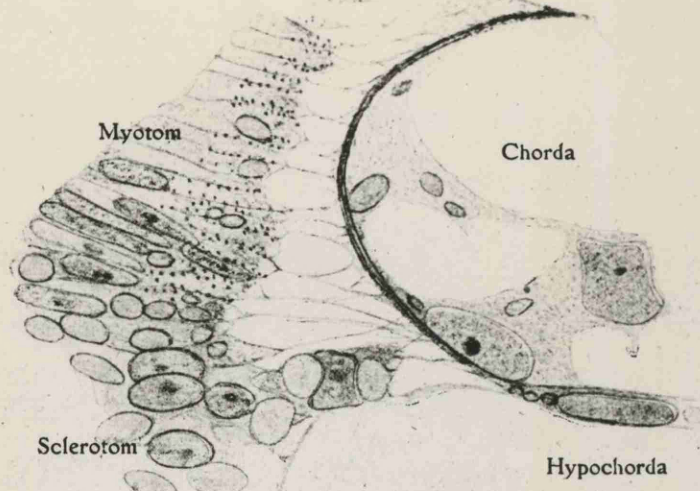


Fig. 1 H

**Acanthias vulgaris.**  
55 Urw.

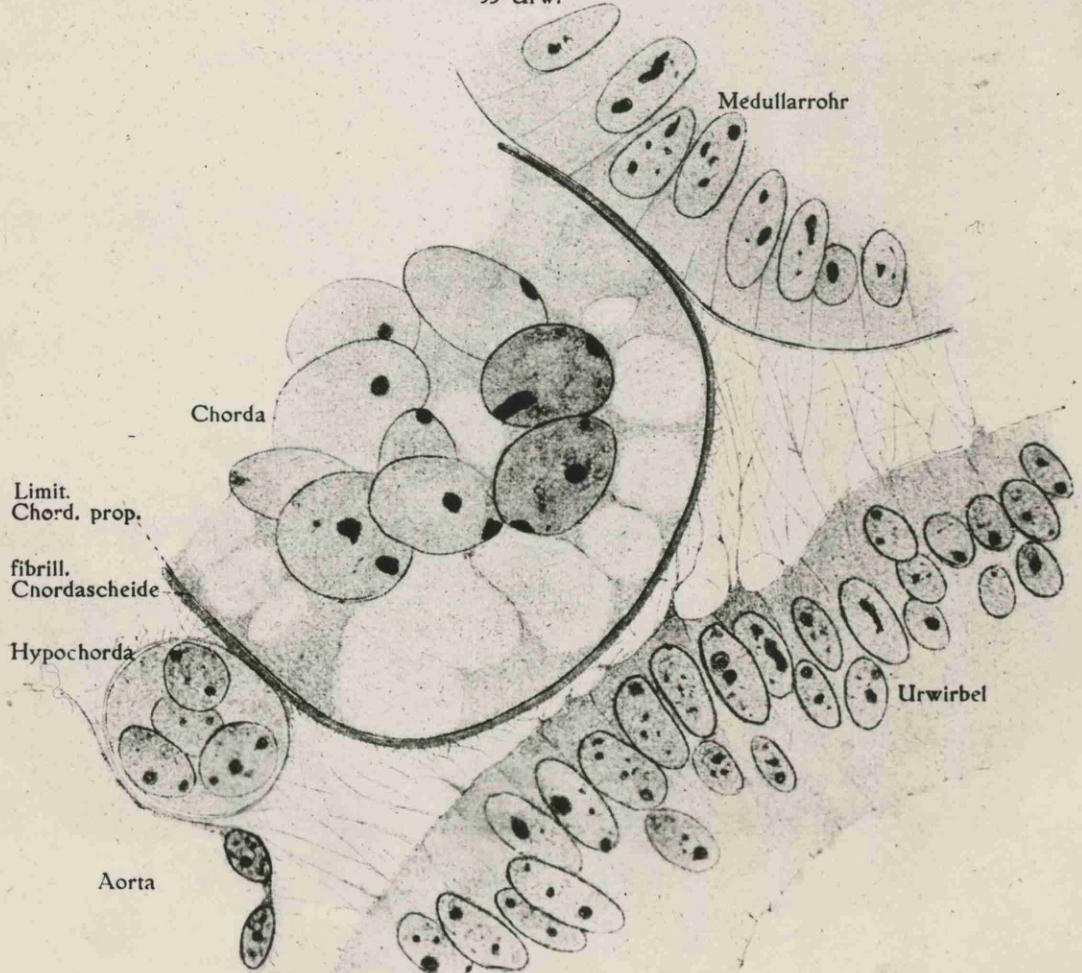


Fig. 2 H

into which penetrate the finest twig endings of the outgrowths of the cells of the opposing myotome. In spite of its appearing more homogeneous than clearly fibrous he calls the membrane the "fibrillar chorda sheath".

Though this description is made of a 13-day Petromyzon embryo the arrangement in the older specimen (Fig. 1 H) is not essentially different. He, however, does not give any indication of the appearance of the primary sheath (elastica externa) in either of the stages quoted.

In an Acanthias embryo of 7 mm. (Fig. 2 H) through the tail region the chorda is already surrounded by a thick fibrillar sheath which stains bright red with molybdenhaematoxylin and picrofuchsin. The enclosed chorda cells by contrast take on a grey colour and immediately external to the chorda cells and internal to the red-staining fibrillar sheath is a fine dark line - the limitans chordae propriae. Held has no difficulty in tracing the origin of the fibrillar sheath; the outgrowths from the myotome cells branch across the space between the myotome and the axial organs forming a network which strengthens the local thickening of "fine intermediate tissue" which closely surrounds the chorda. This intermediate tissue is in existence, according to Held, at a time when the cell layers of the body are still

undifferentiated, and it is situated between myotome and axial organs. At first, though being in the nature of a protoplasmic network it is replaced later by a fibrillar network and Held considers it is derived from the mesoderm.

The actual bodies of the myotome cells stain greyish with the molybdenhaematoxylin but their finest outgrowths pick up the red picrofuchsin. Held accounts for this contrast by the fact that the cytoplasm of the cell-body is granular, the outgrowth becomes finer, loses its granular nature becoming homogeneous and thread-like and showing only reaction to picrofuchsin. This reaction to picrofuchsin is characteristic also of collagenous tissue.

Testing Acanthias material of the same stage of development (7 mm.) with other stains Held considers that the substance or the sheath and the fibrils contained in it are collagenous in nature. By means of appropriate stains he then detects in the collagenous tissue the first beginnings of elastic material which he considers the incipient elastica externa (= primary sheath).

At another part of the body of an Acanthias at the same stage of development he finds only collagenous material surrounding the other axial organs and concludes that elastic tissue - and therefore the elastica externa - is

**Acanthias vulgaris.**

15 mm. Schwanz.

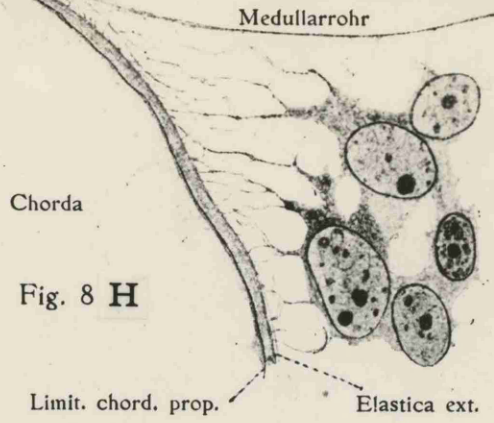


Fig. 8 H

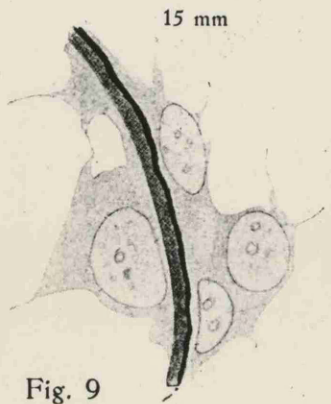


Fig. 9

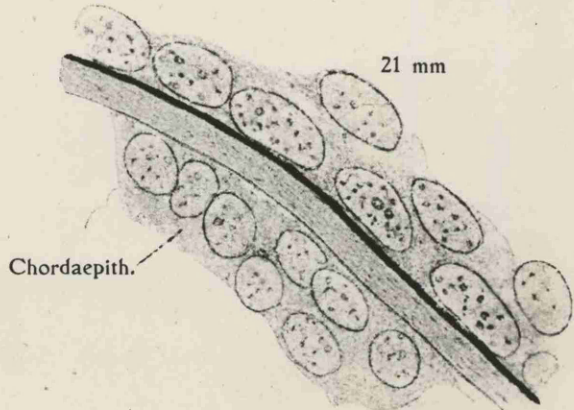


Fig. 10 H

**Acanthias vulgaris.**

40 mm. Schwanz.

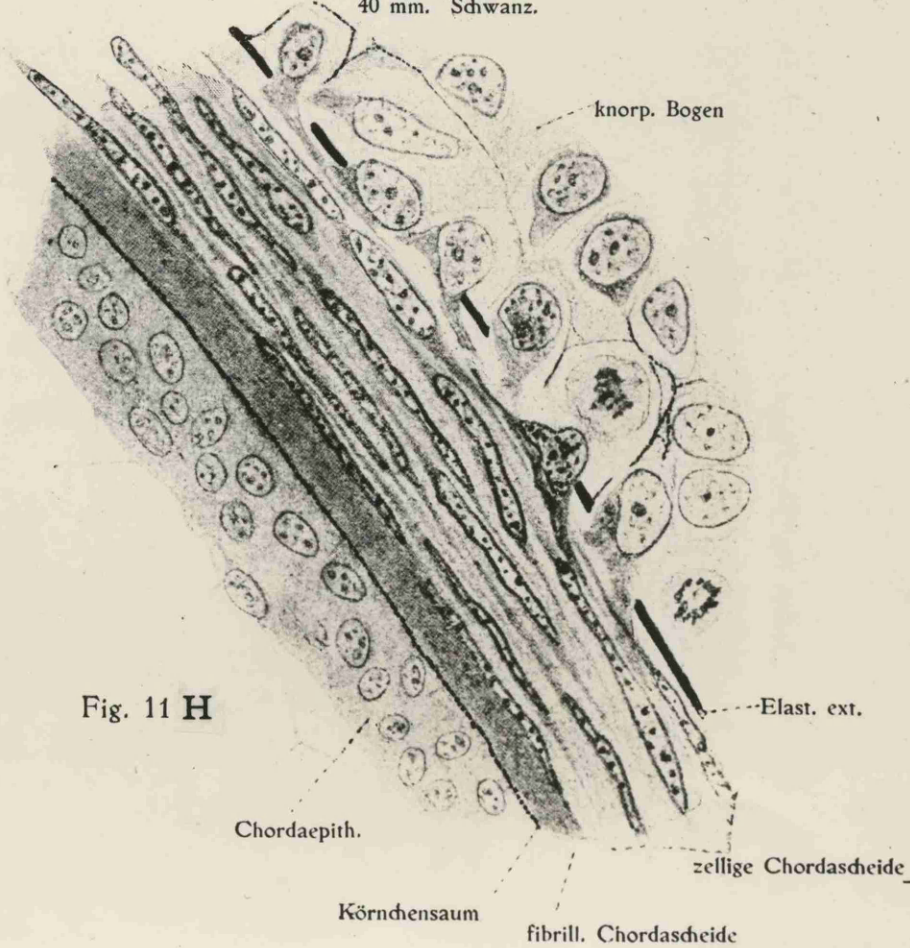


Fig. 11 H

differentiated later than the collagenous fibrous sheath. In an Acanthias at a still later stage (15 mm.) Held figures a distinct strong "limitans chordae propria" (Fig. 8 H) (Limit. chord. pro.) in conspicuous contrast to the thin feeble elastica externa (Elastica ext.). The next stage figured, a 21 mm. Acanthias (Fig. 10 H), shows the elastica externa as a much more definite element and the "limitans" though present is not even labelled. In the 40 mm. Acanthias (Fig. 11 H) Held labels as "Körnchensaum" the row of granules now occupying the position presumably of the "limitans".

Held then proceeds to view critically the work of certain investigators already mentioned (pp. 11 et seq.). He points out that Kölliker has not demonstrated with any direct observations his contention that the notochordal cells secrete the structureless sheaths. He criticises Gegenbaur's assertion that the inner fibrillar sheath is formed as the result of gradual depositions from the peripheral cells of the notochord, though Gegenbaur cites numerous examples among the invertebrates of this ability to form cuticular material, the result of substance passed out from the bodies of the cells. Held considers that there is here a fundamental error in Gegenbaur's reasoning since all cuticular formations are exclusively the product of a layer of free cells; and the circumference of the

notochordal epithelium covered by its sheaths corresponds embryologically with the basal side. Held dismisses on grounds of lack of evidence, Müller's suggestion (1871) that the sheath is derived from certain connective tissue cells from the periphery of the primitive aorta. Claus quoting work done on Acanthias (1894) finds both elastica externa and fibrillar sheath are the result of the activity of the notochordal cells. Held criticises this finding on account of failure to produce accurate analysis of the alleged secretion process; neglect to notice the rôle of the connective tissue; inadequate illustration of the work and then he finds an error in a histological detail, namely that in the fibrillar sheath what Claus describes as fibrils, are, in fact, rows of granules; and the whole sheath fails to show up as a product separate from the chorda. Then, in spite of extensive researches on Petromyzon from an early stage - 7.5 mm up to 20 cms. - v. Ebner (1895) does not succeed according to Held in producing convincing proof for his assertion that the notochord cells may function in such a way as to construct connective tissue.

Two other investigators may be mentioned as having made some attempt to demonstrate the power of the notochordal cells to produce the secretion which might ultimately form the sheaths. The first, Ussow (1900) working on Gasterosteus, detects "segmental swellings in the fibrous sheath which

coincide precisely with the corresponding swellings of the chordal epithelium". Further he mentions, "also there appear 'drops of clear fluid' in the chordal epithelium which discharge and together with the still-homogeneous fibrous sheath flow in a layer to the cuticula chordae". The second, O. Schneider (1913), finds appearing in the chordal epithelium, vacuoles with characteristic inclusions which become applied to what he calls the elastica interna; these are thickly packed and take on resorcin-fuchsin stain similar to the fibrous sheath. Since these inclusions dissolve in caustic potash Schneider contends that these are not elastic but mucin-containing substances.

Held considers that Ussow's findings might be confirmed to the extent that the chordal epithelium produces a characteristic secretion from the vacuoles: that such material forms the chorda sheaths Held is not prepared to allow. He dismisses as not affecting the main issue the fact that Schneider found fibres of the elastica interna penetrating the chordal epithelium. Held then criticises, as a result of the discussion on the literature, the basis on which the Kölliker-Gegenbaur theory rests. The first series of investigations were upon the notochord as a structure isolated from considerations of the rest of the embryo body, the second assumes that as soon as its outer

surface appears the entire sheath is homogeneous and later on will be built up. He agrees that cell-less chorda sheaths are produced and resemble genuine cuticular formations, this formation, however, has originated not from the inside outwards but out of the accumulation of connective tissue and of the fibrillae supplied by the mesodermal epithelial cells.

DESCRIPTION OF THE DEVELOPMENT OF THE NOTOCHORDAL  
SHEATHS IN EMBRYOS OF LEPIDOSIREN FROM  
STAGE 12 TO STAGE 36

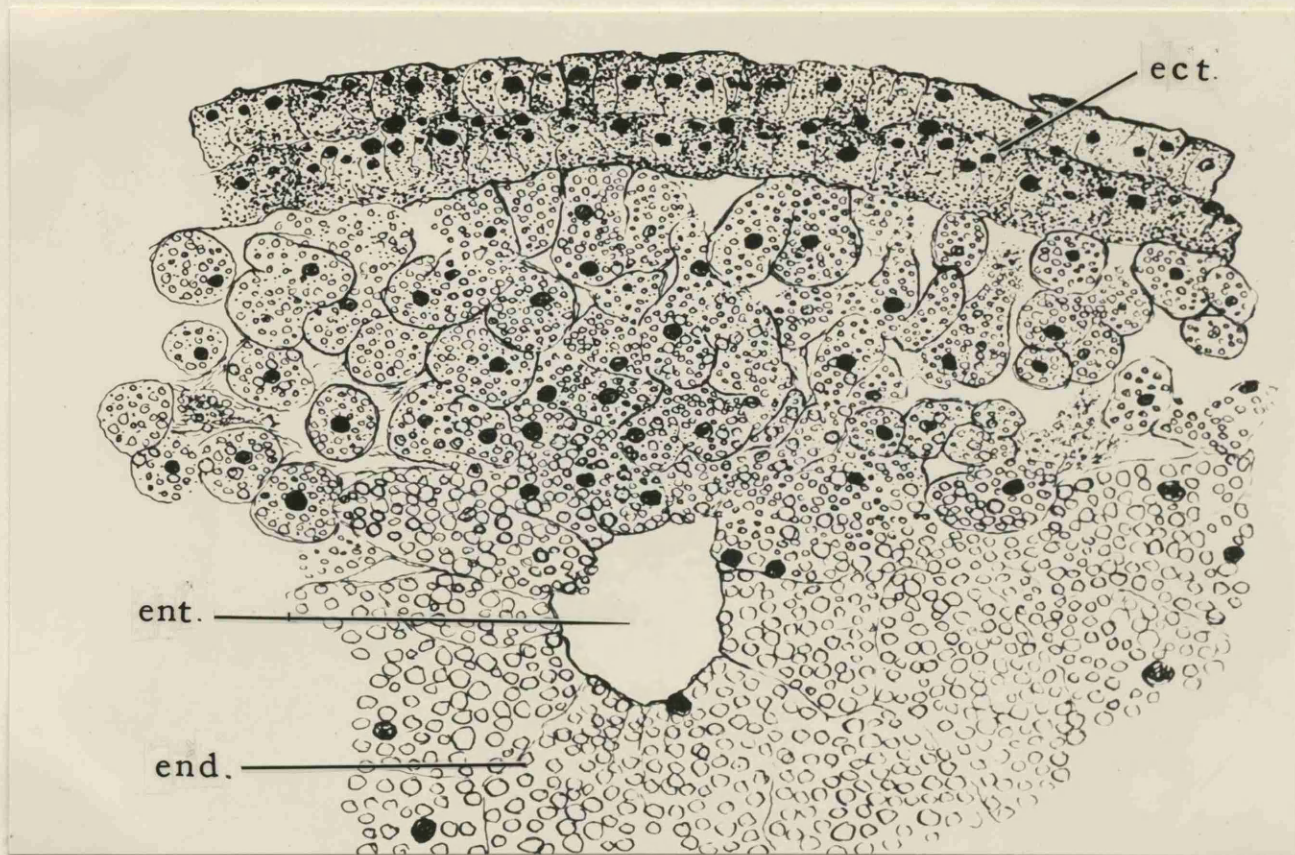


Fig. 1. Lepidosiren embryo, stage 12. T.S. through anterior part of body. ect. ectoderm; end. endoderm; ent. archenteron.

( Obj. 2/3rds. Oc. K6 - 7x. )

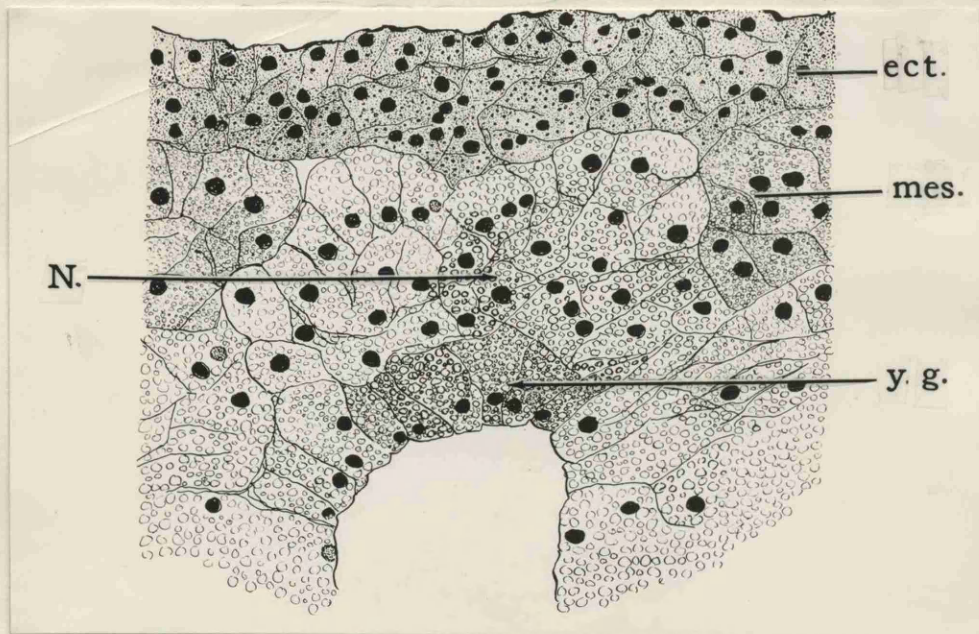


Fig. 2. Lepidosiren embryo, stage 14. T.S. N. notochord; ect. ecto-  
:derm; mes. mesoderm; y.g. yolk granules.

( Obj. 1\_th. Oc. K6-7x. )

## DESCRIPTION OF DRAWINGS

In a section through a developing embryo of Lepidosiren at stage 12 (Fig. 1) transverse to the long axis of the body, the ectoderm of the medullary plate region is well defined (ect.). The cavity of the archenteron (ent.) is surrounded on either side and ventrally by endoderm cells (end.) - their cytoplasm composed of yolk granules of large size, the boundaries of most of the cells being still incomplete and with an occasional nucleus showing here and there.

Dorsal to the archenteron and ventral to the ectoderm lies a mass of cells closely packed together in the middle region but loosening out on either side where spaces develop between the cells in consequence of which they become rounded. This mass of cells comprises the rudiments of the mesoderm and notochord and has manifestly arisen from the endoderm. The noticeable feature is that due to more active cell division the yolk granules in their cytoplasm are smaller than those of the definitive endoderm. Certain of these cells in the mid-ventral part of the mass have become fitted together to form the roof of the archenteron.

At a slightly later stage - stage 14 - of an embryo

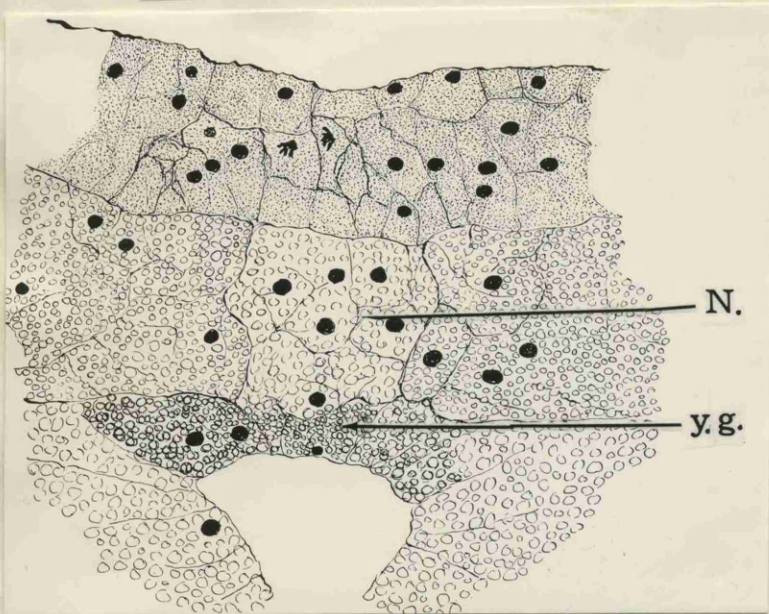


Figure 3. c. stage 15. N. notochord;  
y.g. yolk granules.

( Obj. 1/6th. Oc. K6 -7x. )

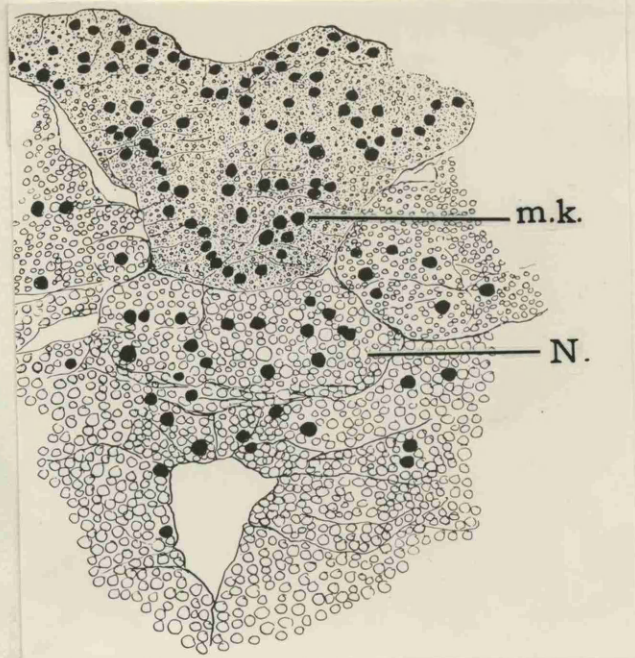


Fig. 4. c. stage 16.

m.k. medullary keel; N. notochord.

( Obj. 2/3rds. Oc. K4-5x. )

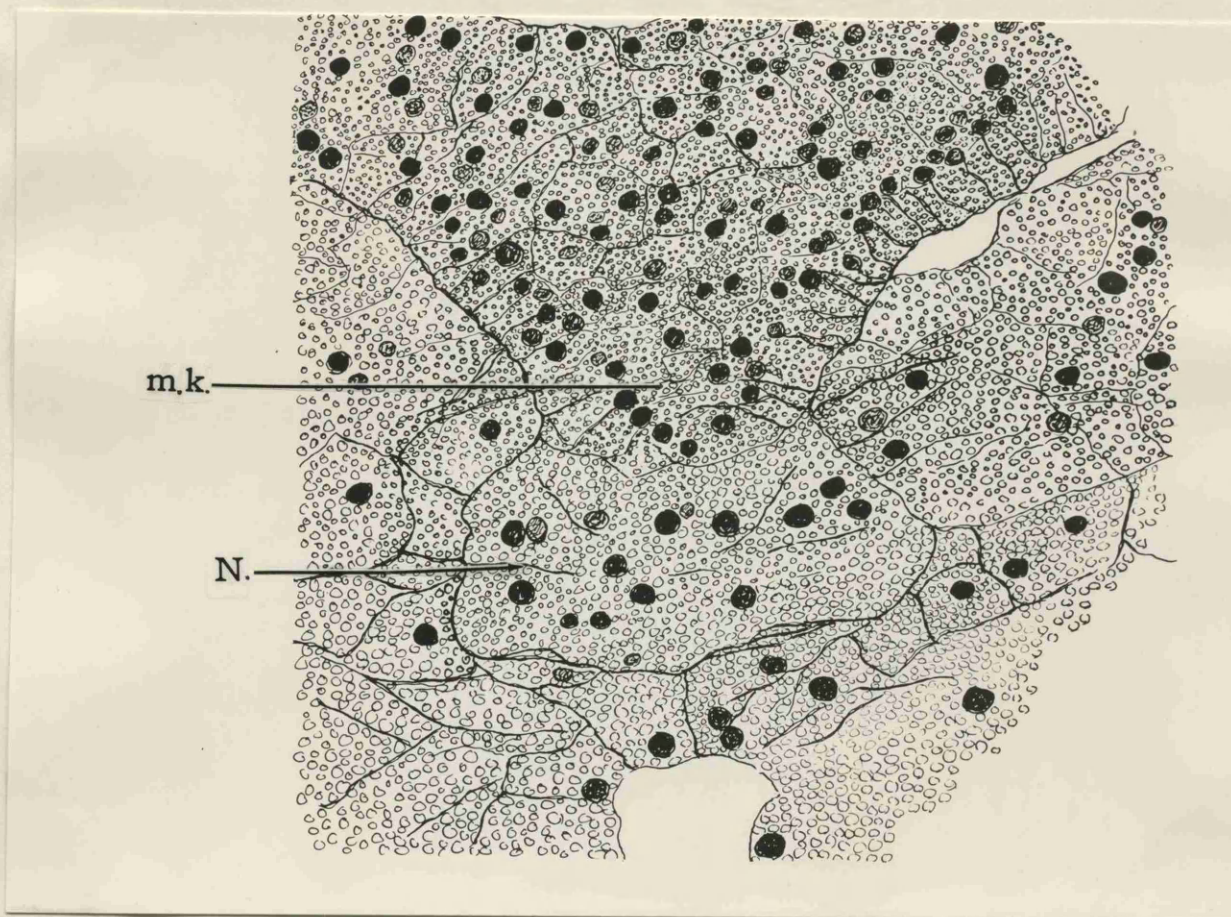


Figure 5. Enlarged drawing of Figure 4.

( Obj. 1/6th. Oc. K6 - 7x. )

51.

(Fig. 2) the mesoderm (mes.) is now clearly marked off from the notochord rudiment (N.) and from the endoderm ventrally in this region. The dorsal limit of the notochord rudiment is marked by the contrast between its cells and the finely granular appearance of the ectoderm cells (ect.). Ventri- laterally there is as yet no separation from the ordinary endoderm cells. Forming the roof of the archenteron are cells which show cytoplasm much more heavily stained (y.g.) so that they stand out conspicuously - their dorsal boundaries indicating the mid-ventral limit of the notochord.

The notochord (Fig. 3 N.) in another specimen slightly older - stage 15 - appears more compact than in the last embryo. Both of these sections (Figs 2 and 3) are cut at the same part, midway down the length of the body. In each specimen the cells of the archenteric roof show a similar appearance of heavily stained yolk granules (Figs 2 and 3 y.g.) and in an examination through the entire series of each embryo almost all sections show this peculiarity. Certain notochordal cells (Fig. 2 N.) have a similar appearance though not so strongly marked.

The most noticeable advance in an embryo at stage 16 is the increase in depth of the medullary keel (Fig. 4 m.k.) towards the anterior end of the body. The notochord by now, though isolated ventri-laterally from the endoderm, is not yet completely marked off from the enteric roof cells.

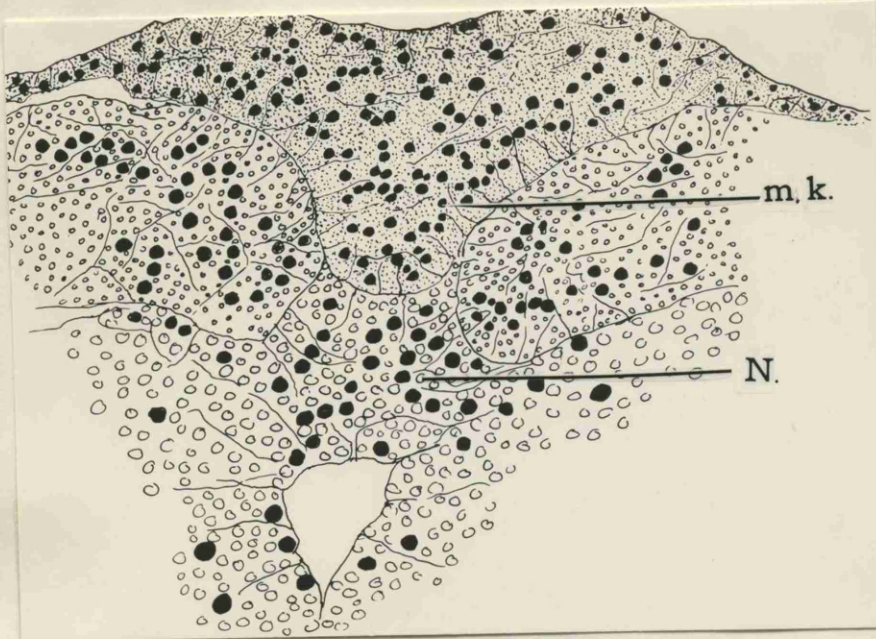


Fig. 6. T.S. through the same embryo as Figs. 4 & 5 but further back from the head. N. area of the notochord; m.k. medullary keel.

(Obj. 2/3rds. Oc. K6 - 7x.)

Figure 7. Enlarged drawing of Fig. 6.

( Obj. 1/6th. Oc.

K6 - 7x.)

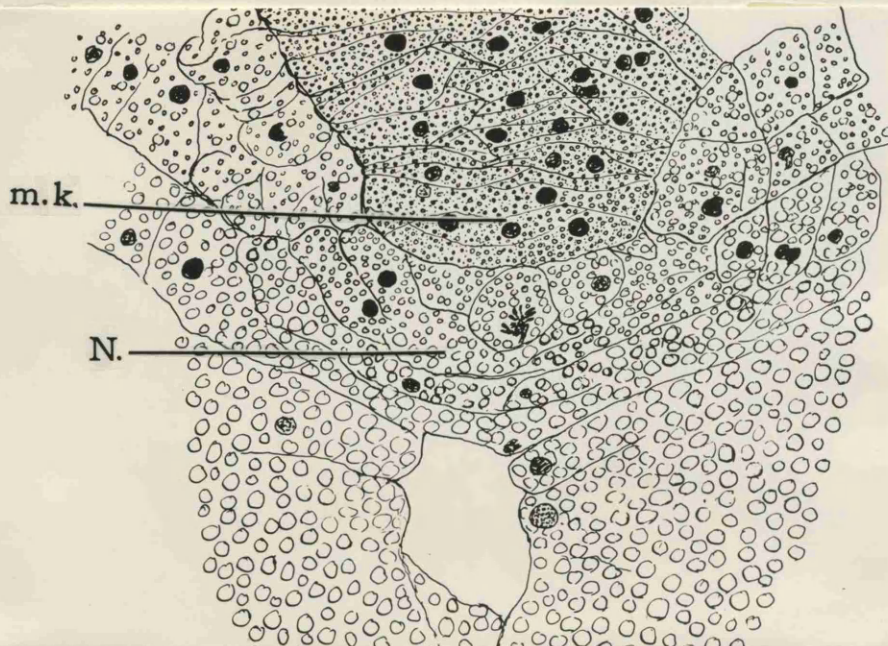
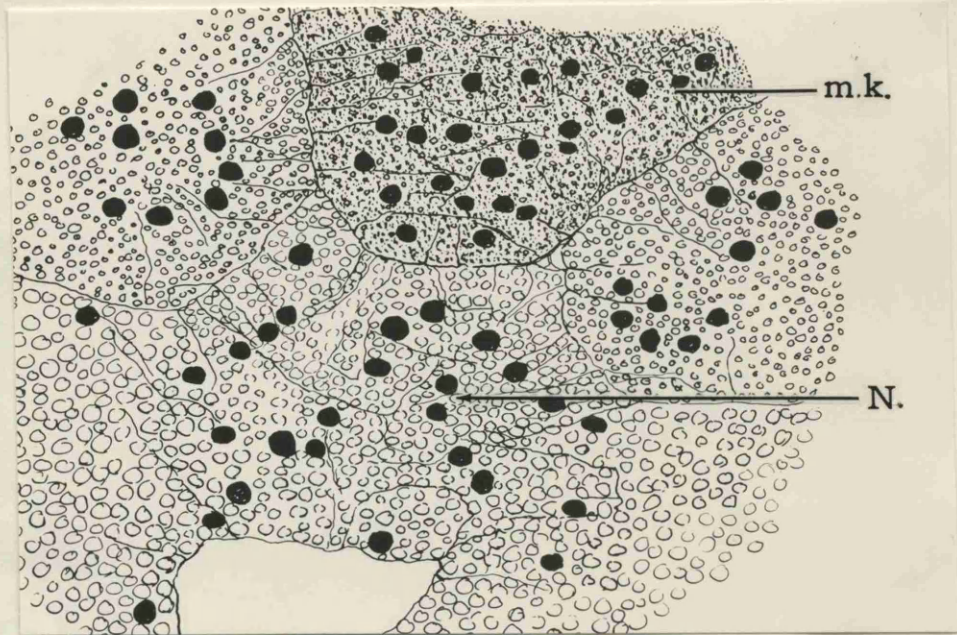


Figure 8. stage 17.

N. notochord; m.k. medullary keel.

( Obj. 1/6th. Oc.

K6 - 7x.)

Even with a high power (Fig. 5) the ventral portion of the thin boundary of the notochordal rudiment is seen to be discontinuous. A second section through the same embryo (Fig. 6) about 0.3 mm. behind that shown in figure 4 reveals the area of the notochord (N.) diffuse and lacking any clear boundary. Even when the section was examined under a higher power there was no obvious sign of any limiting line, though the neighbouring structures are normal and clearly defined (Fig. 7).

This failure might not have been so surprising had it occurred nearer the tail region where the notochordal cells and those of the endoderm tend to merge into one another ventrally. Certain notes made regarding the aspect of the specimen before sectioning disclosed that though most of the organs appeared normal there was a fading of the medullary folds towards the tail and also a similar fading at the head end of the body. The notochord in the anterior region (Fig. 5 N.) is normal, so it is not apparent that there is any connexion between these two peculiarities. The phenomenon does however demonstrate the irregularities of development in early stages.

Up till now the notochord has shown little sign of active development but in a section through an embryo of stage 17 (Fig. 8) a change has come over the yolk granules of the notochordal cells which are now appreciably smaller and more



Fig. 9. c. stage 21. T.S. through notochord now enclosed by continuous primary sheath. p.sh. primary sheath; ent. endoderm cells.

( Obj. 2/3rds. Oc. K8 - 10x. )

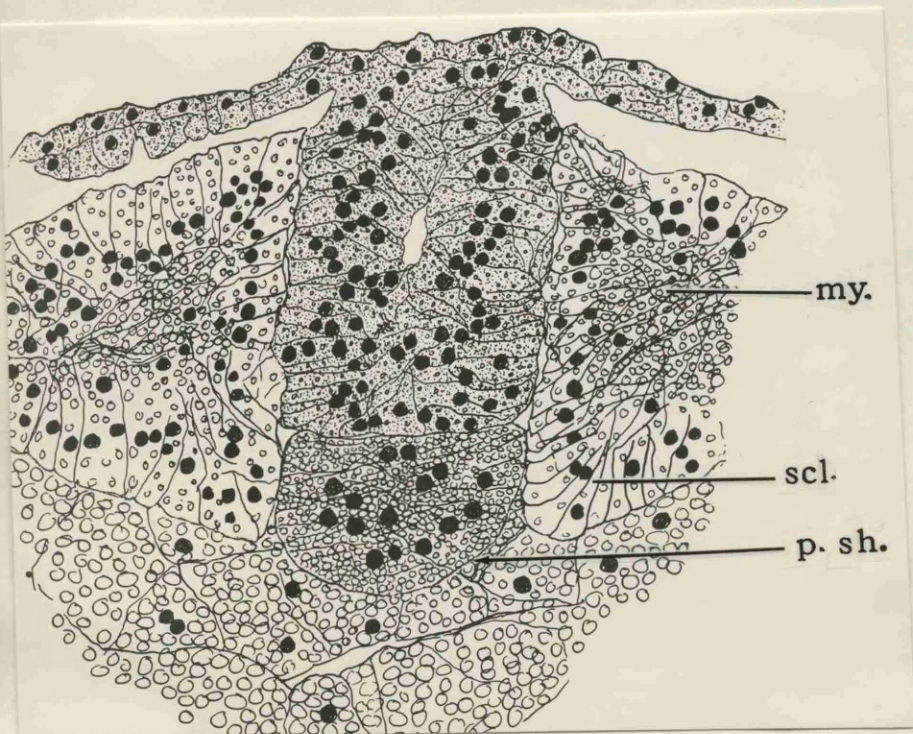


Fig. 10. Another embryo at stage 21. my. myotome; p.sh. primary sheath; scl. sclerotome.

( Obj. 1/6th. Oc. K6 - 7x. )

numerous, in conspicuous contrast to those of the cells of the enteric roof. The presence of these smaller yolk granules in the cytoplasm suggests more active cell division and therefore a more rapid development. The bounding membrane surrounding the notochord is still however incomplete ventrally. A feature of this section is the nucleus of one of the notochordal cells displaying the chromosomes in preparation for mitosis.

Between stage 17 and the next stage figured - about stage 21 - the notochord displays no progress towards isolation from the roof cells of the enteron but now for the first time (Fig. 9) a clear bounding sheath, identifiable as the primary sheath, (p.sh.) surrounds the notochord completely. The enteric roof cells also show growth since they are now two-layered and their granules are slightly smaller than those of the definitive endoderm cells (end.).

In a transverse section of another specimen at the same stage of development (Fig. 10) through the notochord, the primary sheath (p.sh.) is continuous. This section passes through the myotome (my.) on either side and on the ventral face of each may be detected the beginning of the sclerotome (scl.).

It is interesting to note that according to Graham Kerr's "Normal Plates" on the development of Lepidosiren and Protopterus, the primary sheath makes its appearance at

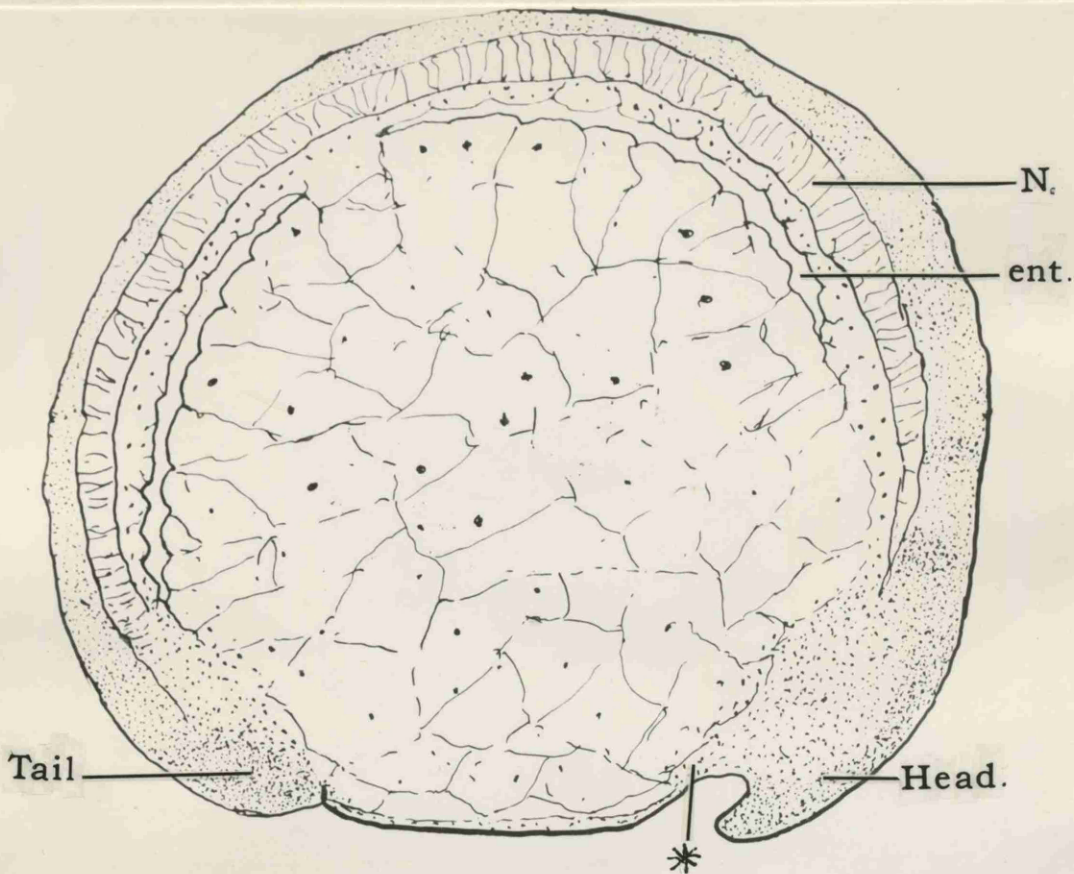


Figure 11. Sagittal section through an embryo at stage 23.

N. notochord; ent. archenteron; \* position of foregut.

( Obj. 2<sup>f</sup> Zeiss. Oc. K6-7x. )

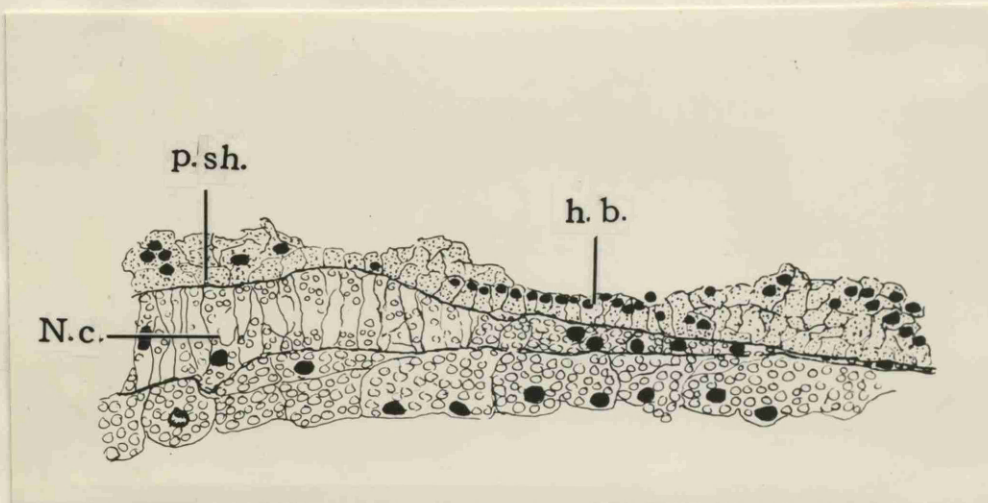


Fig. 12. Reconstruction of anterior end of notochord of the same embryo as Fig. 11. h.b. hind brain; p.sh.

primary sheath; N.c. notochord cells. (Obj. 1/6th. Oc. K8-10x. )

stage 23. Here, however, is evidence from these two Lepidosiren embryos that the primary sheath is already established by stage 21.

A median sagittal section (Fig. 11) through the body of an embryo shows the development and extent of the notochord at stage 23. The notochordal cells show the elongated form typical of this stage of development\* and there is a well-marked primary sheath enclosing it. While this section is median for the notochord it is not so for the nervous system which consists of a series of casual spaces which have not been represented in detail; similarly the small space indicating the appearance of the fore-gut is not obvious but is indicated (\*).

In an attempt to ascertain its anterior limit a reconstruction of the head end of the notochord was made. There is an appearance (Fig. 12) of a sheath continuing anterior to the region of the floor of the hind brain (h.b.) and enclosing cells with none of the characteristics of typical notochord cells (N.c.) but containing large yolk granules resembling those of endoderm cells. Further forward the "sheath" narrows to such an extent that cells are no longer

---

\* Though not shown in the drawing the nuclei of the notochordal cells conform to the arrangement in other stages (p. 62) and are concentrated about the long axis.

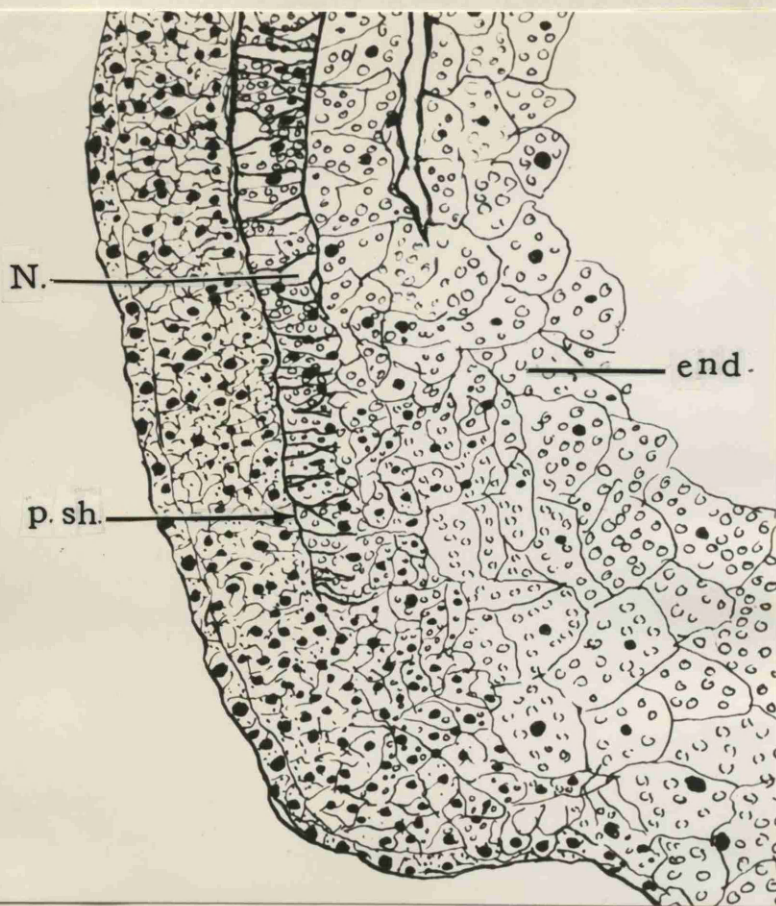


Fig. 13. Tail end of same embryo as Figs. 11 & 12. end. endoderm; N. notochord; p. sh. primary sheath.

( Obj.  $1\frac{1}{2}$ " Watson. Oc. K8-10x. )

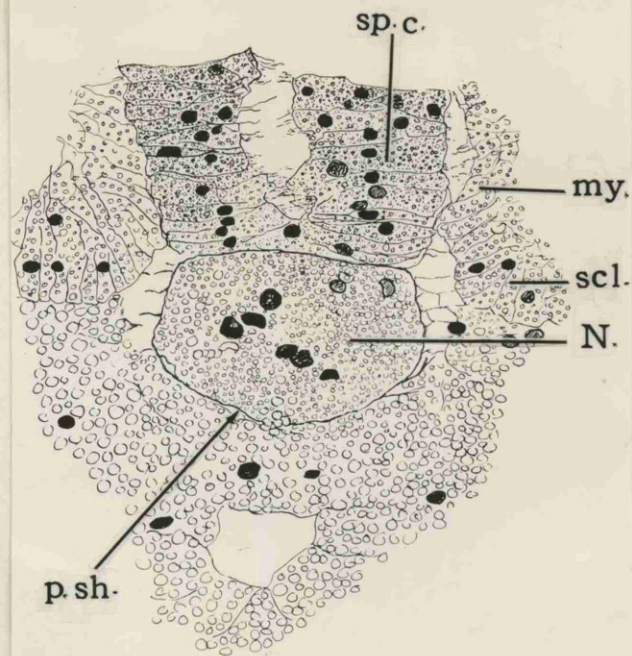


Fig. 14. T.S. through an embryo at stage c. 23. N. notochord; p. sh. primary sheath; my. myotome; scl. sclerotome; sp. c. spinal cord.

( Obj.  $1/6$ th. Oc. K6-7x. )

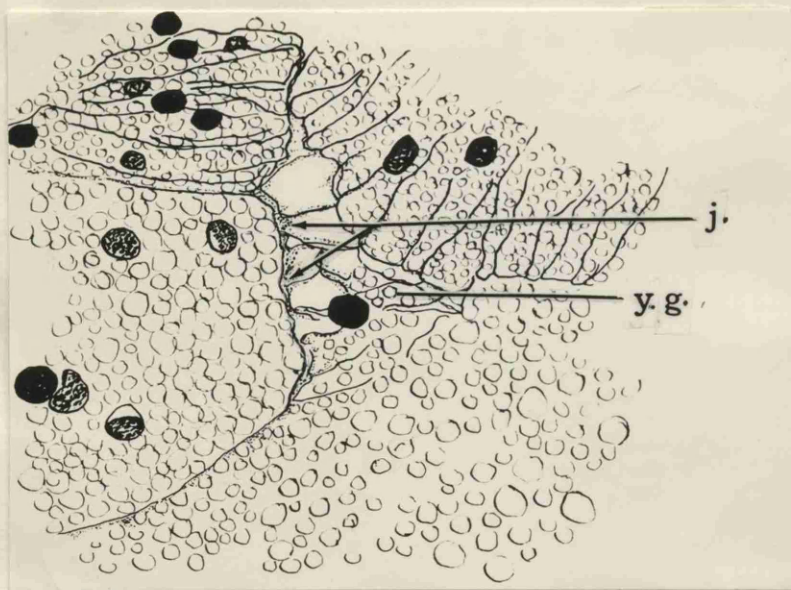


Fig. 15. Part of Fig. 14 with higher magnification.

( Obj.  $1/6$ th. Oc. K8-10x. )

recognisable and only here and there is a nucleus visible. An exactly similar appearance is characteristic of Protopterus at this stage.

A drawing of the notochord at the tail end of the same specimen (Fig. 13) shows the primary sheath (p.sh.) continuing dorsally for some distance beyond the point at which ventrally it has come to an end. As far, however, as the primary sheath is present so far the cells show the typical elongated shape of notochordal cells. The slower growth in the tail region of the ventral part of the primary sheath has a parallel in the course of development in earlier stages (v. Figs 4 and 8).

In a transverse section through another embryo of the same stage - stage 23 - the notochord is seen (Fig. 14) to be completely enclosed by the primary sheath which appears more definite than any of the other bounding membranes. At this stage also the myotomes and developing sclerotomes are evident. On the right hand of the section between the developing sclerotome and the primary sheath (p.sh.) is a reticulum of fine strands. The reticulum on this side is continuous and is shown in more detail under a higher power (Fig. 15) these strands are few and only occasionally reach the primary sheath. Yolk granules (y.g.) obviously from the endoderm cells are to be seen occupying some of the spaces of the reticulum, while among the meshes there appear shreds of structureless jelly (j.) which take on a faint

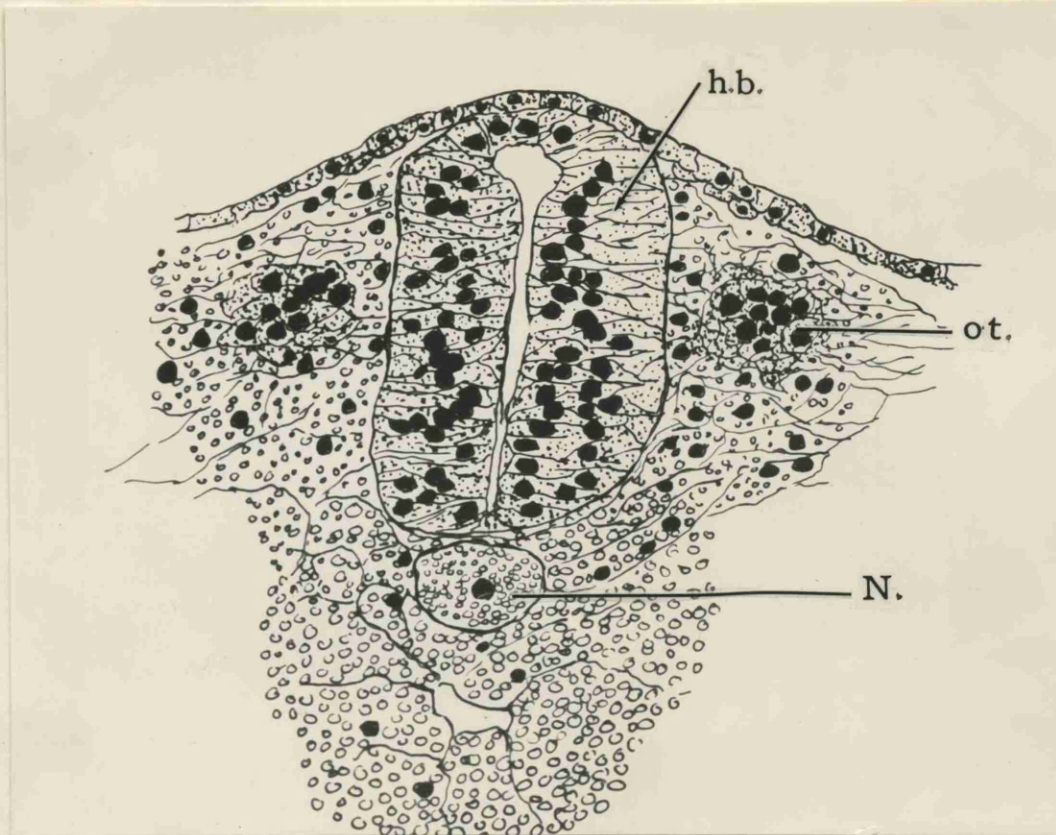


Figure 16. Embryo at stage 24. T.S. through hind brain region and anterior end of notochord. h.b. hind brain; N. notochord; ot. hind wall of otocyst. ( Obj. 2/3rds. Oc. K6 - 7x.)

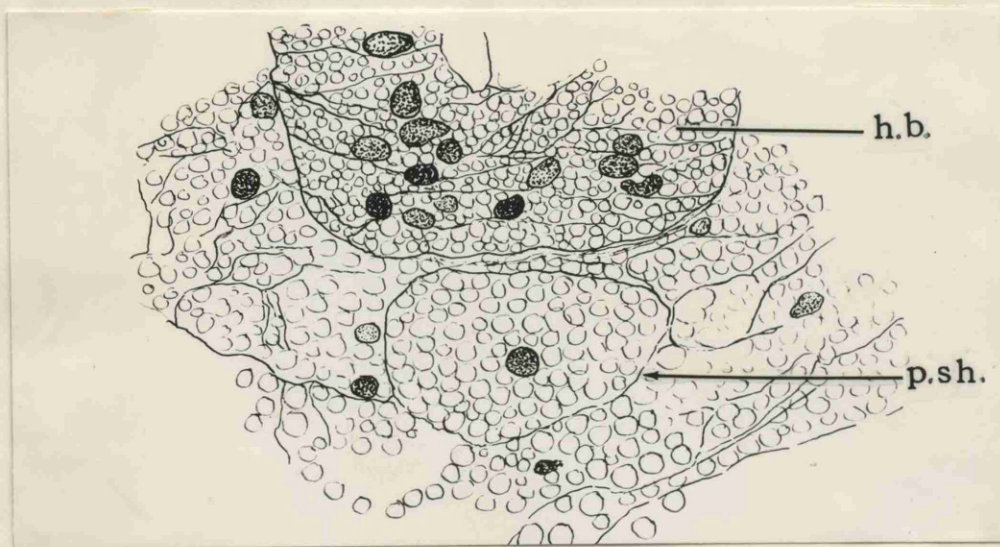


Figure 17. Anterior end of notochord (Fig.16) magnified.

h.b. hind brain; p.sh. primary sheath.

( Obj. 1/6th. Oc. K8 - 10x)

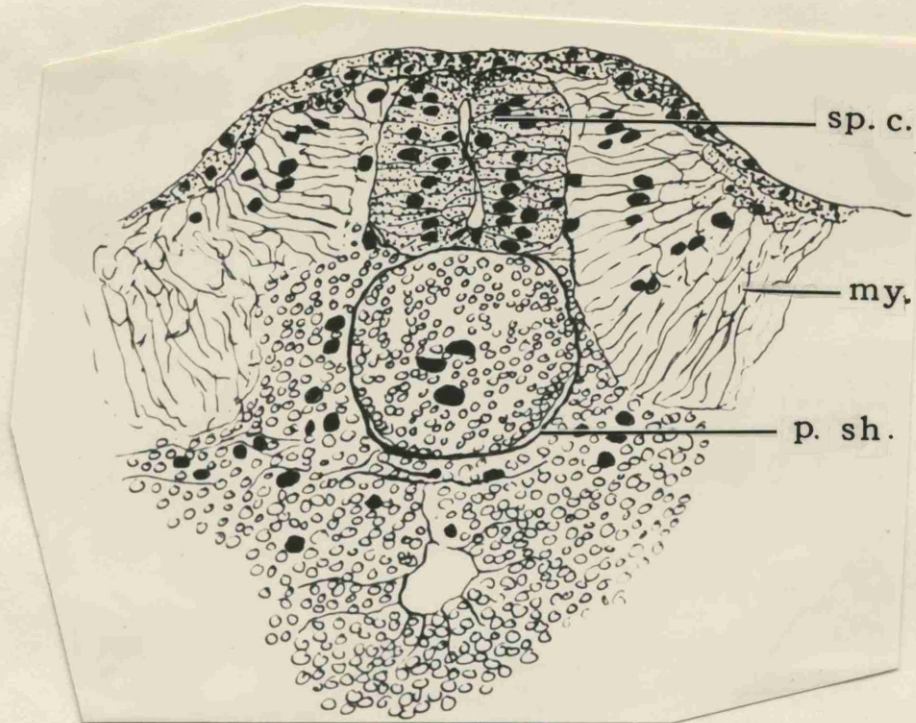


Fig. 18. Section through the same embryo as Fig. 16 but further back in the body. my. myotome; p. sh. primary sheath; sp. c. spinal cord. ( Obj. 2/3rds. Oc. K6 - 7x. )

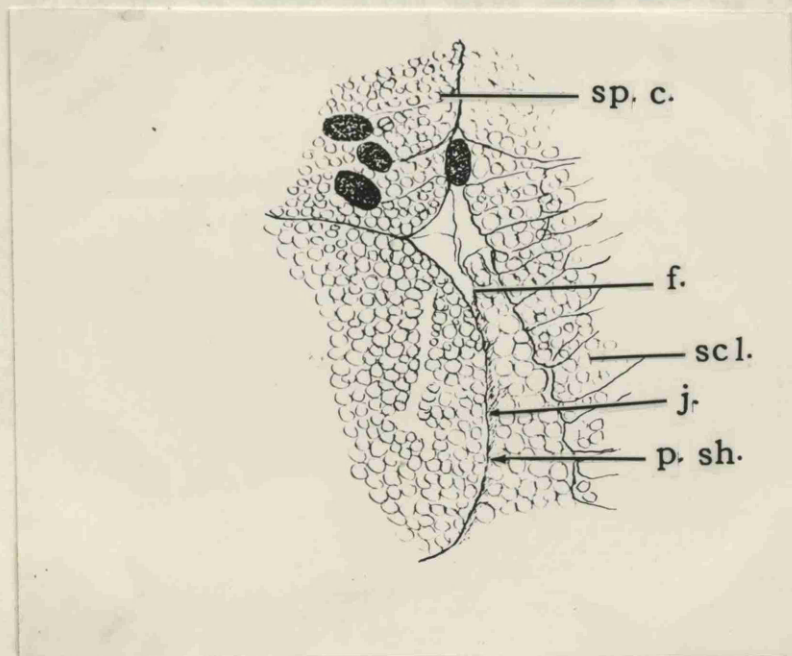


Figure 19. Part of section of Fig. 18 showing myotome and portion of notochord on the right side. f. fibre; j. jelly; scl. sclerotome. ( Obj. 1/6th. Oc. K8 - 10x. )

stain.

Figure 16 shows a section through the anterior end of the notochord of an embryo three days before hatching - stage 24. There are visible on either side of the hind brain (h.b.) the posterior walls of the otocysts (ot.). No clear evidence of notochord is to be found anterior to this section so presumably it is still in a state of more or less active growth forwards towards the position it will ultimately occupy close against the wall of the infundibulum. As will be seen from the drawing under higher power (Fig. 17) the thin primary sheath (p.sh.) is continuous enclosing few cells, the yolk granules of which show little difference in size from those of the surrounding endoderm cells.

A section at the same stage of development taken at one-third of the length of the body from the head shows the notochord (Fig. 18) at its maximum size for this stage. Posterior to this section it gradually diminishes though even at the point where it merges into the endoderm in the tail region, it is still twice as large as it is at the anterior end (v. Fig. 16).

In an enlarged drawing (Fig. 19) of part of Figure 18 between the primary sheath and cells of the sclerotome (scl.) the space is filled mostly with yolk granules manifestly of endodermal origin. There is, however, a clear space dorsal to these granules which is traversed

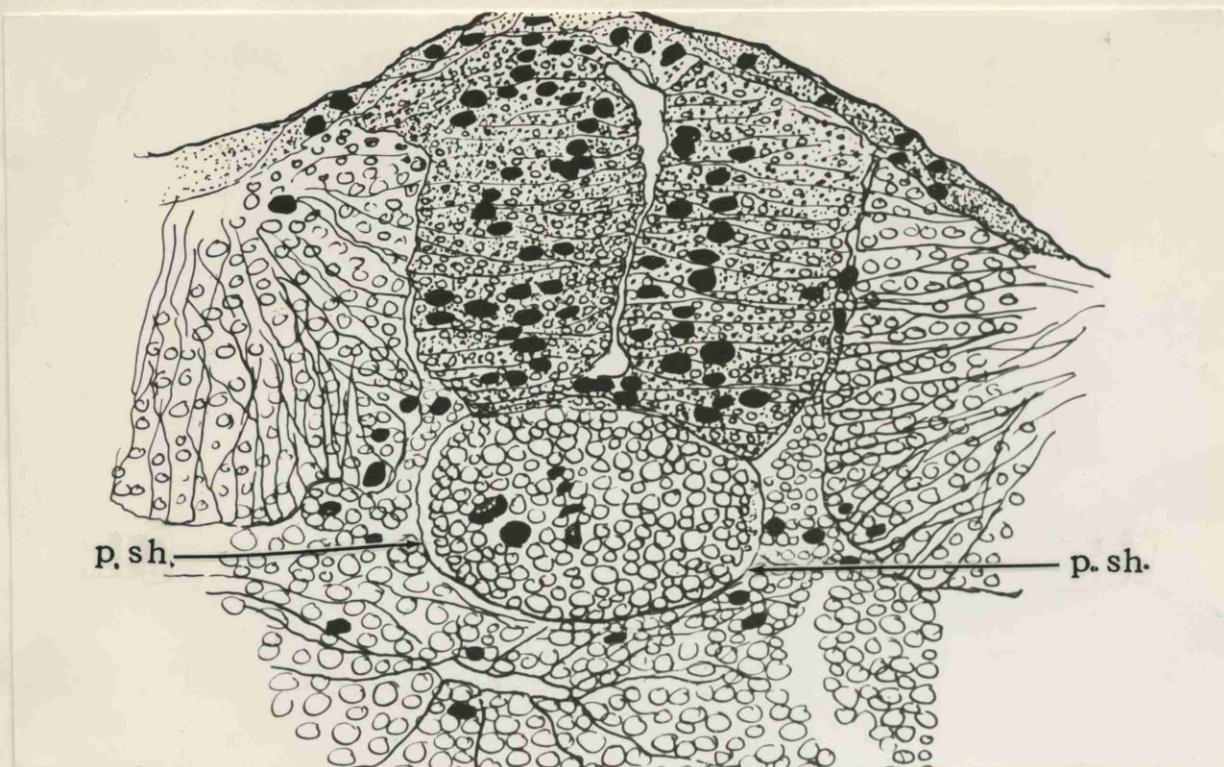


Figure 20. T.S. through the same embryo as Fig. 16. p.sh. primary sheath. ( Obj. 2/3rds. Oc. K8 - 10x.)

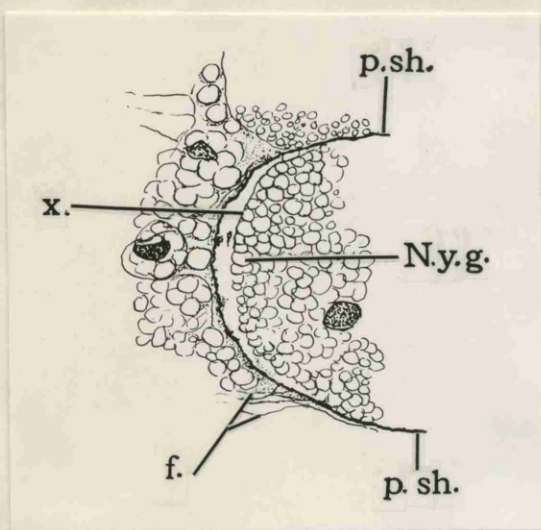


Fig. 21. Left side of Fig. 20  
N.y.g. yolk granules of notochord cells; f. fibres; x. inner "membrane".

( Obj. 1/6th. Oc. K8 - 10x.)

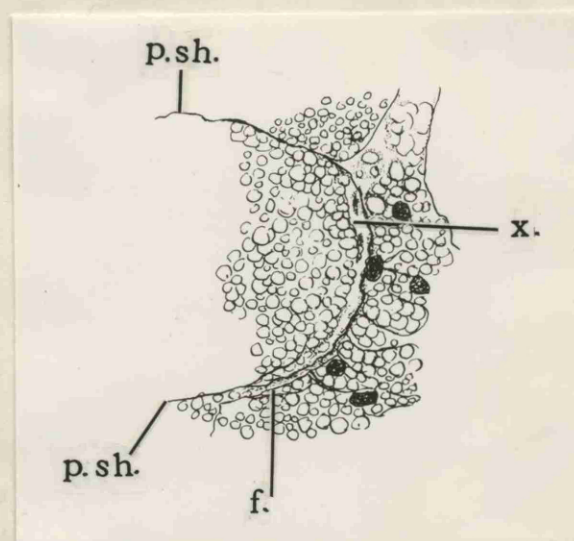


Fig. 22. Right side of Fig. 20.  
p.sh. primary sheath; x. inner "membrane"; f. fibres.

( Obj. 1/6th. Oc. K8 - 10x.)

by thin fibres. These are very few and none seem to approach the primary sheath but stretch rather towards the outer layer of the spinal cord; one fibre (f.) at first directed towards the sheath turns and runs parallel in a ventral direction and disappears in the structureless jelly (j.) coating the outside of the primary sheath.

Equidistant between these two last sections (Figs 16 and 18) lies one which seems to exhibit features of interest. In a low power drawing (Fig. 20) the primary sheath is slightly separated on each side (p.sh. and p. sh.) from the actual cells of the notochord. In an enlarged drawing (Fig. 21) of the left side of Figure 20 there appears to be an inner fine membrane (x.) bounding the yolk granules of the notochord cells (N. y.g.) and between it and the primary sheath exists a more or less clear space. This membrane (x.) does not continue and the free edge from the point where it ceases is carried on towards the ventral side by the boundaries of the yolk granules of the notochord cells themselves as far as the gap lasts. On the right side (Fig. 22) of the notochord in Figure 20 there also occurs a fine membrane (x.) but here it is fainter and extends for a shorter distance than that on the left side. The edge formed by the yolk granules of the notochord cells is less regular so that the space between yolk granules and primary sheath is not so clear. In both sections (Figs 21 and 22)

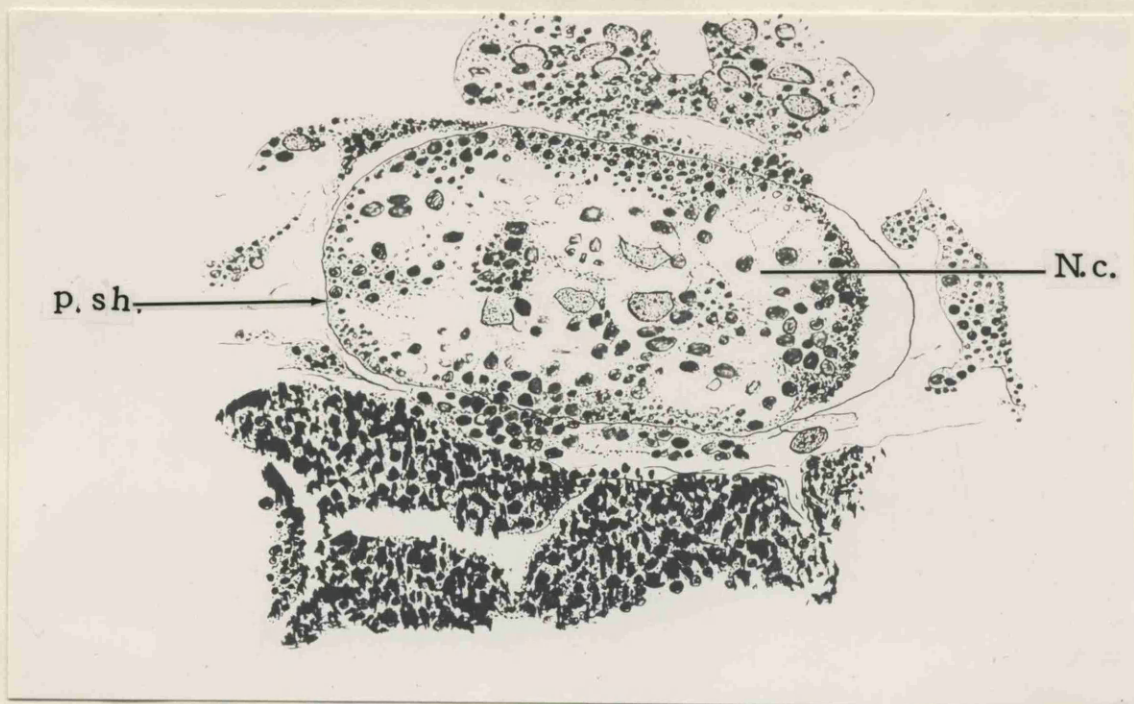


Fig. 23 T.S. through an embryo at stage 27, just before hatching.

N.c. Notochord cells; p.sh. primary sheath.

( Obj. 1/6th. Oc. K6 - 7x. )

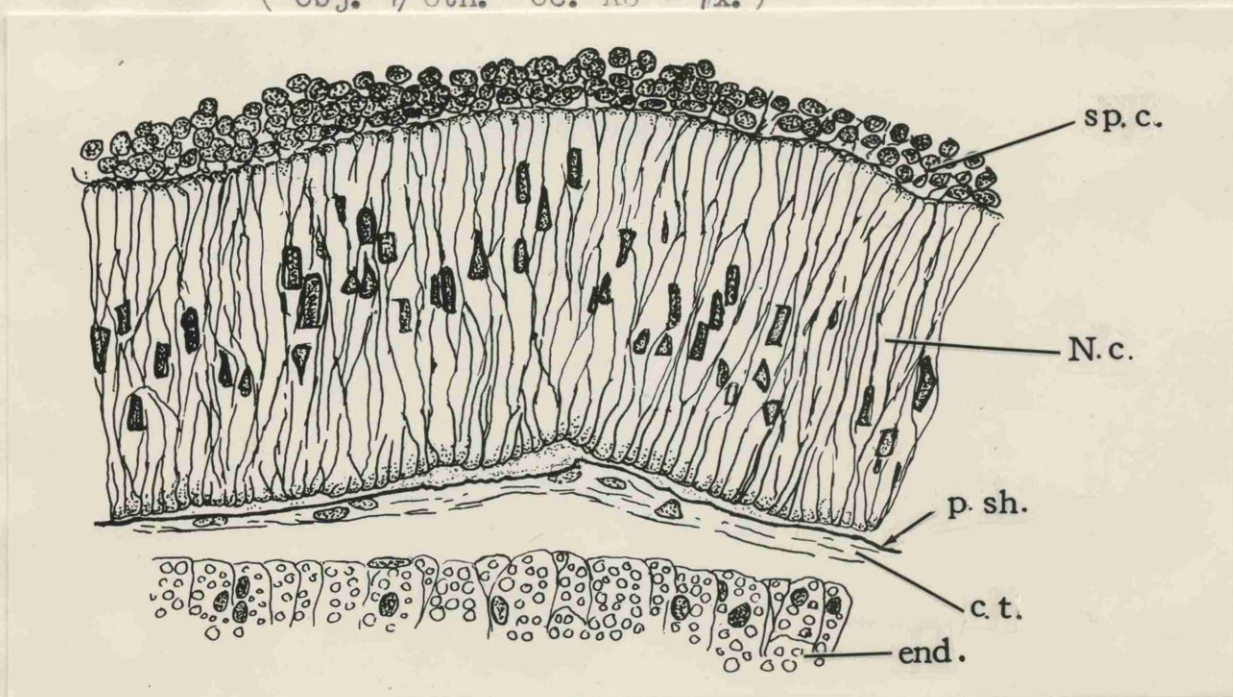


Fig. 24. Sagittal section through another embryo of same stage as

Fig. 23. end. endoderm cells; N.c. Notochord cells; c.t. connective tissue; p.sh. primary sheath; sp.c. spinal cord.

( Obj. 1/6th. Oc. K8 -10x. )

are a few fibres (f.) external to the primary sheath, particularly ventrally, and some of these seem to terminate in the sheath, others appear to run parallel with the sheath.

An embryo of stage 27, just before hatching, shows the notochord greatly increased in volume (Fig. 23) compared with stage 24. The continuity of the primary sheath (p.sh.) may be clearly traced even in those areas where yolk granules congregate thickly. These sections were counterstained with eosin and immediately internal to the primary sheath there occurs a faint border of pink stain; at the point on the right of the section where the primary sheath is separated from the notochord cells the narrow strip of stain continues along the edge of the notochord cells (N.c.).

In an attempt to find out if there might be some cause for the appearance of such a band of stain in that particular part, another embryo (Fig. 24) at the same stage - stage 27 - and sectioned sagittally, was inspected. As a result there seemed to be two points worth noting, the first, that the cytoplasm of the cells (N.c.) which appears more than usually granular, seems to be concentrated at their outer extremities and there are also present very fine granules scattered between the limits of the notochord cells and the primary sheath; the second point, the connective tissue (c.t.) surrounding the primary sheath and more easily seen here than in a thin transverse section, is remarkable for its sparseness and the fewness of its cells.

and in all likelihood for the shark-like elasmobranchs in general. Comparison with the Ceratodus fin revealed that the axis of the fin in Acanthias had increased enormously in thickness especially towards the basal end. It was possible to demonstrate however that in a fossil shark, Pleuracanthus, a similar thickening was beginning to show in the same region, though the fossil still showed the axis fully segmented - a segmentation which is largely lost in Acanthias. The result of this investigation has been to produce evidence suggesting that the pectoral fin of the actively swimming sharks has passed through a phase of skeletal evolution from the primitive archipterygium represented today by the pectoral fin skeleton of Ceratodus.