II. *On the Development of the Spinal Nerves in Elasmobranch Fishes.* *By* F. M. Balfour, *B.A., Fellow of Trinity College, Cambridge. Communicated by Dr.* Michael Foster, *F.R.S., Prælector in Physiology and Fellow of Trinity College, Cambridge.*

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In the course of an inquiry into the development of Elasmobranch Fishes, my attention has recently been specially directed to the first appearance and early stages of the spinal nerves, and I have been led to results which differ so materially from those of former investigators that I venture at once to lay them before the Society. I have employed in my investigations embryos of *Scyllium canicula*, *Scyllium stellare*, *Pristiurus*, and *Torpedo*. The embryos of the latter animal, especially those hardened in osmic acid, have proved by far the most favourable for my purpose, though, as will be seen from the sequel, I have been able to confirm the majority of my conclusions on embryos of all the above-mentioned genera.

A great part of my work was done at the Zoological Station founded by Dr. Dohrn at Naples; and I have to thank both Dr. Dohrn and Dr. Eisig for the uniformly obliging manner in which they have met my requirements for investigation. I have more recently been able to fill up a number of lacunæ in my observations by the study of embryos bred in the Brighton Aquarium; for these I am indebted to the liberality of Mr. Lee and the Directors of that institution.

The first appearance of the Spinal Nerves in Pristiurus.

In a *Pristiurus*-embryo, at the time when two visceral clefts become visible from the exterior (though there are as yet no openings from without into the throat), a transverse section through the dorsal region exhibits the following features (Plate 16. fig. A):—

The external epiblast is formed of a single row of flattened elongated cells. Vertically above the neural canal the cells of this layer are more columnar and form the rudiment of the primitively continuous dorsal fin.

The neural canal (*nc*) is elliptical in section, and its walls are composed of oval cells two or three deep. The wall at the two sides is slightly thicker than at the ventral and dorsal ends, and the cells at the two ends are also smaller than elsewhere. A typical cell from the side walls of the canal is about $\frac{1}{1900}$ inch in its longest diameter. The outlines of the cells are for the most part distinctly marked in the specimens hardened in either chromic or picric acid, but more difficult to see in those prepared with osmic acid; their protoplasm is clear, and in the interior of each is an oval nucleus very large in proportion to the size of its cell. The long diameter of a typical nucleus is about $\frac{1}{3000}$ inch, or about two thirds of that of the cell.

The nuclei are granular, and very often contain several especially large and deeply stained granules; in other cases only one such is present, which may then be called a nucleolus.

In sections there may be seen round the exterior of the neural tube a distinct hyaline membrane: this becomes stained of a brown colour with osmic acid, and purple or red with hæmatoxylin or carmine respectively. Whether it is to be looked upon as a distinct membrane differentiated from the outermost portion of the protoplasm of the cells, or as a layer of albumen coagulated by the reagents applied, I am unable to decide for certain. It makes its appearance at a very early period, long before that now being considered; and similar membranes are present around other organs as well as the neural tube. The membrane is at this stage perfectly continuous round the whole exterior of the neural tube *as well on the dorsal surface as on the ventral.*

The section figured, whose features I am describing, belongs to the middle of the dorsal region. Anteriorly to this point the spinal cord becomes more elliptical in section, and the spinal canal more lanceolate; posteriorly, on the other hand, the spinal canal and tube become more nearly circular in section. Immediately beneath the neural tube is situated the notochord (*ch*). It exhibits at this stage a central area rich in protoplasm, and a peripheral layer very poor in protoplasm; externally it is invested by a distinct cuticular membrane.

Beneath the notochord is a peculiar rod of cells, constricted from the top of the alimentary canal*. On each side and below this are the two aortæ, just commencing to be formed, and ventral to these is the alimentary canal.

On each side of the body two muscle-plates are situated; their upper ends reach about one third of the way up the sides of the neural tube. The two layers which together constitute the muscle-plates are at this stage perfectly continuous with the somatic and splanchnic layers of the mesoblast, and the space between the two layers is continuous with the body-cavity. In addition to the muscle-plates and their ventral continuations, there are no other mesoblast-cells to be seen. The absence of all mesoblastic cells dorsal to the superior extremities of the muscles is deserving of special notice.

Very shortly after this period and, as a rule, before a third visceral cleft has become visible, the first traces of the spinal nerves make their appearance.

First Stage.—The spinal nerves do not appear at the same time along the whole length of the spinal canal, but are formed first of all in the neck and subsequently at successive points posterior to this.

Their mode of formation will be most easily understood by referring to Plate 16. figs. B I, B II, B III, which are representations of three sections taken from the same embryo. B I is from the region of the heart; B II belongs to a part of the body posterior to this, and B III to a still posterior region.

* *Vide* BALFOUR, "Preliminary account of the Development of Elasmobranch Fishes," Quart. Journ. of Microsc. Science, Oct. 1874, p. 33.

In most points the sections scarcely differ from Plate 16. fig. A, which, indeed, might very well be a posterior section of the embryo to which these three sections belong.

The chief point, in addition to the formation of the spinal nerves, which shows the greater age of the embryo from which the sections were taken is the complete formation of the aortæ.

The upper ends of the muscle-plates have grown no further round the neural canal than in fig. A, and no scattered mesoblastic connective-tissue cells are visible.

In fig. A the dorsal surface of the neural canal was as completely rounded off as the ventral surface; but in fig. B III this has ceased to be the case. The cells at the dorsal surface of the neural canal have become rounder and smaller and begun to proliferate, and the uniform outline of the neural canal has here become broken (fig. B III, *pr*). The peculiar membrane completely surrounding the canal in fig. A now terminates just below the point where the proliferation of cells is taking place.

The prominence of cells which springs in this way from the top of the neural canal is the commencing rudiment of a pair of spinal nerves. In fig. B II, a section anterior to fig. B III, this formation has advanced much further (fig. B II, *pr*). From the extreme top of the neural canal there have now grown out two club-shaped masses of cells one on each side; they are perfectly continuous with the cells which form the extreme top of the neural canal, and necessarily also are in contact with each other dorsally. Each grows outwards in contact with the walls of the neural canal; but, except at the point where they take their origin, they are not continuous with its walls, and are perfectly well separated by a sharp line from them.

In fig. B I, though the club-shaped processes still retain their attachment to the summit of the neural canal, they have become much longer and more conspicuous.

Specimens hardened in both chromic acid (Plate 16. fig. C) and picric acid give similar appearances as to the formation of these bodies.

In those hardened in osmic acid, though the mutual relations of the masses of cells are very clear, yet it is difficult to distinguish the outlines of the individual cells.

In the chromic-acid specimens (fig. C) the cells of these rudiments appear rounded, and each of them contains a large nucleus.

I have been unable to prepare longitudinal sections of this stage, either horizontal or vertical, to show satisfactorily the extreme summit of the spinal cord; but I would call attention to the fact that the cells forming the proximal portion of the outgrowth are seen in every transverse section at this stage, and therefore exist the whole way along, whereas the *distal* portion is seen only in every third or fourth section, according to the thickness of the sections. It may be concluded from this that there appears a continuous outgrowth from the spinal canal, from which discontinuous processes grow out.

In specimens of a very much later period (Plate 18. fig. L) the proximal portions of the outgrowth are unquestionably continuous with each other, though their actual junctions with the spinal cord are very limited in extent. The fact of this continuity at a later period is strongly in favour of the view that the posterior branches of the spinal nerves arise from the first as a continuous outgrowth of the spinal cord, from which a series of distal processes take their origin. I have, however, failed to demonstrate this point absolutely. The processes, which we may call the nerve-rudiments, are, as appears from the later stages, equal in number to the muscle-plates.

It may be pointed out, as must have been gathered from the description above, that the nerve-rudiments have at this stage but one point of attachment to the spinal cord, and that this one corresponds with the dorsal or posterior root of the adult nerve.

The rudiments are, in fact, those of the posterior root only.

The next or second stage in the formation of these structures to which I would call attention occurs at about the time when three to five visceral clefts are present. The disappearance from the notochord in the anterior extremity of the body of a special central area rich in protoplasm serves as an excellent guide to the commencement of this epoch.

Its investigation is beset with far greater difficulties than the previous one. This is owing partly to the fact that a number of connective-tissue cells, which are only with great difficulty to be distinguished from the cells which compose the spinal nerves, make their appearance around the latter, and partly to the fact that the attachment of the spinal nerves to the neural canal becomes much smaller, and therefore more difficult to study.

Fortunately, however, in *Torpedo* these peculiar features are not present to nearly the same extent as in *Pristiurus* and *Scyllium*.

The connective-tissue cells, though they appear earlier in *Torpedo* than in the two other genera, are much less densely packed, and the large attachment of the nerves to the neural canal is retained for a longer period.

Under these circumstances I consider it better, before proceeding with this stage, to give a description of the occurrences in *Torpedo*, and after that to return to the history of the nerves in the genera *Pristiurus* and *Scyllium*.

The development of the Spinal Nerves in Torpedo.

The youngest *Torpedo*-embryo in which I have found traces of the spinal nerves belongs to the earliest part of what I called the second stage.

The segmental duct* is just appearing, but the cells of the notochord have not become completely vacuolated. The rudiments of the spinal nerves extend half of the way towards the ventral side of the spinal cord; they grow out in a most distinct manner from the dorsal surface of the spinal cord (Plate 16. fig. D a, *pr*); but the nerve-rudiments of the two sides are no longer continuous with each other at the dorsal median line, as in the earlier *Pristiurus*-embryos. The cells forming the proximal portion of the rudiment have the same elongated form as the cells of the spinal cord, but the remaining cells are more circular.

* *Vide* BALFOUR, "Origin and History of Urino-genital Organs of Vertebrates," Journal of Anatomy and Physiology, Oct. 1875.

From the summit of the muscle-plates (*mp*) an outgrowth of connective-tissue has made its appearance (*c*), which eventually fills up the space between the dorsal surface of the cord and the external epiblast. There is not the slightest difficulty in distinguishing the connective-tissue cells from the nerve-rudiment. I believe that in this embryo the origin of the nerves from the neural canal was a continuous one, though naturally the peripheral ends of the nerve-rudiments were separate from each other.

The most interesting feature of the stage is the commencing formation of the anterior roots. Each of these arises (Plate 16. fig. D a, *ar*) as a small but distinct outgrowth from the epiblast of the spinal cord, near the ventral corner of which it appears as a conical projection. Even from the very first it has an indistinct form of termination and a fibrous appearance, while the protoplasm of which it is composed becomes very attenuated towards its termination.

The points of origin of the anterior roots from the spinal cords are separated from each other by considerable intervals. In this fact, and also in the nerves of the two sides never being united with each other in the ventral median line, the anterior roots exhibit a marked contrast to the posterior.

There exists, then, in *Torpedo*-embryos by the end of this stage distinct rudiments of both the anterior and posterior roots of the spinal nerves. These rudiments are at first quite independent of and disconnected with each other, and both take their rise as outgrowths of the epiblast of the neural canal.

The next *Torpedo*-embryo (Plate 16. fig. D b), though taken from the same female, is somewhat older than the one last described. The cells of the notochord are considerably vacuolated; but the segmental duct is still without a lumen. The posterior nerve-rudiments are elongated, pear-shaped bodies of considerable size, and, growing in a ventral direction, have reached a point nearly opposite the base of the neural canal. They still remain attached to the top of the neural canal, though the connexion has in each case become a pedicle so narrow that it can only be observed with great difficulty.

It is fairly certain that by this stage each posterior nerve-rudiment has its own separate and independent junction with the spinal cord; their dorsal extremities are nevertheless probably connected with each other by a continuous commissure.

The cells composing the rudiments are still round, and have, in fact, undergone no important modifications since the last stage.

The important feature of the section figured (fig. D b), and one which it shares with the other sections of the same embryo, is the appearance of connective-tissue cells around the nerve-rudiment. These cells arise from two sources; one of these is supplied by the vertebral rudiments, which at the end of the last stage (Plate 16. fig. C, *vr*) become split off from the inner layer of the muscle-plates. The vertebral rudiments have in fact commenced to grow up on each side of the neural canal, in order to form the mass of cells out of which the neural arches are subsequently developed.

The dorsal extremities of the muscle-plates form the second source of these connective-tissue cells. These latter cells lie dorsal and external to the nerve-rudiments.

The presence of this connective-tissue, in addition to the nerve-rudiments, removes the possibility of erroneous interpretations in the previous stages of the *Pristiurus*-embryo.

It might be urged that the two masses which I have called nerve-rudiments are nothing else than mesoblastic connective-tissue commencing to develop around the neural canal, and that the appearance of attachment to the neural canal which they present is due to bad preparation or imperfect observation. The sections of both this and the last *Torpedo*-embryo which I have been describing clearly prove that this is not the case. We have, in fact, in the same sections the developing connective-tissue as well as the nerve-rudiments, and at a time when the latter still retains its primitive attachment to the neural canal. The anterior root (fig. D b, *ar*) is still a distinct conical prominence, but somewhat larger than in the previously described embryo; it is composed of several cells, and the cells of the spinal cord in its neighbourhood converge towards its point of origin.

In a *Torpedo*-embryo (Plate 16. fig. D c) somewhat older than the one last described, though again derived from the oviduct of the same female, both the anterior and the posterior rudiments have made considerable steps in development.

In sections taken from the hinder part of the body I found that the posterior rudiments nearly agreed in size with those in fig. D b.

It is, however, still less easy than there to trace the junction of the posterior rudiments with the spinal cord, and the upper ends of the rudiments of the two sides do not nearly meet.

In a considerable series of sections I failed to find any case in which I could be absolutely certain that a junction between the nerve and the spinal cord was effected; and it is possible that in course of the change of position which this junction undergoes there may be for a short period a break of continuity between the nerve and the cord. This, however, I do not think probable. But if it takes place at all, it takes place before the nerve becomes functionally active, and so cannot be looked upon as possessing any physiological significance.

The rudiment of the posterior nerve in the hinder portion of the body is still approximately homogeneous, and no distinction of parts can be found in it.

In the same region of the body the anterior rudiment retains nearly the same condition as in the previous stage, though it has somewhat increased in size.

In the sections taken from the anterior part of the same embryo the posterior rudiment has both grown in size and also commenced to undergo histological changes by which it has become divided into a root, a ganglion, and a nerve.

The root (fig. D c, *pr*) consists of small round cells which lie close to the spinal cord, and ends dorsally in a rounded extremity.

The ganglion (*g*) consists of larger and more elongated cells, and forms an oval mass enclosed on the outside by the downward continuation of the root, having its inner side nearly in contact with the spinal cord.

From its ventral end is continued the nerve, which is of considerable length, and has a course approximately parallel to that of the muscle-plate. It forms a continuation of the root rather than of the ganglion.

Further details in reference to the histology of the nerve-rudiment at this stage are given later in this paper, in the description of *Pristiurus*-embryos, of which I have a more complete series of sections than of the *Torpedo*-embryos.

When compared with the nerve-rudiment in the posterior part of the same embryo, the nerve-rudiment last described is, in the first place, considerably larger, and has secondly undergone changes, so that it is possible to recognize in it parts which can be histologically distinguished as nerve and ganglion.

The developmental changes which have taken place in the anterior root are not less important than those in the posterior. The anterior root now forms a very conspicuous cellular prominence growing out from the ventral corner of the spinal cord (fig. D c, *ar*). It has a straight course from the spinal cord to the muscle-plate, and there shows a tendency to turn downwards at an open angle: this, however, is not represented in the specimen figured. The cells of which it is composed each contain a large oval nucleus, and are not unlike the cells which form the posterior rudiment. The anterior and posterior nerves are still quite unconnected with each other; and in those sections in which the anterior root is present the posterior root of the same side is either completely absent or only a small part is to be seen. The cells of the spinal cord exhibit a slight tendency to converge towards the origin of the anterior nerve-root.

In the spinal cord itself the epithelium of the central canal is commencing to become distinguished from the grey matter, but no trace of the white matter is visible.

I have succeeded in making longitudinal vertical sections of this stage, which prove that the ends of the posterior roots adjoining the junction with the cord are all connected with each other (Plate 16. fig. D d).

If the figure representing a transverse section of the embryo (fig. D c) be examined, or better still the figure of a section of the slightly older *Scyllium*-embryo (Plate 17. fig. H I or I I), the posterior root will be seen to end dorsally in a rounded extremity, and the junction with the spinal cord to be effected, not by the extremity of the nerve, but by a part of it at some little distance from this.

It is from these upper ends of the rudiments beyond the junction with the spinal cord that I believe the commissures to spring which connect together the posterior roots.

My sections showing this for the stage under consideration are not quite as satisfactory as is desirable; nevertheless they are sufficiently good to remove all doubt as to the presence of these commissures.

A figure of one of these sections is represented (Plate 16. fig. D d). In this figure *pr* points to the posterior roots and *x* to the commissures uniting them.

In a stage somewhat subsequent to this I have succeeded in making longitudinal sections, which exhibit these junctions with a clearness which leaves nothing to be desired.

It is there effected (Plate 18. fig. L) in each case by a protoplasmic commissure with imbedded nuclei*. Near its dorsal extremity each posterior root dilates, and from the dilated portion is given off on each side the commissure uniting it with the adjoining roots.

Considering the clearness of this formation in this embryo, as well as in the embryo belonging to the stage under description, there cannot be much doubt that at the first formation of the posterior rudiments a continuous outgrowth arises from the spinal cord, and that only at a later period do the junctions of the roots with the cord become separated and distinct for each nerve.

I now return to the more complete series of *Pristiurus*-embryos, the development of whose spinal nerves I have been able to observe.

Second Stage of the Spinal Nerves in Pristiurus.

In the youngest of these (Plate 17. fig. E) the notochord has undergone but very slight changes, but the segmental duct has made its appearance, and is as much developed as in the *Torpedo*-embryo from which fig. D b was taken.

(The embryo from which fig. E a was derived had three visceral clefts.)

There have not as yet appeared any connective-tissue cells dorsal to the top of the muscle-plates, so that the posterior nerve-rudiments are still quite free and distinct.

The cells composing them are smaller than the cells of the neural canal; they are round and nucleated; and, indeed, in their histological constitution the nerve-rudiments exhibit no important deviations from the previous stage, and they have hardly increased in size. In their mode of attachment to the neural tube an important change has, however, already commenced to be visible.

In the previous stage the two nerve-rudiments met above the summit of the spinal cord and were broadly attached to it there; now their points of attachment have glided a short distance down the sides of the spinal cord†.

The two nerve-rudiments have therefore ceased to meet above the summit of the canal; and in addition to this they appear in section to narrow very much before becoming united with its walls, so that their junctions with these appear in a transverse section to be effected by at most one or two cells, and are, comparatively speaking, very difficult to observe.

In an embryo but slightly older than that represented in fig. E a the first rudiment of the anterior root becomes visible. This appears, precisely as in *Torpedo*, in the form of a small projection from the ventral corner of the spinal cord (fig. E b, *ar*).

The second step in this stage (Plate 17. fig. F) is comparable, as far as the connective-tissue is concerned, with the section of *Torpedo* (Plate 16. fig. D d). The notochord (the

* This commissure is not satisfactorily represented in the figure. *Vide* Explanation of Plate 18.

† [May 18, 1876.—Observations I have recently made upon the development of the cranial nerves incline me to adopt an explanation of the change which takes place in point of attachment of the spinal nerves to the cord differing from that enunciated in the text. I look upon this change as being apparent rather than real, and as due to a growth of the roof of the neural canal in the median dorsal line, which tends to separate the roots of the two sides more and more, and cause them to assume a more ventral position.]

histological details of whose structure are not inserted in this figure) is rather more developed, and the segmental duct, as was the case with the corresponding *Torpedo*-embryo, has become hollow at its anterior extremity.

The embryo from which the section was taken possessed five visceral clefts, but no trace of external gills.

In the section represented, though from a posterior part of the body, the dorsal nerve-rudiments have become considerably larger than in the last embryo; they now extend beyond the base of the neural canal. They are surrounded to a great extent by mesoblastic tissue, which, as in the case of the *Torpedo*, takes its origin from two sources, (1) from the commencing vertebral bodies, (2) from the summits of the muscle-plates.

It is in many cases very difficult, especially with chromic-acid specimens, to determine with certainty the limits of the rudiments of the posterior root.

In the best specimens a distinct bordering line can be seen, and it is, as a rule, possible to state the characters by which the cells of the nerve-rudiments and vertebral bodies differ. The more important of these are the following:—(1) The cells of the nerve-rudiment are distinctly smaller than those of the vertebral rudiment; (2) the cells of the nerve-rudiment are elongated, and have their long axis arranged parallel to the long axis of the nerve-rudiment, while the cells surrounding them are much more nearly circular.

The cells of the nerve-rudiment measure about $\frac{1}{1600}\times\frac{1}{4500}$ to $\frac{1}{1600}\times\frac{1}{3200}$ inch, those of the vertebral rudiment $\frac{1}{1600}\times\frac{1}{1900}$ inch. The greater difficulty experienced in distinguishing the nerve-rudiment from the connective-tissue in *Pristiurus* than in *Torpedo* arises from the fact that the connective-tissue is much looser and less condensed in the latter than in the former.

The connective-tissue cells which have grown out from the muscle-plates form a continuous arch over the dorsal surface of the neural tube (*vide* Plate 17. fig. F); and in some specimens it is difficult to see whether the arch is formed by the rudiment of the posterior root or by connective-tissue. It is, however, quite easy with the best specimens to satisfy one's self that it is from the connective-tissue, and not the nerve-rudiment, that the dorsal investment of the neural canal is derived.

As in the previous case, the upper ends of each pair of posterior nerve-rudiments are quite separate from one another, and appear in sections to be united by a very narrow root to the walls of the neural canal at the position indicated in fig. F*.

The cells forming the nerve-rudiments have undergone slight modifications; they are for the most part more distinctly elongated than in the earlier stage, and appear slightly smaller in comparison with the cells of the neural canal.

They possess as yet no distinctive characters of nerve-cells. They stain more deeply with osmic acid than the cells around them, but with hæmatoxylin there is but a very slight difference in intensity between their colouring and that of the neighbouring connective-tissue cells.

* The artist has not been very successful in rendering this figure.

The anterior roots have grown considerably in length, but their observation is involved in the same difficulties with chromic-acid specimens as that of the posterior rudiments.

There is a further difficulty in observing the anterior roots, which arises from the commencing formation of white matter in the cord. This is present in all the anterior sections of the embryo from which fig. F is taken. When the white matter is formed the cells constituting the junction of the anterior nerve-root with the spinal cord undergo the same changes as the cells which are being converted into the white matter of the cord, and become converted into nerve-fibres; these do not stain with hæmatoxylin, and thus an apparent space is left between the nerve-root and the spinal cord. This space by careful examination may be seen to be filled up with fibres. In osmic-acid sections, although even in these the white matter is stained less deeply than the other tissues, it is a matter of comparative ease to observe the junction between the anterior nerve-root and the spinal cord.

I have been successful in preparing satisfactory longitudinal sections of embryos somewhat older than that shown in fig. F, and they bring to light several important points in reference to the development of the spinal nerves. Three of these sections are represented in Plate 17. figs. G 1, G 2, & G 3.

The sections are approximately horizontal and longitudinal. G 1 is the most dorsal of the three; it is not quite horizontal though nearly longitudinal. The section passes exactly through the point of attachment of the posterior roots to the walls of the neural canal.

The posterior rudiments appear as slight prominences of rounded cells projecting from the wall of the neural canal. From transverse sections the attachment of the nerves to the wall of the neural canal is proved to be very narrow, and from these sections it appears to be of some length in the direction of the long axis of the embryo. A combination of the sections taken in the two directions leads to the conclusion that the nerves at this stage thin out like a wedge before joining the spinal cord.

The independent junctions of the posterior rudiments with the spinal cord at this stage are very clearly shown, though the rudiments are probably united with each other just dorsal to their junction with the spinal cord.

The nerves correspond in number with the muscle-plates, and each arises from the spinal cord, nearly opposite the middle line of the corresponding muscle-plates (figs. G 1 & G 2).

Each nerve-rudiment is surrounded by connective-tissue cells, and is separated from its neighbours by a considerable interval.

At its origin each nerve-rudiment lies opposite the median portion of a muscle-plate (figs. G 1 & G 2); but, owing to the muscle-plate acquiring an oblique direction, at the level of the dorsal surface of the notochord it appears in horizontal sections more nearly opposite the interval between two muscle-plates (figs. G 2 & G 3).

In horizontal sections I find masses of cells which make their appearance on a level with the ventral surface of the spinal cord. I believe I have in some sections successfully traced these into the spinal cord, and I have little doubt that they are the anterior roots of the spinal nerves; they are opposite the median line of the muscle-plates, and do not appear to join the posterior roots (*vide* fig. G 3, *ar*).

At the end of this period or second stage the main characters of the spinal nerves in *Pristiurus* are the following:—

(1) The posterior nerve-rudiments form somewhat wedge-shaped masses of tissue attached dorsally to the spinal cord.

(2) The cells of which they are composed are typical undifferentiated embryonic cells, which can hardly be distinguished from the connective-tissue cells around them.

(3) The nerves of each pair no longer meet above the summit of the spinal canal, but are independently attached to its sides.

(4) Their dorsal extremities are probably united by commissures.

(5) The anterior roots have appeared; they form small conical projections from the ventral corner of the spinal cord, but have no connexion with the posterior rudiments.

The Third Stage of the Spinal Nerves in Pristiurus.

With the *third stage* the first distinct histological differentiations of the nerve-rudiments commence. Owing to the changes both in the nerves themselves and in the connective-tissue around them, which becomes less compact and its cells stellate, the difficulty of distinguishing the nerves from the surrounding cells vanishes; and the difficulties of investigation in the later stages are confined to the modes of attachment of the nerves to the neural canal, and the histological changes which take place in the rudiments themselves.

The stage may be considered to commence at the period when the external gills first make their appearance as small buds from the walls of the visceral clefts. Already, in the earliest rudiments of the posterior root of this period now figured, a number of distinct parts are visible (Plate 17. fig. H I).

Surrounding nearly the whole structure there is present a delicate investment similar to that which I mentioned as surrounding the neural canal and other organs; it is quite structureless, but becomes coloured with all staining reagents. I must again leave open the question whether it is to be looked upon as a layer of coagulated protoplasm or as a more definite structure. This investment completely surrounds the proximal portion of the posterior root, but vanishes near its distal extremity.

The nerve-rudiment itself may be divided into three distinct portions:—(1) the proximal portion, in which is situated the pedicle of attachment to the wall of the neural canal; (2) an enlarged portion, which may conveniently, from its future fate, be called the ganglion; (3) a distal portion beyond this. The proximal portion presents a fairly uniform diameter, and ends dorsally in a rounded expansion; it is attached remarkably enough, not by its extremity, but by its side, to the spinal cord. The dorsal extremities of the posterior nerves are therefore free; as was before mentioned, they probably serve as the starting-point of the longitudinal commissures between the posterior roots.

The spinal cord at this stage is still made up of fairly uniform cells, which do not differ in any important particulars from the cells which composed it during the last stage. The outer portion of the most peripheral layer of cells has already begun to be converted into the white matter.

The delicate investment spoken of before still surrounds the whole spinal cord, except at the points of junction of the cord with the nerve-rudiments. Externally to this investment, and separated from it for the most part by a considerable interval, a mesoblastic sheath (Plate 17. fig. H I, *i*) for the spinal cord is beginning to be formed.

The attachment of the nerve-rudiments to the spinal cord, on account of its smallness, is still very difficult to observe. In many specimens where the nerve is visible a small prominence may be seen rising up from the spinal cord at a point corresponding to *x* (Plate 17. fig. H I). It is, however, rare to see this prominence and the nerve continuous with each other: as a rule they are separated by a slight space, and frequently one of the cells of the mesoblastic investment of the spinal cord is interposed between the two. In some especially favourable specimens, similar to the one figured, there can be seen a distinct cellular prominence (fig. H I, *x*) from the spinal cord, which becomes continuous with a small prominence on the lateral border of the nerve-rudiment near its free extremity. The absence of a junction between the two in a majority of sections is only what might be expected, considering how minute the junction is.

Owing to the presence of the commissure connecting the posterior roots, some part of a nerve is present in every section.

The proximal extremity of the nerve-rudiment itself is composed of cells, which, by their smaller size and a more circular form, are easily distinguished from cells forming the ganglionic portion of the nerve.

The ganglionic portion of the nerve, by its externally swollen configuration, is at once recognizable in all the sections in which the nerve is complete. The delicate investment before mentioned is continuous around it. The cells forming it are larger and more elongated than the cells forming the upper portion of the nerve-rudiment: each of them possesses a large and distinct nucleus.

The remainder of the nerve-rudiment forms the commencement of the true nerve. It can in this stage be traced only for a very small distance, and gradually fades away, in such a manner that its absolute termination is very difficult to observe.

The connective-tissue cells which surround the nerve-rudiment are far looser than in the last stage, and are commencing to throw out processes and become branched.

The anterior root-nerve has grown very considerable since the last stage. It projects from the same region of the cord as before, but on approaching the muscle-plate takes a sudden bend downwards (fig. H II, *ar*).

I have failed to prove that the anterior and posterior roots are at this stage united.

Fourth Stage.

In an embryo but slightly more advanced than the one last described, important steps have been made in the development of the nerve-rudiment. The spinal cord itself now possesses a covering of white matter; this is thickest at the ventral portion of the cord, and extends to the region of the posterior root of the spinal nerve.

The junction of the posterior root with the spinal cord is easier to observe than in the last stage.

It is still effected by means of unaltered cells, though the cells which form the projection from the cord to the nerve are commencing to undergo changes similar to those of the cells which are being converted into white matter.

In the rudiment of the posterior root itself there are still three distinct parts, though their arrangement has undergone some alteration and their distinctness has become more marked (Plate 17. fig. I I).

The root of the nerve (fig. I I, *pr*) consists, as before, of nearly circular cells, each containing a nucleus, very large in proportion to the size of the cell. The cells have a diameter of about $\frac{1}{3000}$ of an inch. This mass forms not only the junction between the ganglion and the spinal canal, but is also continued into a layer investing the outer side of the ganglion and continuous with the nerve beyond the ganglion.

The cells which compose the ganglion (fig. I I, *sp.g*) are easily distinguished from those of the root. Each cell is elongated with an oval nucleus, large in proportion to the cell; and its protoplasm appears to be continued into an angular, not to say fibrous process, sometimes at one and more rarely at both ends. The processes of the cells are at this stage very difficult to observe: figs. I a, I b, I c represent three cells provided with them and placed in the positions they occupied in the ganglion.

The relatively very small amount of protoplasm in comparison to the nucleus is fairly represented in these figures, though not in the drawing of the ganglion as a whole. In the centre of each nucleus is a nucleolus.

Fig. I b, in which the process points towards the root of the nerve, I regard as a commencing nerve-fibre: its more elongated shape seems to imply this. In the next stage special bundles of nerve-fibres become very conspicuous in the ganglion. The long diameter of an average ganglion-cell is about $\frac{1}{1600}$ of an inch. The whole ganglion forms an oval mass, well separated both from the nerve-root and the nerve, and is not markedly continuous with either. On its outer side lies the downward process of the nerve-root before mentioned.

The nerve itself is still, as in the last case, composed of cells which are larger and more elongated than either the cells of the root or the ganglion.

The condition of the anterior root at this stage is hardly altered from what it was; it is composed of very small cells, which with hæmatoxylin stain more deeply than any other cell of the section. A figure of it is given in I II.

Horizontal longitudinal sections of this stage are both easy to make and very instructive. On Plate 18. fig. K I is represented a horizontal section through a plane near the dorsal surface of the spinal cord: each posterior root is seen in this section to lie nearly opposite the anterior extremity of a muscle-plate.

In a more ventral plane (fig. K II) this relation is altered, and the posterior roots lie opposite the hinder parts of the muscle-plates.

The nerves themselves are invested by the hyaline membrane spoken of above; and surrounding this again there is present a delicate mesoblastic investment of spindle-shaped cells.

Longitudinal sections also throw light upon the constitution of the anterior nerve-roots (*vide* fig. K II, *ar*). In the two segments on the left-hand side in this figure the anterior roots are cut through as they are proceeding, in a more or less horizontal course, from the spinal cord to the muscle-plates.

Where the section (which is not quite horizontal) passes through the plane of the notochord, as on the right-hand side, the anterior roots are cut transversely. Each root, in fact, changes its direction, and takes a downward course.

The anterior roots are situated nearly opposite the middle of the muscle-plates: their section is much smaller than that of the posterior roots, and with hæmatoxylin they stain more deeply than any of the other cells in the preparation.

The anterior roots, so far as I have been able to observe, do not at this stage unite with the posterior; but on this point I do not speak with any confidence.

The period now arrived at forms a convenient break in the development of the spinal nerves; and I hope to treat the remainder of the subject, especially the changes in the ganglion, the development of the ganglion-cells, and of the nerve-fibres, in a subsequent paper.

I will only add that, not long after the stage last described, the posterior root unites with the anterior root at a considerable distance below the cord: this is shown in Plate 18. fig. L. Still later the portion of the root between the ganglion and the spinal cord becomes converted into nerve-fibres, and the ganglion becomes still further removed from the cord, while at the same time it appears distinctly divided into two parts.

As regards the development of the cranial nerves, I have made a few observations, which, though confessedly incomplete, I would desire to mention here, because, imperfect as they are, they seem to show that in Elasmobranch Fishes the cranial nerves resemble the spinal nerves in arising as outgrowths from the central nervous system.

I have given a figure of the development of a posterior root of a cranial nerve in fig. M I. The section is taken from the same embryo as figs. B I, B II, and B III.

It passes through the anterior portion of a thickening of the external epiblast, which eventually becomes involuted as the auditory vesicle.

The posterior root of a nerve (VII) is seen growing out from the summit of the hind brain in precisely the same manner that the posterior roots of the spinal nerves grow out from the spinal cord: it is the rudiment of the seventh or facial nerve. The section behind this (fig. M II), still in the region of the ear, has no trace of a nerve, and thus serves to show the early discontinuity of the posterior nerve-rudiments which arise from the brain.

I have as yet failed to detect any cranial anterior roots like those of the spinal nerves*. The similarity in development between the cranial and spinal nerves is especially interesting, as forming an important addition to the evidence which at present exists that the cranial nerves are only to be looked on as spinal nerves, especially modified in connexion with the changes which the anterior extremity of the body has undergone in existing vertebrates.

My results may be summarized as follows:—

Along the extreme dorsal summit of the spinal cord there arises on each side a continuous outgrowth.

From each outgrowth processes corresponding in number to the muscle-plates grow downwards. These are the posterior nerve-rudiments.

The outgrowths, at first attached to the spinal cord throughout their whole length, soon cease to be so, and remain in connexion with it in certain spots only, which form the junction of the posterior roots with the spinal cords.

The original outgrowth on each side remains as a bridge, uniting together the dorsal extremities of all the posterior rudiments. The points of junction of the posterior roots with the spinal cord are at first situated at the extreme dorsal summit of the latter, but eventually travel down, and are finally placed on the sides of the cord.

After these events the posterior nerve-rudiments grow rapidly in size, and become differentiated into a root (by which they are attached to the spinal canal), a ganglion, and a nerve.

The anterior roots, like the posterior, are outgrowths from the spinal cord; but the outgrowths to form them are from the first discontinuous, and the points from which they originally spring remain as those by which they are permanently attached to the spinal cord, and do not, as in the case of the posterior roots, undergo a change of position. The anterior roots arise, not vertically below, but opposite the intervals between the posterior roots.

The anterior roots are at first quite separate from the posterior roots; but soon after the differentiation of the posterior rudiment into a root, ganglion, and nerve a junction is effected between each posterior nerve and the corresponding anterior root. The junction is from the first at some little distance from the ganglion.

Investigators have hitherto described the spinal nerves as formed from part of the mesoblast of the protovertebræ. HIS alone, so far as I know, takes a different view.

HIS's† observations lead him to the conclusion that the posterior roots are developed as ingrowths from the external epiblast into the space between the protovertebræ and the neural canal. These subsequently become constricted off, unite with the neural canal and form spinal nerves.

* [May 18, 1876.—Subsequent observations have led me to the conclusion that no anterior nerve-roots are to be found in the brain.]

† Erste Anlage des Wirbelthier-Leibes.

These statements, which have not been since confirmed, diverge nearly to the same extent from my own results as does the ordinary account of the development of these parts.

HENSEN (VIRCHOW'S 'Archiv,' vol. xxxi. 1864) also looks upon the spinal nerves as developed from the epiblast, but not as a direct result of his own observations*.

Without attempting, for the present at least, to explain this divergence, I venture to think that the facts which I have just described have distinct bearings upon one or two important problems.

One point of general anatomy upon which they throw considerable light is the primitive origin of nerves.

So long as it was admitted that the spinal and cerebral nerves developed in the embryo independently of the central nervous system, their mode of origin always presented to my mind considerable difficulties.

It never appeared clear how it was possible for a state of things to have arisen in which the central nervous system, as well as the peripheral terminations of nerves, whether motor or sensory, were formed independently of each other, while between them a third structure was developed which, growing in both directions (towards the centre and towards the periphery), ultimately brought the two into connexion.

That such a condition could be a primitive one seemed scarcely possible.

Still more remarkable did it appear, on the supposition that the primitive mode of formation of these parts was represented in the developmental history of vertebrates, that we should find similar structural elements in the central and in the peripheral nervous systems.

The central nervous system arises from the epiblast, and yet contains precisely similar nerve-cells and nerve-fibres to the peripheral nervous system, which, if derived, as is usually stated, from the mesoblast, was necessarily supposed to have a completely different origin from the central nervous system.

Both of these difficulties are to a great extent removed by the facts of the development of these parts in Elasmobranchs.

If it be admitted that the spinal roots develop as outgrowths from the central nervous system in Elasmobranch Fishes, the question arises, how far it can be supposed to be possible that in other vertebrates the spinal roots and ganglia develop independently of the spinal cord, and only subsequently become united with it.

I have already insisted that this cannot be the primary condition; and though I am of opinion that the origin of the nerves in higher vertebrates ought to be worked over again, yet I do not think it impossible that, by a secondary adaptation, the nerve-roots might develop in the mesoblast†.

* [May 18, 1876.—Since the above was written HENSEN has succeeded in showing that in mammals the rudiments of the posterior roots arise in a manner closely resembling that described in the present paper; and I have myself, within the last few days, made observations which incline me to believe that the same holds good for the chick. My observations are as yet very incomplete.]

† [May 18, 1876.—HENSEN'S observations, as well as those recently made by myself on the chick, render it almost certain that the nerves in all Vertebrates spring from the spinal cord.]

The presence of transverse commissures connecting the central ends of all the posterior roots is very peculiar. The commissures may possibly be looked on as outlying portions of the cord, rather than as parts of the nerves.

I have not up to this time followed their history beyond a somewhat early period in embryonic life, and am therefore unacquainted with their fate in the adult.

As far as I am aware, no trace of similar structures has been met with in other vertebrates.

The commissures have a very strong resemblance to those by which in Elasmobranch Fishes the glossopharyngeal nerve and the branches of the pneumogastric are united in an early embryonic stage*.

I think it not impossible that the commissures in the two cases represent the same structures. If this is the case, it would seem that the junction of a number of nerves to form the pneumogastric is not a secondary state, but the remnant of a primary one, in which all the spinal nerves were united, as they embryonically are in Elasmobranchs.

One point brought out in my investigations appears to me to have bearings upon the origin of the central canal of the Vertebrate nervous system, and in consequence upon the origin of the Vertebrate group itself.

The point I allude to is the posterior nerve-rudiments making their first appearance at the *extreme dorsal summit* of the spinal cord.

The transverse section of the ventral nervous cord of an ordinary segmented worm consists of two symmetrical halves placed side by side.

If by a mechanical folding the two lateral halves of the nervous cord became bent towards each other, while into the groove formed between the two the external skin became pushed, we should have an approximation to the Vertebrate spinal cord. Such a folding might take place to give extra rigidity to the body in the absence of a vertebral column.

If this folding were then completed in such a way that the groove, lined by external skin and situated between the two lateral columns of the nervous system, became converted into a canal, above and below which the two columns of the nervous system united, we should have in the transformed nervous cord an organ strongly resembling the spinal cord of Vertebrates.

This resemblance would even extend beyond mere external form. Let the ventral nervous cord of the common earthworm, *Lumbricus agricola*, be used for comparison †, a transverse section of which is represented by LEYDIG ‡ and CLAPARÈDE. In this we find that on the ventral surface (the Annelidan ventral surface) of the nervous cord the ganglion-cells (grey matter) (*k*) are situated, and on the dorsal side the nerve-fibres or

* BALFOUR, "A Preliminary Account of the Development of Elasmobranch Fishes," Q. J. Micros. Sc. 1874, plate xv. fig. 14, *v.g.*

† The nervous cords of other Annelids resemble that of *Lumbricus* in the relations of the ganglion-cells of the nerve-fibres.

‡ Tafeln zur vergleichenden Anatomie, Taf. iii. fig. 8.

white matter (*h*). If the folding that I have supposed were to take place, the grey and white matters would have very nearly the relative situations which they have in the Vertebrate spinal cord.

The grey matter would be situated in the interior and surround the epithelium of the central canal, and the white matter would nearly surround the grey and form the anterior white commissure. The nerves would then arise, not from the sides of the nervous cord as in existing Vertebrates, but from its extreme ventral summit.

One of the most striking features which I have brought to light with reference to the development of the posterior roots, is the fact of their growing out from the extreme dorsal summit of the neural canal—a position analogous to the ventral summit of the Annelidan nervous cord. Thus the posterior roots of the nerves in Elasmobranchs arise in the exact manner which might have been anticipated were the spinal cord due to such a folding as I have suggested. The argument from the nerves becomes the stronger, from the great peculiarity in the position of the outgrowth, a feature which would be most perplexing without some such explanation as I have proposed. The central epithelium of the neural canal according to this view represents the external skin; and its ciliation is to be explained as a remnant of the ciliation of the external skin now found amongst many of the lower Annelids.

I have, however, employed the comparison of the Vertebrate and Annelidan nervous cords, not so much to prove a genetic relation between the two as to show the *à priori* possibility of the formation of a *spinal canal* and the *à posteriori* evidence we have of the Vertebrate spinal canal having been formed in the way indicated.

I have not made use of what is really the strongest argument for my view, viz. that the embryonic mode of formation of the spinal canal, by a folding in of the external epiblast, is the very method by which I have supposed the spinal canal to have been formed in the ancestors of Vertebrates.

My object has been to suggest a meaning for the peculiar primitive position of the posterior roots, rather than to attempt to explain in full the origin of the spinal canal.

Explanation of the Plates*.

PLATE 16.

Fig. A. Section through the dorsal region of an embryo of *Scyllium stellare*, with the rudiments of two visceral clefts. The section illustrates the general features at a period anterior to the appearance of the posterior nerve-roots.

nc, neural canal; *mp*, muscle-plate; *ch*, notochord; *x*, subnotochordal

* The figures on these Plates give a fair general idea of the appearance presented by the developing spinal nerves; but the finer details of the original drawings have in several cases become lost in the process of copying.

The figures which are tinted represent sections of embryos hardened in osmic acid; those without colour sections of embryos hardened in chromic acid.

rod; *ao*, rudiment of dorsal aorta; *so*, somatopleure; *sp*, splanchnopleure; *al*, alimentary tract. All the parts of the section except the spinal cord are drawn somewhat diagrammatically.

Figs. B I, B II, B III. Three sections of a *Pristiurus*-embryo. B I is through the heart, B II through the anterior part of the dorsal region, and B III through a point slightly behind this. Drawn with a camera. (Zeiss CC ocul. 2.)

In B III there is visible a slight proliferation of cells from the dorsal summit of the neural canal.

In B II this proliferation definitely constitutes two club-shaped masses of cells (*pr*), both attached to the dorsal summit of the neural canal. The masses are the rudiments of the posterior nerve-roots.

In B I the rudiments of the posterior roots are of considerable length.

pr, rudiment of posterior roots; *nc*, neural canal; *mp*, muscle-plate; *ch*, notochord; *x*, subnotochordal rod; *ao*, dorsal aorta; *so*, somatopleure; *sp*, splanchnopleure; *al*, alimentary canal; *ht*, heart.

Fig. C. Section from a *Pristiurus*-embryo, slightly older than B. Camera. (Zeiss CC ocul. 2.) The embryo from which this figure was taken was slightly distorted in the process of removal from the blastoderm.

vr, rudiment of vertebral body. Other reference letters as in previous figures.

Fig. D a. Section through the dorsal region of a *Torpedo*-embryo with three visceral clefts. (Zeiss CC ocul. 2.) The section shows the formation of the dorsal nerve-rudiments (*pr*) and of a ventral anterior nerve-rudiment (*ar*), which at this early stage is not distinctly cellular.

ar, rudiment of an anterior nerve-root; *y*, cells left behind on the separation of the external skin from the spinal cord; *c*, connective-tissue cells springing from the summit of the muscle-plates. Other reference letters as above.

Fig. D b. Section from dorsal region of a *Torpedo*-embryo somewhat older than D a. Camera. (Zeiss CC ocul. 2.) The posterior nerve-rudiment is considerably longer than in fig. D a, and its pedicle of attachment to the spinal cord is thinner. The anterior nerve-rudiment, of which only the edge is present in the section, is distinctly cellular.

m, mesoblast growing up from vertebral rudiment; *sd*, segmental duct.

Fig. D c. Section from a still older *Torpedo*-embryo. Camera. (Zeiss CC ocul. 2.) The connective-tissue cells are omitted. The rudiment of the ganglion (*g*) on the posterior root has appeared. The rudiment of the posterior nerve is much longer than before, and its junction with the spinal cord is difficult to detect. The anterior root is now an elongated cellular structure.

g, ganglion.

Fig. D d. Longitudinal and vertical section through a *Torpedo*-embryo of the same age as D c.

The section shows the commissures uniting the posterior roots.

PLATE 17.

Fig. E a. Section of a *Pristiurus*-embryo belonging to the second stage. Camera. (Zeiss CC ocul. 2.) The section shows the constriction of the pedicle which attaches the posterior nerve-rudiments to the spinal cord.

pr, rudiment of posterior nerve-root; *nc*, neural canal; *mp*, muscle-plate; *vr*, vertebral rudiment; *sd*, segmental duct; *ch*, notochord; *so*, somatopleure; *sp*, splanchnopleure; *ao*, aorta; *al*, alimentary canal.

Fig. E b. Section of a *Pristiurus*-embryo slightly older than E a. Camera. (Zeiss CC ocul. 2.) The section shows the formation of the anterior nerve-root (*ar*).

ar, rudiment of the anterior nerve-root.

Fig. F. Section of a *Pristiurus*-embryo with the rudiments of five visceral clefts. Camera. (Zeiss CC ocul. 2.)

The rudiment of the posterior root is seen surrounded by connective-tissue, from which it cannot easily be distinguished. The artist has not been very successful in rendering this figure.

Figs. G 1, G 2, G 3. The longitudinal and horizontal section of an embryo somewhat older than F. The embryo from which these sections were taken was hardened in osmic acid, but the sections have been represented without tinting. G 1 is most dorsal of the three sections. Camera. (Zeiss CC ocul. 1.)

nc, neural canal; *sp.c*, spinal cord; *pr*, rudiment of posterior root; *ar*, rudiment of anterior root; *mp*, muscle-plate; *c*, connective-tissue cells; *ch*, notochord.

Fig. H I. Section through the dorsal region of a *Pristiurus*-embryo in which the rudimentary external gills are present as very small knobs. Camera. (Zeiss CC ocul. 2.)

The section shows the commencing differentiation of the posterior nerve-rudiment into root (*pr*), ganglion (*sp.g*), and nerve (*n*), and also the attachment of the nerve-root to the spinal cord (*x*). The variations in the size and shape of the cells in the different parts of the nerve-rudiment are completely lost in the figure.

pr, posterior nerve-root; *sp.g*, ganglion of posterior root; *n*, nerve of posterior root; *x*, attachment of posterior root to spinal cord; *w*, white matter of spinal cord; *i*, mesoblastic investment to the spinal cord.

Fig. H II. Section through the same embryo as H I. (Zeiss CC ocul. 1.)

The section contains an anterior root, which takes its origin at a point opposite the interval between two posterior roots.

The white matter has not been very satisfactorily represented by the artist.

Figs. I I, I II. Two sections of a *Pristiurus*-embryo somewhat older than H. Camera. (Zeiss CC ocul. 1.)

The connective-tissue cells are omitted.

Figs. I a, I b, I c. Three isolated cells from the ganglion of one of the posterior roots of the same embryo.

PLATE 18.

Figs. K I, K II. Two horizontal longitudinal sections through an embryo in which the external gills have just appeared. K I is the most dorsal of the two sections. Camera. (Zeiss CC ocul. 1.)

The sections show the relative positions of the anterior and posterior roots at different levels.

pr, posterior nerve-rudiment; *ar*, anterior nerve-rudiment; *sp.c*, spinal cord; *n.c*, neural canal; *mp*, muscle-plate; *mp'*, first-formed muscles.

Fig. L. Longitudinal and vertical section through the trunk of a *Scyllium*-embryo after the external gills have attained their full development. Camera. (Zeiss CC ocul. 1.)

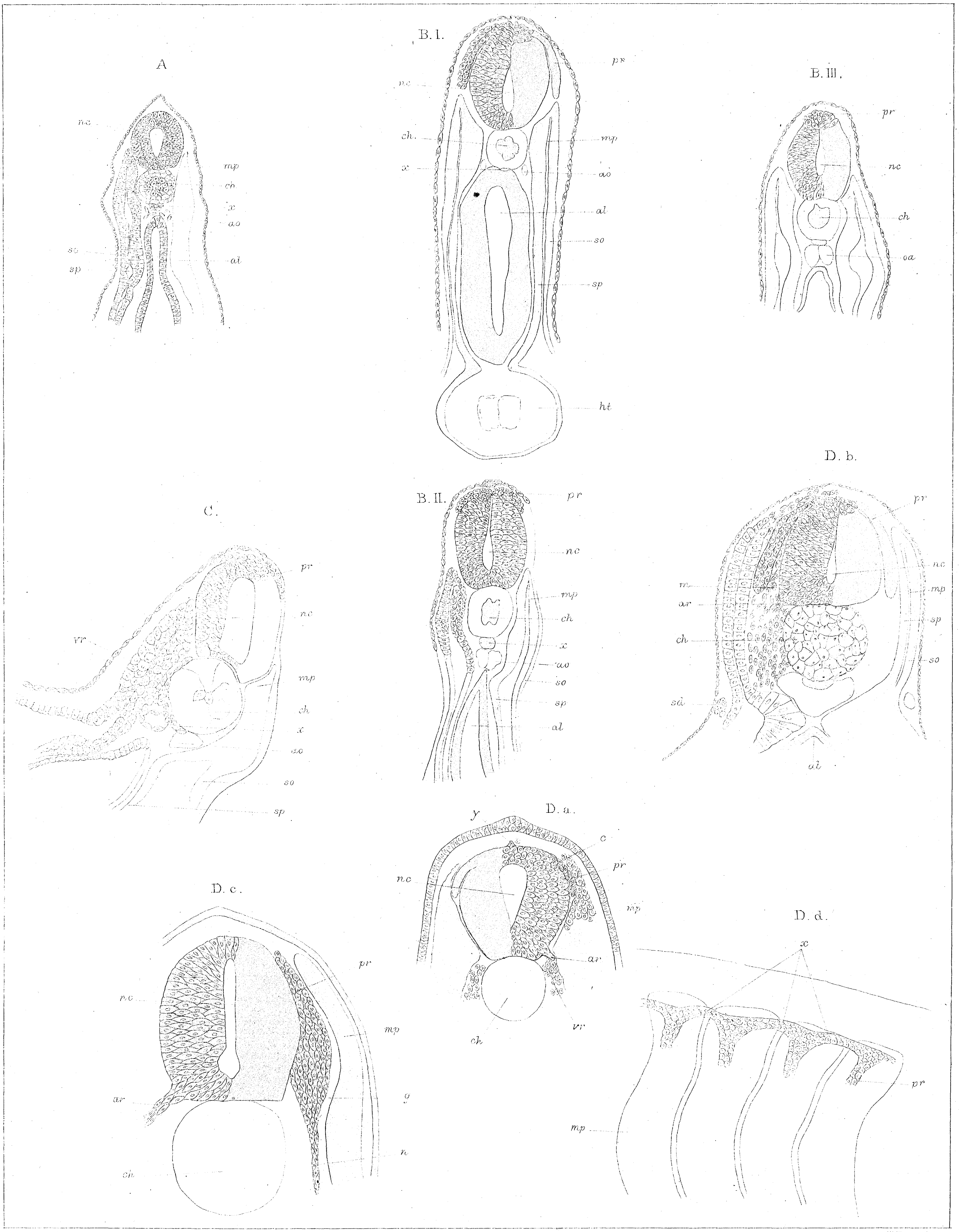
The embryo was hardened in a mixture of chromic acid and osmic acid.

The section shows the commissures which dorsally unite the posterior roots, and also the junction of the anterior and posterior roots. The commissures are unfortunately not represented in the figure with great accuracy; their outlines are in nature perfectly regular, and not, as in the figure, notched at the junctions of the cells composing them. Their cells are apparently more or less completely fused, and certainly not nearly so clearly marked as in the figure. The commissures stain very deeply with the mixture of osmic and chromic acid, and form one of the most conspicuous features in successful longitudinal sections of embryos so hardened. In sections hardened with chromic acid only they cannot be seen with the same facility.

sp.c, spinal cord; *gr*, grey matter; *w*, white matter; *ar*, anterior root; *pr*, posterior root; *x*, commissure uniting the posterior roots.

Figs. M I, M II. Two sections through the head of the same embryo as fig. B. M I, the foremost of the two, passes through the anterior part of the thickening of epiblast, which becomes involuted as the auditory vesicle. It contains the rudiment of the seventh nerve, VII. Camera. (Zeiss CC ocul. 2.)

VII, rudiment of seventh nerve; *au*, thickening of external epiblast, which becomes involuted as the auditory vesicle; *n.c*, neural canal; *ch*, notochord; *pp*, body-cavity in the head; *so*, somatopleure; *sp*, splanchnopleure; *al*, throat, exhibiting an outgrowth to form the first visceral cleft.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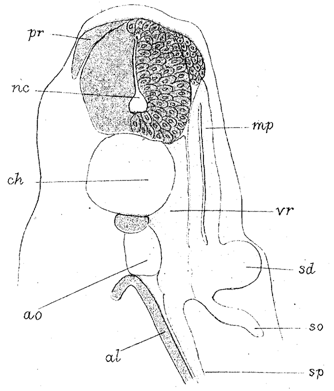

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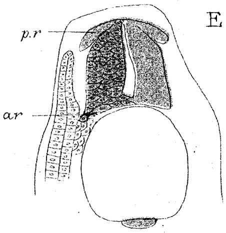

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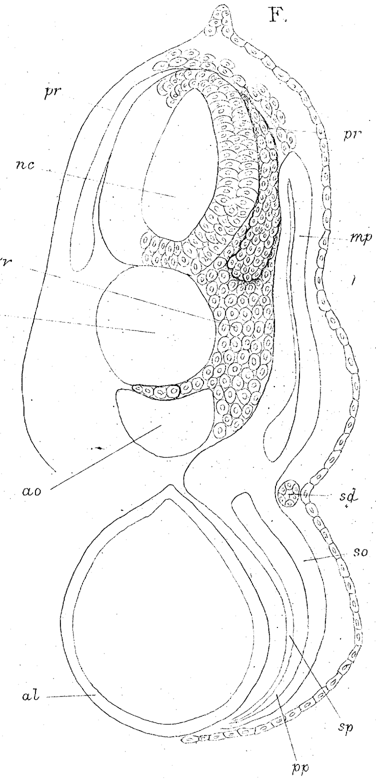

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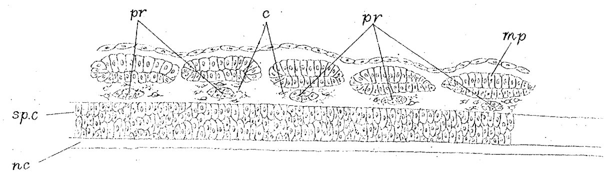

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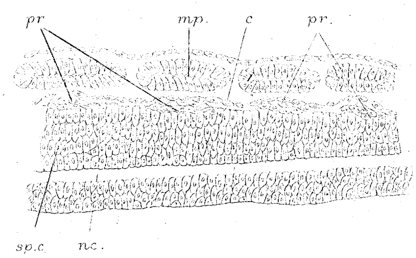

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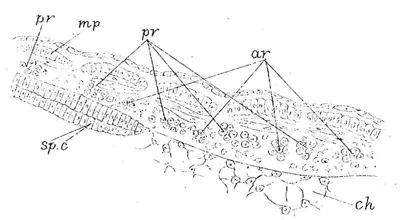
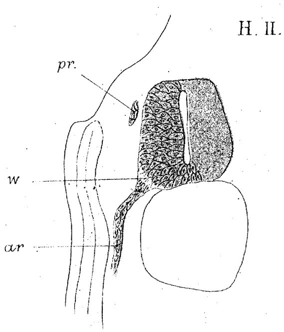


I. a.

I. b.

I. c.

H. II.



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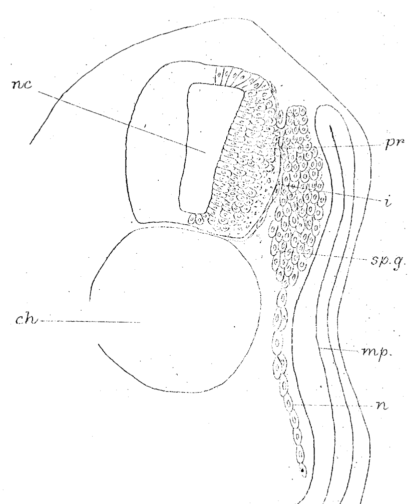

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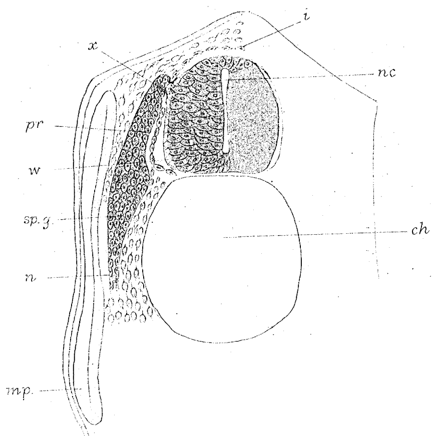

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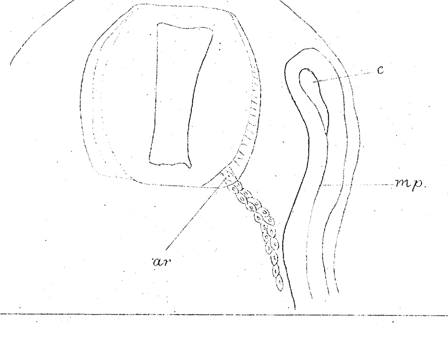

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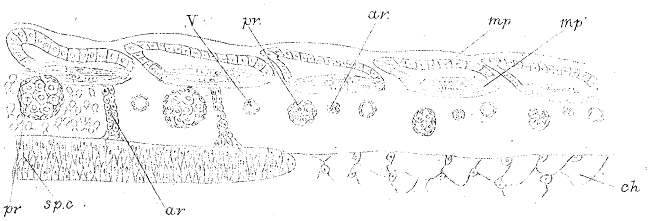

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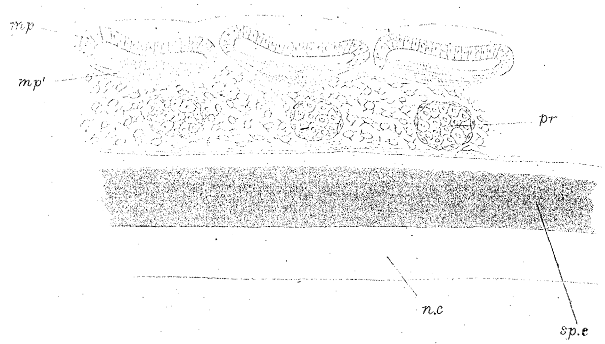

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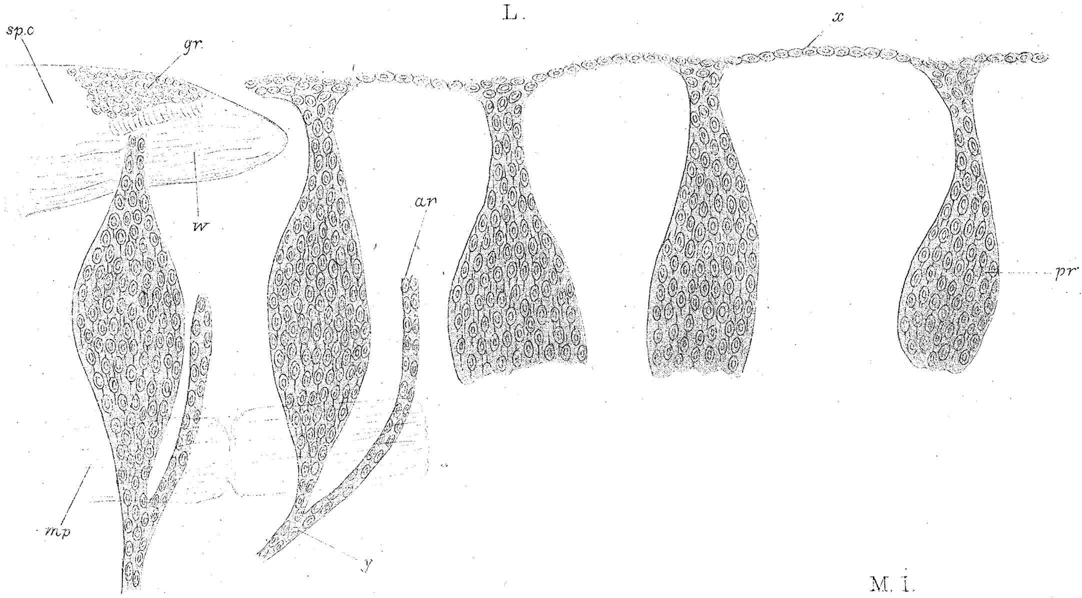

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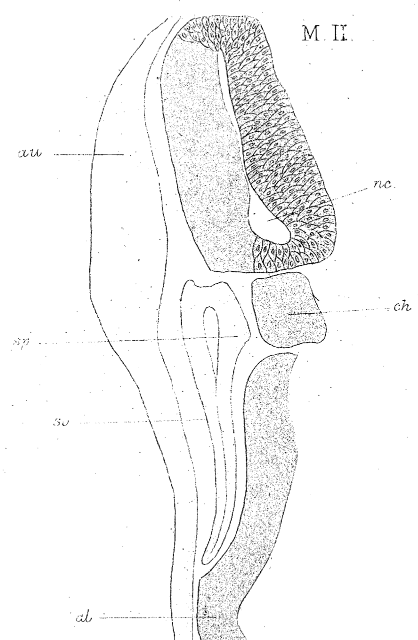
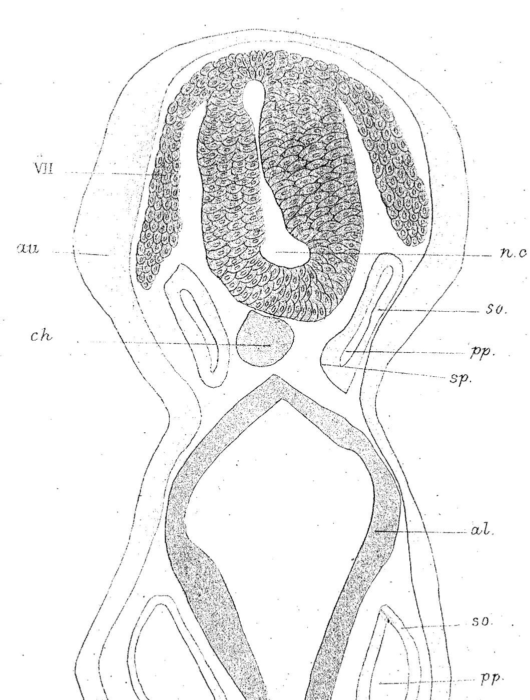


M.I.



W. West & C° imp.

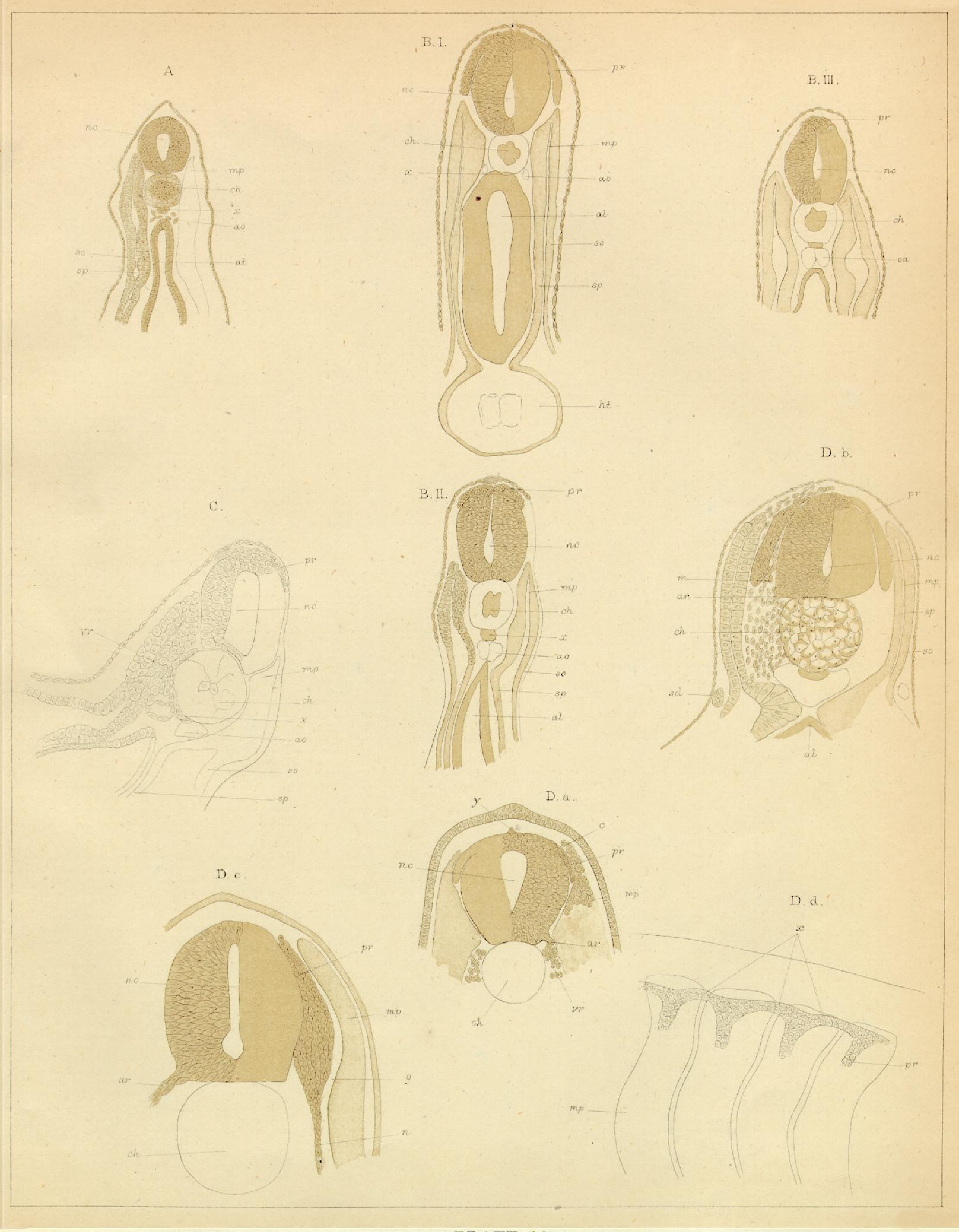


PLATE 16.

Fig. A. Section through the dorsal region of an embryo of *Scyllium stellare*, with the rudiments of two visceral clefts. The section illustrates the general features at a period anterior to the appearance of the posterior nerve-roots.

nc, neural canal; *mp*, muscle-plate; *ch*, notochord; *x*, subnotochordal rod; *ao*, rudiment of dorsal aorta; *so*, somatopleure; *sp*, splanchnopleure; *al*, alimentary tract. All the parts of the section except the spinal cord are drawn somewhat diagrammatically.

Figs. B I, B II, B III. Three sections of a *Pristiurus*-embryo. B I is through the heart, B II through the anterior part of the dorsal region, and B III through a point slightly behind this. Drawn with a camera. (Zeiss CC ocul. 2.)

In B III there is visible a slight proliferation of cells from the dorsal summit of the neural canal.

In B II this proliferation definitely constitutes two club-shaped masses of cells (*pr*), both attached to the dorsal summit of the neural canal. The masses are the rudiments of the posterior nerve-roots.

In B I the rudiments of the posterior roots are of considerable length.

pr, rudiment of posterior roots; *nc*, neural canal; *mp*, muscle-plate; *ch*, notochord; *x*, subnotochordal rod; *ao*, dorsal aorta; *so*, somatopleure; *sp*, splanchnopleure; *al*, alimentary canal; *ht*, heart.

Fig. C. Section from a *Pristiurus*-embryo, slightly older than B. Camera. (Zeiss CC ocul. 2.) The embryo from which this figure was taken was slightly distorted in the process of removal from the blastoderm.

vr, rudiment of vertebral body. Other reference letters as in previous figures.

Fig. D a. Section through the dorsal region of a *Torpedo*-embryo with three visceral clefts. (Zeiss CC ocul. 2.) The section shows the formation of the dorsal nerve-rudiments (*pr*) and of a ventral anterior nerve-rudiment (*ar*), which at this early stage is not distinctly cellular.

ar, rudiment of an anterior nerve-root; *y*, cells left behind on the separation of the external skin from the spinal cord; *c*, connective-tissue cells springing from the summit of the muscle-plates. Other reference letters as above.

Fig. D b. Section from dorsal region of a *Torpedo*-embryo somewhat older than D a. Camera. (Zeiss CC ocul. 2.) The posterior nerve-rudiment is considerably longer than in fig. D a, and its pedicle of attachment to the spinal cord is thinner. The anterior nerve-rudiment, of which only the edge is present in the section, is distinctly cellular.

m, mesoblast growing up from vertebral rudiment; *sd*, segmental duct.

Fig. D c. Section from a still older *Torpedo*-embryo. Camera. (Zeiss CC ocul. 2.) The connective-tissue cells are omitted. The rudiment of the ganglion (*g*) on the posterior root has appeared. The rudiment of the posterior nerve is much longer than before, and its junction with the spinal cord is difficult to detect. The anterior root is now an elongated cellular structure.

g, ganglion.

Fig. D d. Longitudinal and vertical section through a *Torpedo*-embryo of the same age as D c.

The section shows the commissures uniting the posterior roots.

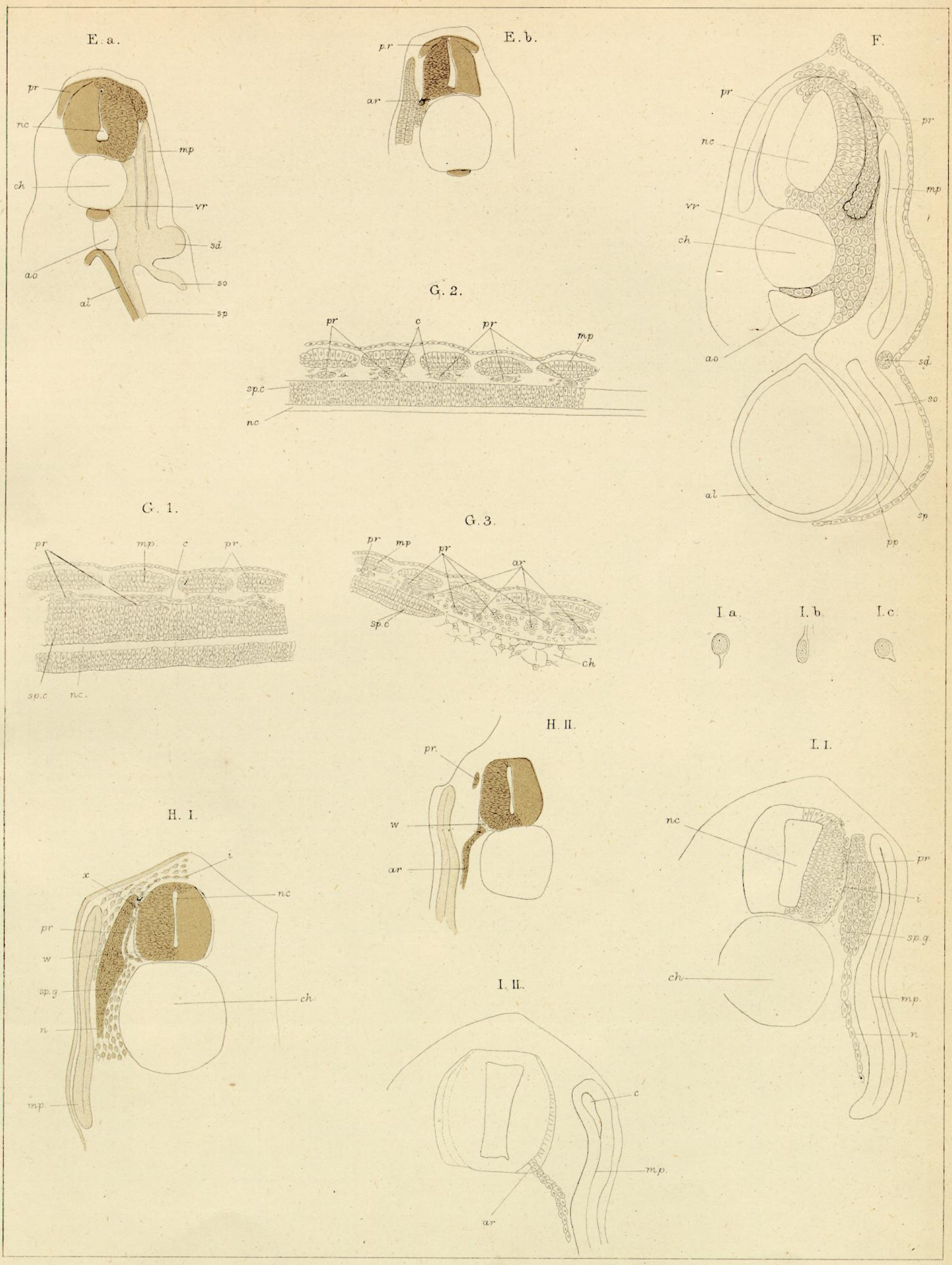


PLATE 17.

Fig. E a. Section of a *Pristiurus*-embryo belonging to the second stage. Camera. (Zeiss CC ocul. 2.) The section shows the constriction of the pedicle which attaches the posterior nerve-rudiments to the spinal cord.

pr, rudiment of posterior nerve-root; *nc*, neural canal; *mp*, muscle-plate; *vr*, vertebral rudiment; *sd*, segmental duct; *ch*, notochord; *so*, somatopleure; *sp*, splanchnopleure; *ao*, aorta; *al*, alimentary canal.

Fig. E b. Section of a *Pristiurus*-embryo slightly older than E a. Camera. (Zeiss CC ocul. 2.) The section shows the formation of the anterior nerve-root (*ar*).

ar, rudiment of the anterior nerve-root.

Fig. F. Section of a *Pristiurus*-embryo with the rudiments of five visceral clefts. Camera. (Zeiss CC ocul. 2.)

The rudiment of the posterior root is seen surrounded by connective-tissue, from which it cannot easily be distinguished. The artist has not been very successful in rendering this figure.

Figs. G 1, G 2, G 3. The longitudinal and horizontal section of an embryo somewhat older than F. The embryo from which these sections were taken was hardened in osmic acid, but the sections have been represented without tinting. G 1 is most dorsal of the three sections. Camera. (Zeiss CC ocul. 1.)

nc, neural canal; *sp.c*, spinal cord; *pr*, rudiment of posterior root; *ar*, rudiment of anterior root; *mp*, muscle-plate; *c*, connective-tissue cells; *ch*, notochord.

Fig. H I. Section through the dorsal region of a *Pristiurus*-embryo in which the rudimentary external gills are present as very small knobs. Camera. (Zeiss CC ocul. 2.)

The section shows the commencing differentiation of the posterior nerve-rudiment into root (*pr*), ganglion (*sp.g*), and nerve (*n*), and also the attachment of the nerve-root to the spinal cord (*x*). The variations in the size and shape of the cells in the different parts of the nerve-rudiment are completely lost in the figure.

pr, posterior nerve-root; *sp.g*, ganglion of posterior root; *n*, nerve of posterior root; *x*, attachment of posterior root to spinal cord; *w*, white matter of spinal cord; *i*, mesoblastic investment to the spinal cord.

Fig. H II. Section through the same embryo as H I. (Zeiss CC ocul. 1.)

The section contains an anterior root, which takes its origin at a point opposite the interval between two posterior roots.

The white matter has not been very satisfactorily represented by the artist.

Figs. I I, I II. Two sections of a *Pristiurus*-embryo somewhat older than H. Camera. (Zeiss CC ocul. 1.)

The connective-tissue cells are omitted.

Figs. I a, I b, I c. Three isolated cells from the ganglion of one of the posterior roots of the same embryo.

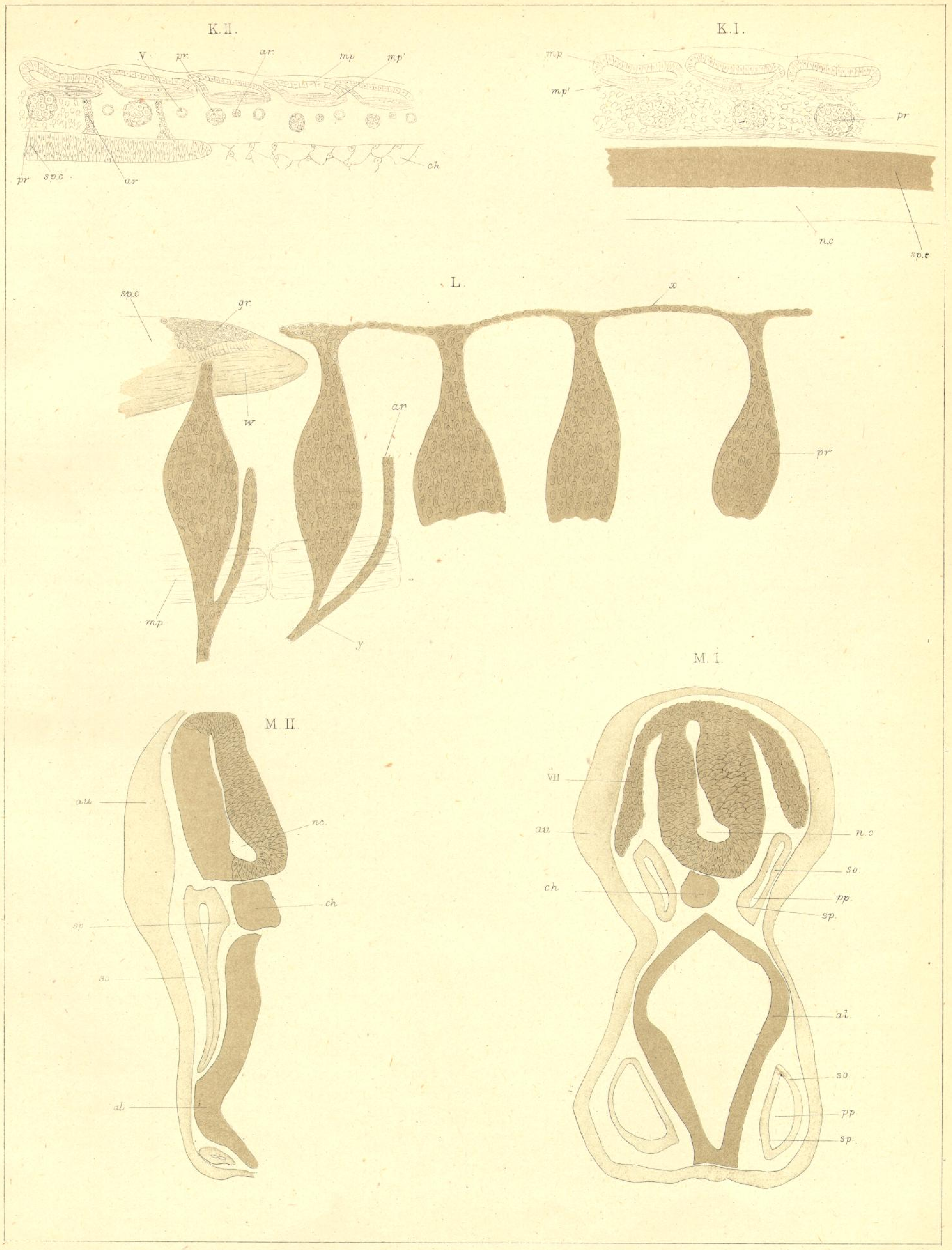


PLATE 18.

Figs. K I, K II. Two horizontal longitudinal sections through an embryo in which the external gills have just appeared. K I is the most dorsal of the two sections. Camera. (Zeiss CC ocul. 1.)

The sections show the relative positions of the anterior and posterior roots at different levels.

pr, posterior nerve-rudiment; *ar*, anterior nerve-rudiment; *sp.c*, spinal cord; *n.c*, neural canal; *mp*, muscle-plate; *mp'*, first-formed muscles.

Fig. L. Longitudinal and vertical section through the trunk of a *Scyllium*-embryo after the external gills have attained their full development. Camera. (Zeiss CC ocul. 1.)

The embryo was hardened in a mixture of chromic acid and osmic acid.

The section shows the commissures which dorsally unite the posterior roots, and also the junction of the anterior and posterior roots. The commissures are unfortunately not represented in the figure with great accuracy; their outlines are in nature perfectly regular, and not, as in the figure, notched at the junctions of the cells composing them. Their cells are apparently more or less completely fused, and certainly not nearly so clearly marked as in the figure. The commissures stain very deeply with the mixture of osmic and chromic acid, and form one of the most conspicuous features in successful longitudinal sections of embryos so hardened. In sections hardened with chromic acid only they cannot be seen with the same facility.

sp.c, spinal cord; *gr*, grey matter; *w*, white matter; *ar*, anterior root; *pr*, posterior root; *x*, commissure uniting the posterior roots.

Figs. M I, M II. Two sections through the head of the same embryo as fig. B. M I, the foremost of the two, passes through the anterior part of the thickening of epiblast, which becomes involuted as the auditory vesicle. It contains the rudiment of the seventh nerve, VII. Camera. (Zeiss CC ocul. 2.)

VII, rudiment of seventh nerve; *au*, thickening of external epiblast, which becomes involuted as the auditory vesicle; *n.c*, neural canal; *ch*, notochord; *pp*, body-cavity in the head; *so*, somatopleure; *sp*, splanchnopleure; *al*, throat, exhibiting an outgrowth to form the first visceral cleft.