

IV. *On the Development of the Vertebral Column of Anura.*

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[PLATES 14–18.]

Introduction.

The justification of the following paper is found in the fact that, notwithstanding the investigations of DUGÉS (1834), GEGENBAUR (1861, 1862, 1876), GOETTE (1875), SCHWEGMANN (1884), HASSE (1892), PETER (1895), GADOW (1896), RIDWOOD (1897), KAPELKIN (1900), SCHAINSLAND (1906), and EMELIANOF (1925), which deal with the vertebral column in Anura, there still remains a great deal of uncertainty as to formation of the centrum. We are not certain, first, whether the Anuran centrum is formed from the dorsal arcualia or has an independent origin; secondly, whether the intervertebral body is formed from the dorsal arcualia, or has an independent origin; thirdly, whether any change takes place in the developing vertebra as it passes from its cartilaginous condition to its ossified stage, for none of the above-mentioned workers have traced it up to its full development in the adult condition; fourthly, how the ninth vertebra, both in the case of *Rana temporaria* and *Bufo melanostictus* comes to have two condyles to articulate with the urostyle; fifthly, how the first (atlas) vertebra develops, and what is its relation to the occipital region; lastly, how the Anuran skull has developed two condyles for articulation with the first vertebra.

The writer desires to acknowledge his indebtedness to Prof. E. W. MACBRIDE, who with valuable advice and criticism has greatly facilitated these researches. He has also to acknowledge his obligations to Mr. H. R. HEWER*, Mr. N. MURTI, Mr. P. GRAY, and Mr. C. JOHN for their kind assistance, which has led to the improvement of this paper.

Material.

The material was collected from various sources. Prof. MACBRIDE gave the writer some tadpoles of *Rana temporaria*, which he reared during the spring of 1926. The embryonic, as well as post-larval stages of *Rana temporaria*, were reared by the writer himself during 1926–28. The whole material of *Bufo melanostictus* was sent to the

* The beautiful microphotographs which illustrate this paper are entirely the work of Mr. HEWER: the drawings of the vertebral columns of *Rana*, *Bombinator* and *Xenopus* were made by Mr. JOHN.—E. W. M.

writer by Mr. J. BHADURI, of the Indian Museum of Calcutta. Most of the *Bombinator igneus* material was purchased from a local dealer. The material for the research on the development of *Xenopus laevis* was contributed partly by Prof. HOGBEN, of Cape Town University, South Africa, and partly by Dr. C. S. GOBBELAAR from the collection of Prof. C. G. S. DE VILLIERS, of the Stellenbosch University.

Now the writer wishes to take the opportunity of expressing his indebtedness to every one who helped him for the materials, but for which it would have been impossible for him to carry on the investigation.

Method.

Owing to the absence of any definite means of ascertaining the age of the animal, the writer has adopted the usual method of indicating age by size; in order to render this more definite, he has mentioned, in addition, the changes in the external morphology, wherever possible. It must, of course, be borne in mind that size can never be the sole criterion in determining age, since there are individual variations. Larvæ were measured from the tip of the snout to the end of the tail. The young adolescent stages of Anura just after the metamorphosis were measured from the tip of the snout to the cloaca. The early embryonic stages of *Rana temporaria* were fixed in Smith's fixative (1912). Most of the larval and post-larval stages were fixed in Bouin's fluid, except some *Xenopus laevis* material sent by Dr. GOBBELAAR, which Prof. DE VILLIERS had fixed in 10 per cent. formaldehyde. The state of preservation was generally very satisfactory.

In order to get the structures intact, the celloidin method of embedding was used for early stages of *Rana temporaria*. Generally, the paraffin used in this investigation was of the melting point 56° C., but for the materials fixed in Smith's fixative, 52° C. paraffin was used. To decalcify the advanced stages, 3 per cent. nitric acid with 70 per cent. alcohol was used for a number of days, according to the size of the specimen. The majority of the specimens were studied by means of serial, transverse, frontal and sagittal sections.

For early embryonic stages, Delafield's hæmatoxylin was used for staining, the stain being differentiated by acid alcohol and the sections afterwards subjected to ammonia vapour, in order to get rid of the last traces of acid. Mallory's triple stain and Borax carmine followed by Picro-indigo-carmine were used for larval as well as the adult stages.

In order to compare the structure of a developing vertebra with its adult condition in relation with other skeletal structures, *in situ*, the alizarine method of staining the skeletal system was used.

PART 2.—ANURA.

Rana temporaria.

The writer has studied the early embryonic stages, the detailed description of which has been omitted, since they merely show the differentiation of the embryonic layers. Regarding the shape of the notochord in its earliest condition, the only observation worthy of record is that the notochord is squarish in cross-section.

A.—Stages before Metamorphosis.

Stage 5 mm.—The notochord is oval in cross-section at the two extremities, and round at the middle region. It is quite vacuolated, although there are some scattered intact notochordal cells here and there, especially at the junctions of the septa, which separate these vacuoles. There is a layer of notochordal cells round the periphery of the notochord. The mesodermal somites have liberated the sclerotomic cells from their ventro-median corners, and should now be called myotomes. The sub-notochord projects on the dorsal wall of the intestine below the notochord. Both the spinal cord and the notochord are surrounded by scattered layers of sclerotomic cells. There is no trace of a notochordal sheath.

Stage 7 mm.—The notochord shows the same condition as in the previous stage. The sclerotomic cells surrounding the notochord have changed their shape and become ordinary fibrous connective tissue cells. The sub-notochord is entirely free from the dorsal wall of the intestine and is present throughout from the level of the duodenum to the anus as a straight rod of two cells thick. The aorta is visible between the sub-notochord and the dorsal wall of the intestine.

Stage 11 mm.—The peripheral notochordal cells form a definite layer which may be termed the *notochordal epithelium*.

The septa are regular and the chordal epithelium in its turn is covered externally by two thin membranes, the *elastica externa* and the *elastica interna*, both products of the secretion of the notochordal cells themselves. The *elastica interna* lies between the *elastica externa* and the chordal epithelium. Anteriorly the notochord is rather bluntly pointed. In the region of the auditory capsule it is laterally compressed, whilst posteriorly it is slightly tapering. At its anterior end it is slightly bent ventrally in accordance with the bend of the spinal cord. The fibrous cells which surrounded the notochord in a scattered manner are now aggregated in the centre of the myotomal regions forming rings—the *perichordal rings*. Corresponding to each myotome there is a spinal nerve ganglion opposite the cranial half and opposite the caudal half a scattered aggregation of sclerotomic cells. The anterior end of the body contains two dorsal aortæ, but they fuse into one at the level of the beginning of the sub-notochord which continues towards the posterior end of the body. Connective tissue cells become aggregated round the aorta and give it a strong support.

Stage 14 mm.—The sclerotomic cells at the dorsolateral corners of the notochord corresponding to the caudal half of each myotome have greatly increased in number and become compact. The rings of fibrous connective tissue have extended in a cranial as well as in a caudal direction so as to form a continuous tube—the *perichordal tube*. In contrast to the previous stage in which the notochord is of uniform diameter throughout, in the present stage it exhibits in its trunk portion moniliform swellings. There are dilatations opposite the middle of each myotome and constrictions opposite the inter-myotomal regions. This moniliform shape of the notochord is, of course, caused by the perichordal tube outside it. The dorsolateral aggregations of sclerotomic cells exert

pressure and constrict the notochord and the perichordal tube in the intermyotomal (vertebral) region. The part of the perichordal tube at the bases of the dorsolateral aggregations is so thin that they seem to rest directly on the notochordal sheath. Closer examination shows, however, that this is not so.

The sub-notochord is still present, but becomes more and more reduced towards the posterior end. Towards the tail end the notochord becomes compressed from side to side. The sheaths of the notochord in the tail are very much folded.

Stage 17 mm.—The anterior portion of the notochord lies between the cartilaginous auditory capsules, behind which on each side is the vagus nerve ganglion. The dorsolateral sclerotomic cells lying above the notochord have become modified into procartilaginous cells. These procartilaginous dorsolateral elements run up as far as half the height of the spinal cord but do not actually enclose it, the rest being covered by connective tissue. These dorsolateral procartilaginous structures are the *basidorsals*, which are the rudiments of the future neural arches. Their bases are quite separate. The fibrous perichordal tube has a thick layer outside it on the ventral side of the notochord in this vertebral region, which GADOW has described as the *membrana reuniens*.

The sub-notochord has in this stage disappeared altogether and the whole space between the notochord and the aorta is filled up with the connective tissue cells. Here the fibrous perichordal tube is very prominent and at the posterior end of the body some sclerotomic cells become aggregated on its mid-ventral surface. It begins from the level where the two aortæ meet to form the dorsal aorta and runs backwards to the cloaca. The sheaths of the notochord in the tail region are very thick.

Stage 20 mm.—The posterior region of the auditory capsule is the hinder limit of a myotome; behind this is the vagus nerve ganglion corresponding to the cranial half of the next myotome, and to the caudal half of this myotome corresponds the occipital arch of the cartilaginous cranium. Behind this there is an arch corresponding to the cranial half of the myotome behind the occipital region, which is followed by another arch corresponding to the caudal half of the same myotome. This 2nd arch corresponding to the caudal half is the atlas arch proper. So between the occipital arch and the atlas arch there is an intercalated arch as well as a nerve, corresponding to the cranial half of the myotome, which is a unique structure in the vertebral column (fig. 1, Plate 14). Connective tissue cells coming from all sides in a slanting direction converge towards a point on the two opposite sides of the arch; they grow into it and sever it into a ball in front and a cup behind. It has already been stated that in the previous stage the notochord is compressed at the anterior region; so that at this stage the two sides of this intercalated arch are not wide apart.

It is very easy to make out the number of the future vertebræ by counting the number of the arches. Altogether there are eleven arches, of which the last two are made up of sclerotomic cells; beyond these arches the spinal cord is covered only by connective tissue. The base of each left half arch is connected with the base of the corresponding right half arch by means of connective tissue.

On the midventral aspect of the perichordal tube in the region from the beginning of the aorta to the commencement of the tail there is a mass of spindle-shaped connective tissue cells. In the centre of this mass a rod of round cells becomes differentiated which afterwards becomes cartilaginous and which should be called the *hypochord*. As will be seen later it probably represents the missing basiventralia.

Stage 22 mm.—The part of the intercalated piece of arch between the occipital arch and the atlas arch proper has fused with the latter forming a big mass of cartilage with two cups at the anterior end. These cups lie on the two lateral aspects of the centrum with little interspace between them. The line of connective tissue cells which penetrated the intercalated arch so as to cut it into the ball and the socket, now splits up into two layers with a cavity between them, but they are still in the connective tissue state.

The first nine neural arches have completely enveloped the spinal cord. In the vertebral region the thin perichordal tube just at the base of each cartilaginous arch changes from a fibrous connective tissue layer to one row of cartilaginous cells. There is a marked difference between the character of these cells and the cells of the arch. These perichordal cells are very small and the intercellular spaces are almost negligible. The mid-dorsal part as well as the ventral part of the perichordal tube is still fibrous.

The perichordal tube in the intervertebral region where there are no basidorsals has the shape of a thick cylinder. The zone of growth has become transferred from the vertebral to the intervertebral region, since the perichordal tube in the vertebral region has cartilaginous basidorsals, which cause a rigidity which impedes its growth in thickness.

In the tail the fibrous perichordal tube is of uniform thickness. There are fibrous arches developed at the posterior (caudal) half of each myotome. These arches are not complete.

Stage 25 mm.—The dorsal part of the fibrous perichordal tube of the vertebral region is now in procartilaginous condition.

The fibrous perichordal tube in the intervertebral region is thickened, and owing to the pressure exerted by the basidorsals on it before and behind it forms an outward bulge in the middle of the intervertebral region with two tapering ends and thus the zone of growth of the perichordal tube is restricted to the intervertebral portions. The bulging constricts the notochord a little, so that the place where originally the notochord was dilated now becomes constricted (fig. 2, Plate 14).

The connective tissue lining the cups of the atlas and the balls of the occipital region becomes cartilaginous, but there is a good deal of difference of character between those cells and the cartilaginous cells of either the cup proper or the ball proper.

The neural arch of the atlas is formed by the fusion of the cups, *i.e.*, part of the intercalated arch with the atlas arch proper. The cups are deflected ventrally so that the spinal cord is not covered mid-dorsally in this region. Only the atlas arch proper completely covers the spinal cord. There is no trace of a diapophysis of this vertebra.

The neural arches of the vertebræ from 2nd to 8th have almost the same appearance. The cartilaginous basidorsals of two sides meet mid-dorsally where there were connective tissue cells in the 20 mm. stage (fig. 3, Plate 14). On the dorsal aspect of the neural arch element there is a slight projection of cartilaginous tissue with a mass of sclerotomic cells at its free end. These are the beginnings of the transverse processes (*diapophyses*) and the pad of cells at the free end is the rudiment of the rib. The dorsal arcualia of the 9th and the 10th vertebra fuse and connective tissue cells migrate in a slanting manner so as to divide the two arcualia on both sides, and the balls go to the end of the 9th and the sockets to the 10th. In between these two articulations there is the intervertebral element which is almost crushed in and also divided in a straight line by an extension of the connective tissue cells which also divided the arcualia.

In the region of the 10th and the 11th vertebræ, that is, in the anterior region of the future urostyle, the basal parts of the dorsolateral sclerotomic cell-masses become changed from ordinary sclerotomic cells to procartilaginous ones.

Below the notochord just where there was a mass of spindle-shaped connective tissue cells in the 20 mm. stage, and at the centre of which there was the sclerotomic hypochord, there is now incipient cartilage. There is no indication of this cartilage being formed from two separate elements.

Stage 30 mm.—The occipital arch with occipital condyles is a massive homogeneous structure, and so also are the cups and the atlas arch proper. The synovial cavity is also prominent between the balls (occipital condyles) on the one hand and the sockets and the intervertebral element between them on the other. The suboccipitalis nerve is almost degenerating.

The neural arches have extended dorsolaterally, the extension of the one arch meeting the extension of the next behind. These extensions of two consecutive arch elements have become continuous procartilaginous rods slanting craniocaudally, and should be called *dorsointervertebrals*. Connective tissue cells grow in so as to divide these rods into two in a horizontal line at the middle of the rods, so that the dorsal portion of each fuses with the posterior portion of each neural arch, and is known as *postzygapophysis*, and the ventral portion fuses with the anterior end of the next neural arch, and is known as *prezygapophysis*. The strands of connective tissue which penetrated the procartilaginous dorsointervertebral bodies have become split up into two with a cavity inside them. This cavity is the synovial cavity between the two zygapophyses.

The ventral part of the perichordal tube of the vertebral region which was fibrous in the previous stage becomes procartilaginous, so that the notochord in the vertebral regions has a ring of which the dorsal and lateral parts are cartilaginous and the ventral is procartilaginous. The neural arches bear diapophyses dorsolaterally with procartilaginous cells at their free ends (fig. 4, Plate 14). These processes are very large in the case of the 9th vertebra.

Stage 32 mm.—The neural arch of the 1st (atlas) vertebra becomes converted into bone, and this ossification commences in the perichondral layer, which thus become periosteum. The transverse processes of the 2nd to the 8th vertebræ are now cartilaginous. The 9th vertebra has bigger transverse processes, which are also cartilaginous. The perichordal tube in the vertebral region is a complete ring of cartilage.

The perichordal tube in the intervertebral regions of the trunk is getting thicker with further growth, and this crushes the notochord. In the trunk region, *i.e.*, from the 1st to 9th vertebræ below the perichordal tube at each vertebral region, there is an aggregation of fibrous connective tissue which is homologous with the hypochordal cartilage of the urostyle and represents the missing basiventrals.

If we examine the side view of the tail, after clearing it in cedarwood oil under the microscope, we find that neither the notochord nor the spinal cord is extended in a straight line, but that at the posterior end of the tail they are both pushed slightly ventrally.

The neural arches of the 10th and the 11th vertebræ are cartilaginous. The articulations of the 9th with that of the 10th, namely, the two balls and the two sockets formed by the division of the arcualia of the 9th and the 10th, are very prominent, and a cavity is formed between the connective tissue cells which divided them into ball and socket.

Beyond the 9th vertebra above the anterior portion of the urostyle there is not a complete neural arch enveloping the spinal cord. In this region the neural arch forms the cups on its two sides for the articulation with the 9th vertebra. Posterior to this region there are two neural arches which completely envelop the spinal cord. These two complete neural arches are now cartilaginous. Behind the last neural arch there are no basidorsals, but only connective tissue surrounding the spinal cord. On the ventral side of the perichordal tube is the cartilaginous hypochord (fig. 5, Plate 14).

Stage 35 mm.—The number of notochordal septa has been increased, so that the intervening spaces have become very much reduced in size. The dorsal portions of the basidorsal cartilages have been replaced by an osseous tissue containing a marrow cavity, but their bases are still cartilaginous. The perichondral layer becomes considerably thicker round the distal end of the basidorsal, and is converted into the periosteal layer. The fibrous perichordal tube of the intervertebral region has been changed into procartilage, and exerts a pressure on the notochord, constricting it slightly dorsoventrally as well as laterally.

In the trunk region the fibrous hypochordal structure (basiventral element) is very prominent.

The procartilaginous pre- and post-zygapophyses of the previous stage are now cartilaginous, and the connective tissue cells bordering the synovial cavity between the pre- and the post-zygapophyses, are now in procartilaginous condition

B.—*Stages during Metamorphosis.*

Stage 26 mm. (in the reduction of the tail during metamorphosis).—The notochordal septa are very thick; both the chordal epithelium and the sheaths are very prominent. In the intervertebral regions owing to the pressure of the perichordal tube, the sheaths are very much folded. The cartilaginous cells of the perichordal tube of the intervertebral region are quite different from the cartilaginous cells of the neural arches.

In the region of the 1st vertebra, the notochordal sheaths are very much folded owing to the pressure exerted by the cups of the 1st vertebra. The septa here are very thick compared with the septa of the other vertebræ of the trunk region.

The neural arch of the 9th vertebra has bulky transverse processes which articulate with the ilium at the side, a little below the tip of the latter. The notochord is flattened dorsoventrally by the pressure of the perichordal tube.

The perichordal tube in the anterior portion of the urostyle becomes thickened laterally owing to the pressure exerted by the upgrowth of the hypochordal cartilage from below. The anterior portion of the hypochordal cartilage as far as the level of the last neural arch is oval in cross-section, and beyond this position as far as the level of the cloaca it is round.

The notochord is very much larger in the tail region than in the trunk or the urostyle region and the spaces between the septa are much wider than in the trunk region. The notochordal sheath is very much folded since there are no rigid structures outside it and the fibrous perichordal tube is very prominent. The spinal cord is gradually reduced and is enveloped by the fibrous neural arches.

Stage 20 mm. (in the reduction of the tail during metamorphosis).—In the urostyle region the notochordal septa are seen to be breaking down, whilst in the tail region, where the notochord is especially large, they are very prominent and still complete. The sheaths in the tail region are much more folded whereas in the urostyle they are not. In a sagittal section the hypochordal cartilage can be seen as a rod-like structure with a bulky posterior and a tapering anterior end. The cut ends of the two neural arches can be seen, behind which there is no arch, since the spinal cord is only enveloped by connective tissue (fig. 6, Plate 14).

Stage 12 mm. (in the reduction of the tail during metamorphosis).—The dorsal parts of the 2nd to the 8th neural arches have become bony. The cartilage cells between the perichondral layers are destroyed and replaced by a mass of bone-marrow. The rest of the arch remains cartilaginous.

The notochord in the 9th vertebral region is flattened ventrally whereas in the anterior, *i.e.*, 1st to 8th vertebral regions, it is circular in cross-section.

There are fibrous connective tissue cells ventral to the cartilaginous perichordal tube on all the trunk vertebræ. This connective tissue projected downwards in the previous stage, but now it has changed its shape and almost touches the ventral portion

of the cartilaginous perichordal tube of the vertebral region. In the urostyle region where there is the hypochordal cartilage the fibrous connective tissue is absent.

In the region of the urostyle the hypochordal cartilage is much strengthened and it pushes upwards, so that the notochord is flattened dorsoventrally, but in the posterior region the notochord is still round in transverse section.

In the tail region the notochord is irregular in shape, the sheaths being much strengthened and folded. The notochordal septa and epithelium are not in contact with the sheaths or the perichordal tube. Based on the perichordal tube on each side and enveloping the spinal cord there is a mass of fibrous connective tissue which represents collectively all the basidorsals of the tail. It is interrupted metamerically by holes for the spinal nerve ganglia. Similar masses are based on the ventral aspect of the notochord and meet below thus enclosing the caudal blood vessels. These masses obviously represent the hæmal arch elements (fig. 7, Plate 15).

C.—*Young Adult Stages.*

Stage 10 mm. (metamorphosis just completed).—The procartilaginous perichordal tube of the intervertebral regions has become changed into cartilage and a regular strand of connective tissue cells is growing into this in a slanting manner from all directions to converge in a single point. We know that *Rana temporaria* has procoelous vertebræ, so that this strand of connective tissue slants caudalwards. The growth of the intervertebral perichordal tube is not uniform. The dorsal portion undergoes greater growth than the ventral and with the growth of the dorsal portion this cartilaginous mass constricts the notochord, so that in a series of cross-sections we find the intervertebral perichordal cartilaginous tube beginning dorsolaterally.

In the anterior region of the urostyle the hypochordal cartilage has completely compressed the notochord dorsoventrally, so that the septa are no longer to be seen. The fibrous perichordal tube has also changed to cartilage. In the posterior region of the urostyle the notochord is unchanged.

Stage 12 mm. (after metamorphosis).—In the trunk region, the intervertebral perichordal cartilaginous tubes have become larger and have greatly constricted the notochord. As a result of this inpushing the intervertebral perichordal cartilaginous discs become shortened and successive basidorsals are thus closely approximated to one another and thus a shortening in length of the vertebral column as a whole is effected (fig. 8, Plate 15).

Stage 20 mm. (about one year old).—The dorsal parts of the neural arches and the transverse processes have become osseous. The cartilage cells of these structures have been destroyed and replaced by bone-marrow. The bases of the neural arches are still cartilaginous. The free ends of the transverse processes have changed from procartilage to cartilage. The dorsal portion of the perichordal cartilaginous tube is now directed downwards and lateralwards. The connective tissue cells which grew in between the 9th and 10th neural arches have split up the intervertebral disc into

distinct pieces which are attached to the 9th vertebra and to the urostyle respectively, and these connective tissue cells are now cartilaginous.

Stage 30 mm. (about two years old).—The neural arches of the trunk region are now almost entirely osseous, only a few scattered patches of cartilage being still present, and the periosteal layer is very thick. In the vertebral region the notochord is still surrounded by the cartilaginous perichordal tube. Only at the beginning as well as the ends of the vertebral region are the bases of the basidorsals of the right and of the left side connected by means of a strip of cartilaginous cells. The notochordal septa in this region are very thick and sheaths are very prominent.

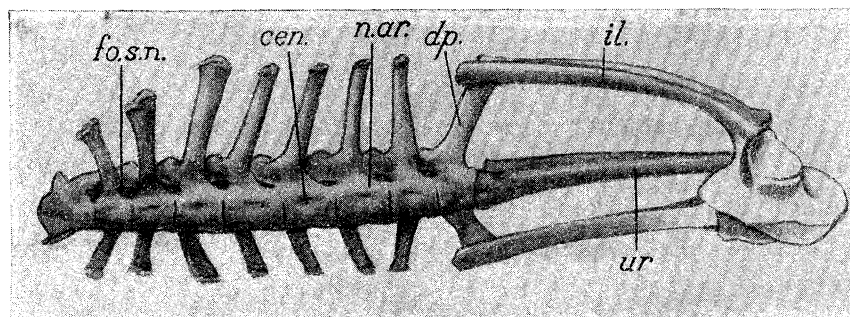
In the intervertebral regions, although the perichordal tube has constricted the notochord to a great extent, still continuity of the notochord is unbroken (fig. 9, Plate 15). The connective tissue cells which grew in the intervertebral perichordal tube have been split up into two, keeping the direction of their line. These connective tissue cells are now cartilaginous. The intervertebral perichordal cartilage is divided into two. The big cranial part forms the socket and the small caudal part forms the ball. Thus the procelous vertebra is formed.

Above the urostyle the notochord is completely obliterated. In the anterior portion of the urostyle the neural arches are almost completely osseous and mostly replaced by bone-marrow, only their mid-dorsal parts which ultimately form the dorsal ridge of the urostyle being still cartilaginous. The hypochordal cartilage has become fused with the basal part of the basidorsals, and the mass so formed has been converted into bone, except for the ventral part. The posterior part of the last neural arch of the urostyle is prolonged posteriorly and fused with hypochordal cartilage and the conjoined mass so formed is converted into bone (fig. 10, Plate 15).

Stage 50 mm. (about three years old).—The notochord persists only in the vertebral regions, having been obliterated in the intervertebral regions. The cranial part of the intervertebral cartilage is now completely fused with the preceding vertebra and it is in process of being replaced by bone-marrow. The two halves of the intervertebral cartilage are now almost completely separated, only a narrow neck of cartilage remaining between them. Thus the vertebra has definitely assumed the procelous character typical of Anura. The cartilage cells of the edge of the ball or the socket are quite different from either the cartilaginous cells of the ball or the socket proper because these edges are derived from the connective tissue cells migrating inside the intervertebral cartilage to cut it into two (fig. 11, Plate 15).

Stage (adult skeleton).—The first (atlas) vertebra has two sockets at the anterior end. These sockets project laterally and are not connected dorsally so that the spinal cord remains uncovered dorsally between the cranium and the 1st vertebra. Each trunk vertebra has a distinct centrum and the arcualia are superimposed on it. There are lateral transverse processes for each vertebra from 2nd to 9th. Each transverse process has a piece of cartilage at its free end. The transverse processes of the 9th vertebra are very large compared to the others. Each articulates with

the ilium on the side a little below the tip keeping the ilium ventral to the transverse processes. All the vertebræ from 1st to 7th are procœlous. The 8th is amphiocœlous and the 9th has a condyle at its anterior end and two condyles at its posterior end. The anterior end of the urostyle has two cups to articulate with the two condyles of the 9th vertebra. The cups of the urostyle are not connected with one another dorsally so that the spinal cord between the 9th vertebra and the urostyle remains unprotected dorsally.



TEXT-FIG. 1.—Lateral view of the entire vertebral column of an adult *Rana temporaria*. *dp.*, diapophysis; *cen.*, centrum; *fo.s.n.*, foramen for a spinal nerve; *il.*, ilium; *n.ar.*, neural arch; *ur.*, urostyle.

Bufo melanostictus.

A.—Stages before Metamorphosis.

The early development of the vertebral column in this species which we have studied in a series beginning with an embryo 3 mm. long, is absolutely identical with that of *Rana temporaria* up till the beginning of metamorphosis. The formation of the mesenchymatous sheath of the notochord, of the basidorsals, of the centra and of the intervertebral discs proceeds in exactly the same way. So, too, the intercalated arch is formed between atlas and the occipital region of the skull, and split into two by penetrating connective tissue, the front half forming the condyles of the skull and the hinder the cups on the atlas vertebra.

The so-called dorso-intervertebral cartilage connecting successive basidorsals also arises in the same way as in *Rana* and is cut into pre- and post-zygapophyses.

There is also formed as in *Rana* a continuous cylindrical mass of fibrous cells beneath the notochord and its perichordal tube, extending from the union of the arches of the aorta to the root of the tail, which as in *Rana* we term the hypochord.

In the tail region in *Bufo* as in *Rana* no distinct basidorsals are formed, but the spinal cord is enveloped in a continuous fibrous tube pierced with a series of holes in which the spinal ganglia are contained. This fibrous investment sends a prolongation into the dorsal fin. A similar fibrous sheath ventral to the notochord surrounds the caudal artery and caudal vein and sends a prolongation into the ventral fin.

B.—Stages during Metamorphosis.

Stage 9 mm. (metamorphosed).—It should be noted here that the disappearance of the tail of *Bufo melanostictus* is very sudden, quite unlike that of *Rana temporaria*.

The connective tissue layer which joined the bases of the right and left halves of the neural arches above the notochord has changed into cartilage. The neural spines are cartilaginous. The pre- and post-zygapophyses are cartilaginous. The connective tissue cells which divided them and which formed the edges of the synovial cavity are now in procartilaginous condition.

The intervertebral portion of the perichordal tube is now procartilaginous and its growth constricts the notochord very much. Thus we find the swellings of the notochord in the regions alternating with those in which it was originally swollen. Originally the dilatations were restricted to the intervertebral regions and constrictions to the vertebral regions ; now they are just reversed.

The connective tissue which entered into the intercalated piece of arch between the occipital arch and the atlas arch proper has been divided into two and the connective tissue cells themselves have become cartilaginous, but between the cartilaginous cells of the arch and these cells there is a good deal of difference in character. The major portion of the intercalated arch goes to the occipital arch and fuses with it forming the occipital condyles. The minor portion is fused with the atlas arch proper as cups to articulate with the occipital condyles (fig. 13, Plate 16). In a frontal section the atlas arch has a massive structure in comparison with the other vertebral arches of the trunk region (fig. 12, Plate 16).

Connective tissue cells also cut into the intervertebral procartilaginous structures in a slanting manner, and the direction of the migration determines the nature of the vertebra whether pro- or opisthocœlous.

The 9th arch and the 10th arch are fused together and the anterior portion of the 10th is not complete dorsally. Connective tissue also cuts in at the bases of these two arches just at the spot where they are joined. These connective tissue cells so cut in as to form two balls and two sockets, as in the occipital region. It should be noted here that these sockets *are not formed from the intervertebral cartilage*, but the intervertebral cartilage divides in a straight line forming the space which intervenes between the two balls, or the two sockets, respectively.

Beneath the ventral region of each cartilaginous vertebral ring there is now to be seen a mass of connective tissue whose position corresponds to that of the hypochordal element of the urostyle and it can be said to be the vestige of the basiventral. The hypochordal cartilage has pressed upwards crushing the notochord.

Stage 15 mm. (after metamorphosis).—The basidorsal develops a flexible region near its base by a modification of the cartilage cells. The cells in this region develop little intercellular substance and remain in an embryonic condition. This region may be regarded as an incipient suture (*s.*, fig. 14, Plate 16), and a comparison is suggested between it and the neuro-central suture in reptiles. It does not persist however and is completely obliterated when ossification sets in.

The intervertebral cartilage constricts the notochord in a most peculiar way. It grows downwards and lateralwards, inwards and upwards, so that in transverse section it has

a quite distinct appearance from that of the transverse section of *Rana temporaria*. There are narrow cavities in the dorsal region of the dorsal arcualia.

C.—*Stages of the Young Toad.*

Stage 27 mm. (about two years old).—The dorsal arcualia together with the transverse processes become osseous and the cartilaginous cells inside them are eaten up by bone-marrow. The neural spines have become osseous. The vertebral rings are also partly ossified. The intervertebral bodies have been completely divided into two portions which are still partly cartilaginous and partly replaced by bone-marrow, but their edges remain as cartilage; this cartilage is formed from the connective tissue cells migrating inside the intervertebral body (fig. 15, Plate 16). The pre- and the post-zygapophyses are evacuated by bone-marrow but the edges of the synovial cavity have cartilaginous cells derived from the connective tissue cells which migrated inside the dorsointervertebral body.

Stage (adult skeleton, and comparison with the adult skeleton of *Rana temporaria*).—The atlas vertebra is just the same as that of *Rana*. All the trunk vertebræ from the 2nd to the 8th are procœlous, but whereas the 8th vertebra in the case of *Rana* is amphicœlous, in *Bufo melanostictus* it is procœlous. The transverse processes of the 9th vertebra in the case of *Rana* are downward processes, but in *Bufo* they are at right angles to the centrum. Their articulation with the ilium is just the same as that of *Rana*. The 9th vertebra has a cup at its anterior end, whereas in *Rana* it has a ball. The posterior ends of the 9th vertebra of both have two condyles.

Bombinator igneus.

A.—*Stages before Metamorphosis.*

(1) 5 mm.—The notochord in this stage is still a vacuolated rod of cells surrounded by the elastica interna and externa, outside which are loose mesenchyme (sclerotomic) cells.

(2) 8 mm.—In this stage, the *notochordal epithelium* is formed, and also regular septa (remnants of the notochordal cells) traversing the gelatinous interior of the notochord. The *perichordal rings* embracing the notochord have appeared, as also the first indications of the mesenchymatous basidorsals.

(3) 10 mm.—The perichordal rings have extended fore and aft, so as to form the perichordal tube. The mesenchymatous basidorsals have grown bigger and denser, and press on the notochord, so as to impede its growth in the intermyotomal regions, and thus give to the rod a moniliform shape. The basidorsals, however, do not rest directly on the notochord, but as in *Rana* and *Bufo* are separated from it by a thin layer of perichordal tube. In the hinder region of the larva, the basidorsals are only beginning to be formed (fig. 16, Plate 16).

(4) 12 mm.—The basidorsals have begun to chondrify and those in front are completely

cartilaginous. The perichordal tube between successive basidorsals has become greatly thickened—these thickenings impede the growth of the notochord more than do the basidorsals—and so now the dilatations of the notochord are found beneath the basidorsals. The intercalated arch between the atlas vertebra and the cranium has appeared, and is already being cut into two pieces (occipital condyles in front — atlas cups behind) by an ingrowing sheet of connective tissue cells.

The formation of definite centra by the chondrification of the perichordal tube has begun. This cartilage appears *dorsally* between the bases of the basidorsals. Basidorsals do not yet meet above the spinal cord, their apices being joined by a transverse belt of connective tissue. The 2nd, 3rd and 4th pairs of basidorsals have developed lateral outgrowths (diapophyses) which are chondrified, but there are pads of mesenchymatous cells at their apices, which are the rudiments of ribs. Ten pairs of basidorsals are formed, and beyond this there are fibrous masses in the tail region. Beneath the notochord behind the 9th arch is the fibrous rudiment of the hypochord.

B.—*Stages during Metamorphosis.*

(1) 10 *mm.* (the tail has begun to shrink).—The perichordal tube in the region of the atlas is completely chondrified, the other vertebral regions are only chondrified dorsally, and the intervertebral rings are also chondrified dorsally. All the neural arches have now diapophyses, and the rudiments of the ribs at the apices of the 2nd, 3rd and 4th pairs of diapophyses are also chondrified, as is the hypochord.

The intervertebral cartilages are beginning to be cut into ball in front and socket behind by ingrowing connective tissue cells. The fibrous basidorsals and hæmal arches in the tail have extended so as to form a continuous investment of the spinal cord above, and the hæmal vessel below.

(2) 8 *mm.* (the tail is reduced to a vestige).—The basidorsals have begun to ossify, and narrow cavities have appeared in their apices.

Dorso-intervertebrals have appeared connecting successive basidorsals, and are in process of being divided into pre- and post-zygapophyses. Where right and left arches meet, a neural spine is developed as a backwardly projecting mass of cells. The ribs attached to the 2nd, 3rd and 4th diapophyses have developed sutures between themselves and the diapophyses.

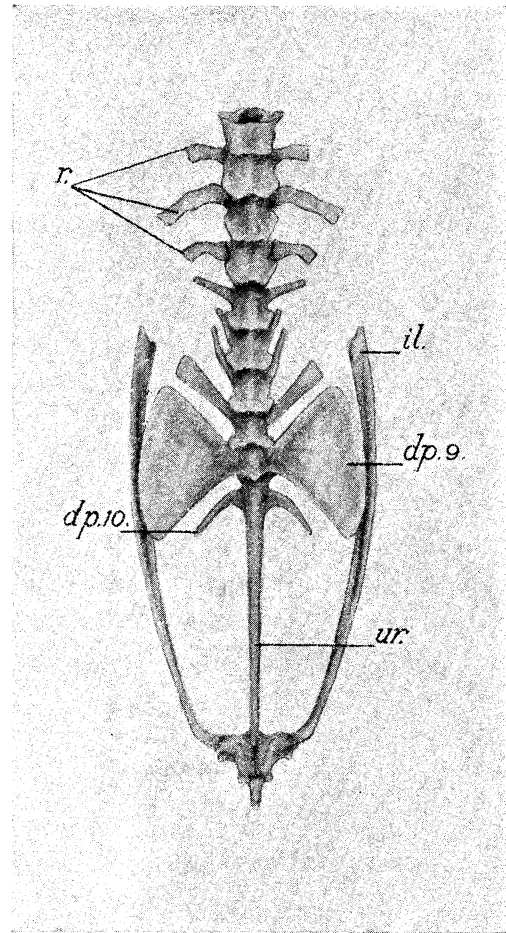
C.—*Stages of the Young Adult.*

(1) 7 *mm.* (immediately after the tail has disappeared).—The notochord in the trunk region is becoming crushed dorso-ventrally. It degenerates as does the ventral portion of the perichordal tube, and so the *bony centra are formed only from the dorsal parts of the perichordal tube.* The hypochord has now fused with the basidorsals above it, and the notochord between has almost disappeared. The intervertebral cartilage between 9th and 10th pairs of basidorsals remains undivided, and so these two vertebræ have become fused with one another.

(2) 12 *mm.*—The diapophyses of the 9th vertebræ have become expanded into crescents. Only isolated remnants of the notochord remain in the vertebral column, both in vertebral and intervertebral regions. In the urostyle region, the notochord has completely disappeared.

(3) 25 *mm.* (about two years old).—The whole neural arch is a mass of bone-marrow. The transverse processes of the 2nd, 3rd and 4th vertebræ and their attached ribs are also evacuated by bone-marrow and look like long transverse processes only. The transverse processes of 5th, 6th, 7th and 8th vertebræ project not exactly in a transverse direction, but rather forwards. The 9th vertebra has the wing-like transverse processes, and the 10th has ordinary transverse processes. The vertebral bodies have also become osseous, and cartilage cells are replaced by bone-marrow. The intervertebral bodies have been completely divided into two, the ball portion articulating in front of a vertebra, and the socket with the posterior end of the previous vertebra. Except the edges the whole mass is osseous. The edges are still cartilaginous.

Adult vertebral column.—The atlas (1st) vertebra has a cylindrical centrum, since the whole perichordal tube chondrifies at this region. The 2nd, 3rd and the 4th vertebræ have long transverse processes which are formed not only from the transverse processes proper, but also from a fusion of the ribs, which lose their identity with ossification. The 5th, 6th, 7th and 8th vertebræ have upwardly directed transverse processes. The 9th has flat, disc-shaped transverse processes to articulate with the ilium at the side, a little below its tip. The urostyle has a pair of transverse processes at its anterior end, which show the position of the 10th vertebra, but the rest of the urostyle is fused with the posterior end of this. (Text-figure 2.)



TEXT-FIG. 2.—Vertebral column of adult *Bombinator igneus* seen from dorsal side. *d.p.9.*, expanded diapophysis with which the ilium articulates; *d.p.10.*, diapophysis of the 10th vertebra fused with the urostyle; *il.*, ilium; *r.*, ribs fused with the diapophyses of the 2nd, 3rd and 4th vertebræ.

but the rest of the urostyle is fused with the

*Xenopus laevis.*A.—*Stages before Metamorphosis.*

(1) 5 mm.—The notochord is a vacuolated rod of cells, laterally compressed in front and behind and cylindrical in the middle.

(2) 7 mm.—The notochordal epithelium and *elastica interna* and *externa* have been formed. Outside this the loose mesenchymatous perichordal rings have appeared which already are beginning to coalesce so as to form the perichordal tube.

(3) 9 mm.—Membranous basidorsals have appeared and have by their pressure made the notochord moniliform.

(4) 12 mm.—The three first pairs of basidorsals have become procartilaginous and the intercalated arch between atlas and occipital condyle has made its appearance. The basidorsals are connected above the spinal cord by fibrous tissue. The 5th, 6th, 7th, 8th and 9th pairs of basidorsals are still fibrous—a 10th pair is not formed. The membranous rudiment of the hypochord has appeared (fig. 17, Plate 16).

(5) 18 mm.—The intercalary arch is divided by immigrating connective tissue cells into occipital condyles in front and atlas cups behind (fig. 18, Plate 17). The intervertebral portions of the perichordal tube have grown thick and compress the notochord so that dilatations now occur beneath the neural arches and its moniliform shape is reversed.

(6) 31 mm.—The halves of the intercalary arch have fused with occipital region in front and atlas behind. The whole perichordal tube in the atlas region is chondrified (as in *Bombinator*). The basidorsals are chondrified, but are still connected above the spinal cord by fibrous tissue. In the vertebral regions the dorsal portions of the perichordal tube are chondrified. The sides of the tube remain fibrous. *Its ventral portion however becomes changed into a rectangular unsegmented cartilage—not to be confused with the hypochord, which is below and outside the perichordal tube* (fig. 19, Plate 17). All the basidorsals (neural arches) are now chondrified: there is no intervertebral region between the 9th vertebra and the urostyle. The hypochord is procartilage. There are two pairs of fibrous basidorsals above the urostyle behind the 9th vertebra and in the tail region there are fibrous dorsal and ventral arches.

(7) 40 mm. *stage.*—Dorso-intervertebrals have appeared between successive 1st, 2nd and 3rd pairs of neural arches and are being divided into pre- and post-zygapophyses. On the 2nd, 3rd and 4th pairs of neural arches very small knob-like diapophyses have appeared. The remaining neural arches are now chondrified, but still incomplete dorsally. The membranous basidorsals above the urostyle are now procartilage. The dorsal portion of the perichordal tube in the vertebral regions is cartilaginous, but in the intervertebral regions it is still procartilage. The ventral rectangular portion of the perichordal tube in the whole trunk region is now procartilage (fig. 20, Plate 17).

(8) 52 *mm.* stage.—The notochord—in former stages compressed laterally—is now compressed dorso-ventrally. In the atlas region the whole perichordal tube is chondrified, forming a ring-shaped centrum which is beginning to be ossified. The ventral part of the perichordal tube is now cartilage, as are also the dorsal intervertebral portions (figs. 21 and 22, Plate 17).

B.—*Stages during Metamorphosis.*

(1) 40 *mm.* (tail reduced).—The atlas vertebra is ossified. The intervertebral cartilages are being divided by connective tissue cells into ball and socket. The basidorsals are all ossified and the notochord is compressed dorso-ventrally, very much shrunken and in process of degeneration. The sides of the perichordal tube and its ventral portion consisting of the rectangular cartilage are also in rapid degeneration, only the dorsal part remains, and from this the trunk centra (as in *Bombinator*) are formed (figs. 23 and 24, Plate 18). In the urostyle region the notochord and perichordal tube also ultimately degenerate and the hypochord presses upwards joining the 9th pair of neural arches.

(2) 32 *mm.* (tail a vestige).—The centra formed from the dorsal portions of the perichordal tube are now being ossified. The intervertebral cartilages are divided into ball and socket. In the 2nd, 3rd and 4th vertebræ flexible regions of young cartilage cells (*i.e.* incipient sutures) (fig. 25, Plate 18) between the small diapophyses and the ribs can be seen. The diapophyses of the 5th, 6th, 7th and 8th arches are directed upwards—those of the 9th vertebra are dilated but do not as yet articulate with the ilium.

The hypochord is fused with the tissue representing basidorsals anteriorly which is now chondrified, but in its posterior portion this tissue is lacking and here the spinal cord is exposed. The ribs are represented by masses of procartilage at the apices of the 2nd, 3rd and 4th vertebræ. The rectangular cartilage formed from the ventral part of the perichordal tube is still persisting beneath the trunk vertebræ and above the hypochord in the urostyle region (figs. 26 and 27, Plate 18). In the tail region the notochord persists elliptical in section and has above and below fibrous arches.

C.—*Young Adult.*

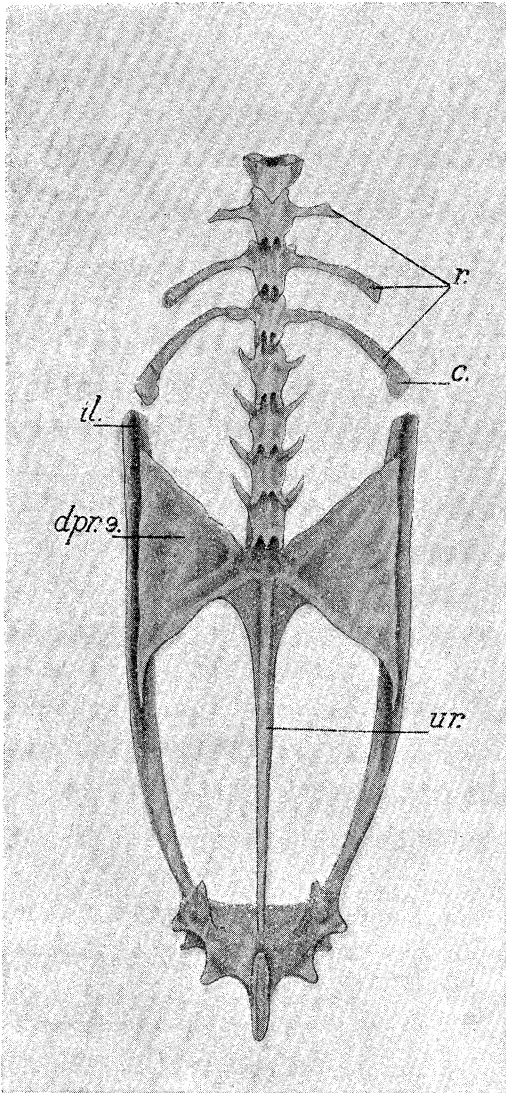
65 *mm.*—The trunk vertebræ are ossified and evacuated by marrow cavities, as are their neural arches and diapophyses. The ribs are ossified and indistinguishably fused with the diapophyses. The 9th diapophysis articulates with the ilium.

D.—*Fully-grown Adult.*

The whole urostyle is fused with the 9th vertebra. The diapophyses of this vertebra are expanded so as to look like butterfly wings and there are grooves on the

ilia into which these crescents fit. The 1st vertebra is cylindrical, the rest flattened.

The 2nd and 3rd have long diapophyses with which ribs are indistinguishably fused. The 5th, 6th, 7th and 8th have diapophyses directed upwards. (Text-figure 3.)



TEXT-FIG. 3.—Vertebral column of adult *Xenopus laevis* seen from the dorsal side. C., Cartilage at the end of the 4th rib; *dpr. 9.*, diapophysis of the 9th vertebra with which the ilium articulates; this vertebra is fused with the urostyle; *il.*, ilium; *r.*, ribs attached to diapophyses of the 2nd, 3rd and 4th vertebrae.

Discussion.

The transverse section of the developing notochord is at first rectangular, subsequently oval and eventually round. The general shape of the notochord is rod-like with two tapering ends. In *Rana temporaria* the notochord in the tail end of a tadpole has a bend, so that it resembles the notochord of a fish. This bend is also found in the spinal cord. The tadpoles of *Rana temporaria* have a big tail, whereas those of *Bufo melanostictus* have a small one.

That the notochord of Anura has a sheath of two layers was first discovered by GEGENBAUR (1862), but subsequently contradicted as well as supported by later workers. GOETTE (1875) failed to notice the two layers of the notochordal sheath in the case of the Unke (*Bombinator igneus*), and tried to explain away statements to the contrary by stating that there was a layer closely surrounding the inner sheath which developed from cells coming from the mesodermal somite and that this layer ultimately became cartilaginous and could be compared with the cartilaginous chordal sheath of Elasmobranch. He has hitherto neither been contradicted nor confirmed in his observation on *Bombinator*, as no one worked on this particular type after him, but the present writer's observation on *Bombinator igneus* clearly shows that he was

mistaken, as there are two distinct layers in the true notochordal sheath which can easily be made out, and the present writer has definitely proved that what GOETTE has called "äusseren chordascheide" is nothing but the perichordal layer which ultimately becomes cartilaginous.

From the observations made on four different types, viz., *Rana*, *Bufo*, *Bombinator* and *Xenopus*, it seems probable that the formation of the two layers in the notochordal sheath is of universal occurrence in the whole group of Anura.

The change of form of the notochord takes place after the formation of its sheaths and of the perichordal rings, which are derived from sclerotomic cells, corresponding to the myotomal regions. Corresponding to each myotomal (intervertebral) region there is a dilatation and corresponding to each intermyotomal (vertebral) region a constriction. The change of form of the notochord is due solely to the external forces. The perichordal rings in the myotomal region once formed protect the notochord in that region, but the condensations of mesenchyme, which give rise to the arches, compress the notochord in the interspaces of the perichordal rings.

After the formation of the dorsal arcualia, and on the completion of the perichordal tube by the fusion of the successive perichordal rings with the mesenchymatous tissue cells in the interspaces, the zone of growth changes from the vertebral to the intervertebral region. The dorsal arcualia, once formed, check the growth of the perichordal tube in the vertebral region, and so the constriction of the notochord ceases, but the intervertebral discs compress the notochord strongly and ultimately destroy it.

The change of form of the notochord, in its first phase of dilatation in the intervertebral region and constriction in the vertebral region, and their reversion in its second phase, has not been recorded in the case of Anura by any previous authors, although it has been recorded in Amniota by several authors.

The notochord in the vertebral region anterior to the urostyle in the case of *Rana temporaria* persists throughout life. In the case of *Bufo melanostictus*, it is ultimately displaced by a mass of bone-marrow. Both in the case of *Bombinator* and *Xenopus*, the notochord in the vertebral, as well as in the intervertebral regions, degenerates, leaving no trace of its existence in the adult form.

The notochord of the urostyle region also degenerates, and the so-called basiventral or hypochordal cartilage, which forces itself dorsalwards, ultimately fuses with the basidorsals forming a triangular piece in the anterior part. The posterior region of the urostyle, where there are no basidorsals, also degenerates.

In the tail region, the notochord has a uniform diameter throughout with a tapering end. It degenerates with the degeneration of the tail itself.

We have found in Urodela, clear indications of resegmentation of sclerotome for the formation of a definite vertebra. The same thing happens in the case of Anura.

It should be emphasised here that the skeletogenous layer outside the notochordal sheath, in the case of Anura in general, has the appearance of fibrous connective tissue cells for which GADOW (1896) suggested the name "membrana reuniens."

According to GEGENBAUR (1862), the vertebral body in Anura develops, as follows: after the formation of the notochordal sheaths, the skeletogenous tissue makes its appearance and fills up the spaces between the notochord and the spinal cord. Ultimately these skeletogenous tissue cells aggregate on the dorsolateral corners of the notochord

to form the neural arches (dorsal arcualia). They rest directly on the sheath of the notochord. These arches correspond to the caudal half of each myotome, while the spinal ganglia correspond to the cranial half. At the same time, another mass of skeletogenous tissue is formed mid-ventral to the notochord. Ultimately these skeletogenous aggregations become cartilaginous. These cartilaginous elements are connected by connective tissue to form a complete ring. The basal portion of each arch extends dorsally, as well as ventrally, so that the two unite to form a complete ring of cartilage which become the osseous vertebral centrum. This method of development of the vertebral column should be called the *perichordal* mode. The development of the vertebral body of both *Rana* and *Bufo* follows this mode. There is another mode of development of the centrum called the *epichordal* mode, in which the ventral element remains membranous and degenerates along together with the notochord. The centrum is thus formed only from the bases of the dorsal arcualia. This mode includes the development of the centrum of *Pseudis*, *Bombinator*, etc.

GADOW (1896) only supported the statement of GEGENBAUR (1862).

RIDEWOOD (1897), who worked only on *Xenopus* and *Pipa*, is of opinion that the arcualia sit directly on the notochordal sheaths. The mesenchymatous tissue below the spinal cord between the bases of the arches becomes cartilaginous, and also there is formed a cartilaginous body, square in section, below the notochord in the case of *Xenopus*. RIDEWOOD was unaware of what ultimately happens to the rectangular cartilage below the notochord, for he says, "What is the ultimate fate of this ventral cartilage of *Xenopus* I cannot say, since I have no late larvæ of the genus in which to study its subsequent history."

SCHAINS LAND (1906), whose work is merely a compilation, adds nothing more than the above statements.

It has already been stated that, outside the notochordal sheath, the sclerotomic cells form a fibrous tube-like structure. Support to this has been given by EMELIANOFF (1925), who worked on *Rana esculenta* and *Pelobates fuscus*, but who failed to observe either the moniliform notochord, or the fact that this fibrous perichordal tube follows the moniliform structure of the notochord outside. The vertebral (centrum) regions are constricted, and the intervertebral regions are dilated, so that in the vertebral region, especially at the dorsolateral corners of the notochord, the perichordal layer of the tube is very thin. Outside this thin layer of perichordal tube there form, on the two dorsolateral corners, aggregations of sclerotomic cells to form the dorsal arcualia. The cause of the thinness of the fibrous perichordal layer on the two dorsolateral corners is quite obvious; it is the pressure exerted by the aggregation of the sclerotomic cells which form the dorsal arcualia. This dorsolateral pressure transfers the growth of the perichordal tube from the dorsolateral towards the ventral side, and thus is formed a thick layer of perichordal tube mass ventral to the notochord.

Therefore the dorsal arcualia have nothing to do with the vertebral centrum. But GEGENBAUR, SCHWEGMANN, GADOW, RIDEWOOD and SCHAINS LAND failed to observe

that between the bases of the arcualia and the notochordal sheath there is a thin layer of mesenchymatous tissue which is quite different from the dorsolateral aggregation of the sclerotomic cells to form the arches.

In the cases of *Rana temporaria* and *Bufo melanostictus*, it is this fibrous part of the perichordal tube, just below the cartilaginous dorsal arcualia, which first becomes chondrified. The cells of this portion are quite distinct from the cartilaginous cells of the dorsal arcualia. This first chondrification of the perichordal tube at the bases of the dorsal arcualia has led to a mistaken idea that cartilaginous arcualia sit directly on the true notochordal sheath. Subsequently, the perichordal layer of the mid-dorsal portion and then of the ventral portion of the perichordal tube become chondrified. The cartilaginous bases of the right and the left arches then extend and meet dorsally, not throughout the whole extent of a vertebra, but only at its extreme ends, near the intervertebral spaces, contrary to the statement of EMELIANOFF (1925), who has asserted that the two arch bases meet dorsally on the notochord outside the perichordal ring. Even here one can easily distinguish the difference of the cartilaginous structures of the extended dorso-median arch bases from the mid-dorsal cartilage of the perichordal tube.

From the description of GEGENBAUR, SCHWEGMANN, GADOW and SCHAINSLAND, it follows that they regarded the condition of a particular region of the vertebral columns of *Rana* and *Bufo* as the general structure of the trunk vertebra, for we cannot otherwise explain why they described the formation of the trunk vertebra almost in the same way as that of the anterior portion of the urostyle. There is no separate ventral cartilage, such as they assumed to represent the united pair of hæmal arch elements.

In the case of *Bombinator igneus*, the perichordal tube chondrifies first beneath the bases of the dorsal arcualia, and then mid-dorsally, but the ventral portion, instead of chondrifying, degenerates along with the notochord. According to EMELIANOFF (1925), *Pelobates fuscus* follows the same sort of development of the vertebral centrum of the trunk vertebra. GOETTE (1875) is the first worker who noticed a difference between the cellular structure of the cartilaginous semi-ring dorsally to the notochord, and the cells of the overlying cartilaginous dorsal arcualia. But he failed to detect the two layers of the sheath of the notochord, and tried to reconcile this fact by saying that this perichordal tube was the outer layer. As almost all the workers in this line found these two layers quite distinct, and as the outer layer is not cartilaginous, nobody took any notice of the statement of GOETTE, and especially as no one has worked on this particular species since his time.

Xenopus lævis is a unique and most interesting case. The chondrification of the perichordal tube starts just as in the other cases first beneath the basidorsals and then mid-dorsally. As already stated, *Xenopus* has a rectangular cartilaginous structure ventral to the notochord which RIDWOOD (1897) thought might be homologous with the hypochordal cartilage of *Bombinator* described by GOETTE in the urostyle region. RIDWOOD emphasised this since nobody previously had described a hypochordal

cartilaginous structure in the trunk vertebræ in an animal with the so-called perichordal type of centrum. He also failed to elucidate the ultimate fate of this.

The present writer holds that this rectangular structure cannot be homologous with the hypochordal structure described by GOETTE. It is not outside the fibrous perichordal tube but forms a part of it; so that in *Xenopus* in the vertebral region there is a semi-circular cartilage at the base of the dorsal arcualia with a rectangular cartilaginous structure ventral to the notochord, and connecting these two structures to complete the ring is the fibrous layer on the two sides. At a later stage this rectangular structure degenerates together with the notochord and the two lateral connective tissue masses. The adult centrum is formed from the upper semi-circular cartilage.

The generally accepted classification of the formation of the vertebral centrum of Anura, viz., perichordal mode and epichordal mode, is sound, although the centra are not formed as previous workers have assumed from the arcualia but from the perichordal tube. In the perichordal mode (*Rana*, *Bufo*) the whole tube becomes chondrified and converted into centra, in the epichordal mode (*Bombinator*, *Xenopus*) only its dorsal portion forms the centra. In all Anura the 1st or atlas vertebra is formed from the whole perichordal tube.

As to the formation of the intervertebral element, GADOW is of opinion that there are interdorsal and interventral cartilages which are quite distinct chondrifications comparable with the basidorsals. According to him, the interdorsal element is very prominent and constricts the notochord dorsolaterally in the case of *Rana*, and in the case of *Bufo* it grows obliquely downwards and inwards. The interventral element takes an insignificant part in this construction.

According to the present writer, after the dorsal arcualia have become cartilaginous the perichordal tube of the vertebral portion is checked in its further growth and so the zone of growth is transferred to the intervertebral portion; and so the perichordal layer between the two cartilaginous arches acquires the form of a thick ring whose middle portion is thickest and the two sides tapering. A little later the pressure exerted by the growing intervertebral fibrous tissue on the notochordal sheath constricts the notochord to a greater extent: but this growth is not uniform all round the notochord. It constricts the notochord first dorsally, and in the case of *Rana temporaria* it presses from the dorsolateral corner towards the ventral side, but in *Bufo melanostictus* it presses directly downwards and inwards and upwards and inwards, so that in the two genera the transverse sections are absolutely different. This perichordal fibrous layer becomes cartilaginous. The transformation from fibrous tissue to cartilage starts first in the dorsal region. As there is no sharp line of distinction of the dorsal and the ventral regions we cannot discriminate interdorsal and interventral. Support for this statement has been given by EMELIANOFF (1925) who also could not find the two distinct masses of chondrification. After the intervertebral body becomes cartilaginous a strand of connective tissue cells grows in from all directions in a slanting manner inside this cartilaginous body forming an arc. It is interesting to find that the intervertebral

disc of *Rana temporaria* retains a vestige of the notochord even in a specimen more than two years old. Subsequently, the growth of the intervertebral cartilage destroys the notochord entirely. The connective tissue cells get inside the intervertebral cartilage forming an arc, split up into two lines following the line of arc. Subsequently, they become cartilaginous. The split becomes the synovial cavity and the ball and the socket fuses with the two consecutive vertebral bodies. In *Rana temporaria*, in the trunk vertebra all except the 1st, 8th and the 9th vertebra are procoelous. In *Bufo melanostictus*, there are some variations, but the general structure of vertebræ is procoelous. So that the connective tissue cells coming in from the side grow from a cranial towards a caudal direction so as to have the socket cranially and the ball caudally. Thus we get in each vertebra a socket in front and a ball behind. The cartilaginous layer derived from the strand of connective tissue remains as cartilage throughout life. The cartilaginous ball as well as the socket becomes osseous and evacuated by a mass of bone marrow.

In the case of the opisthocelous type the direction of the slant of the connective tissue cells is just the reverse of that of the procoelous type. The 8th vertebra of *Rana temporaria* is amphicoelous, but in *Bufo melanostictus* it is procoelous, so that the 9th vertebra of the former has one ball in front but the latter has got a socket in front. In both cases the 9th vertebra has two balls at the back in order to articulate with the urostyle. NICHOLLS (1914) reported these variations but could not give any explanation for them.

In *Bombinator igneus*, where only the dorsal portion of the perichordal tube of the intervertebral region becomes very thick, it squeezes the notochord dorsoventrally to a great extent. This thickened portion of the perichordal tube becomes cartilaginous. Subsequently a strand of connective tissue grows in slanting towards the cranial-end in the middle and the intervertebral cartilage becomes divided into a ball and a socket. The ball fuses in front of a vertebra and the socket behind. The ventral portion of the membranous semi-ring of the perichordal tube forming the intervertebral region degenerates without transforming into cartilage.

In the case of *Xenopus laevis* in the intervertebral region the dorsal portion of the perichordal tube becomes very thick and eventually becomes cartilaginous. As RIDWOOD (1897) had failed to observe the perichordal tube, he has stated that the intervertebral body was formed from the extension of the dorsal arcualia. The ventral portion of the perichordal tube, instead of remaining a fibrous layer, becomes a rectangular cartilage. This piece of cartilage is connected on two sides by means of fibrous tissue to the upper cartilaginous portion of the perichordal tube. The ventral cartilage is not metamericly segmented but is continuous throughout the vertebral column both in the vertebral as well as in the intervertebral regions. It eventually degenerates completely.

In both *Bombinator igneus* and *Xenopus laevis* the intervertebral cartilage proper, *i.e.*, both the ball and the socket portions, become evacuated by bone-marrow, but the

cartilaginous lining of the articular surfaces derived by the connective tissue strand which entered into the intervertebral cartilage in a slanting way remain as cartilage throughout life.

The present writer could not find any reference as to the formation of the double articulation between the 9th vertebra and the urostyle in the case of *Rana temporaria* and *Bufo melanostictus* in any previous work. The general conception is that these articulations are formed from intervertebral cartilages as in the case of the other vertebræ, but it is very interesting to know that the intervertebral cartilage is divided in a vertical plane instead of in a curve. The dorsal arcualia of the 9th vertebra fuse with the anterior portion of the dorsal arcualia of the urostyle. This latter portion is flattened laterally, and from the division of this arch we get the two balls and two sockets just as in the case of the occipital condyles and the sockets of the 1st vertebra. The intervertebral cartilage only persists in the interspace between the two condyles. The evidence of this is obvious from the adult dried skeleton in which we can see that the anterior portion of the urostyle has a gap in the place of the neural arch although there is a centrum. The complete arch can be seen at a place a little posterior to the anterior region of the urostyle.

In the anterior region of the urostyle there are dorsal arcualia on the two dorsolateral corners of the notochord and a hypochordal cartilage ventrally. These three pieces of cartilages are united together by means of connective tissue on the two sides.

The dorsal arcualia compress the perichordal tube to such an extent that apparently they appear to rest direct on the notochordal sheath and so also on the hypochordal (EMELIANOFF, 1925), but the whole notochord in this region as elsewhere is surrounded by the perichordal tube as we have already stated.

One can easily find that the former workers from GEGENBAUR (1862) to SCHAINSLAND (1906) had the idea that there are two dorsolateral cartilages forming the dorsal arcualia, and a ventral representing the hæmal arch element throughout the whole length of the vertebral column. Their descriptions of the formation of the vertebral column can only tally to a certain extent with the structure of the anterior portion of the urostyle, so that one cannot imagine that these workers ever cut sections of the actual trunk vertebra. As a matter of fact, SCHWEGMANN (1884) has given two figures, one for the trunk vertebra and another for the urostyle, and they resemble each other, the only variation being in their size.

In the urostyle region there are two pairs of dorsal arcualia at the anterior portion and a hypochordal cartilage starts from the beginning of the urostyle and continues up to its hinder end. It has a bulky posterior portion where there is no neural arch or cartilaginous dorsal arcualia. The spinal cord in this portion is enveloped by connective tissue arches.

GADOW (1896) has stated in the case of frog that the anterior portion of the urostyle has two lateral basidorsals and a pair of basiventrals (or hypochordals) for two vertebræ, the so-called 10th and 11th. The recent worker EMELIANOFF (1925) has not contradicted him.

The present worker could not find a pair of cartilages ventral to the notochord in the anterior portion of the urostyle, but found only one median cartilage.

In the cases of *Rana temporaria* and *Bufo melanostictus* there are two dorsal arcualia at the anterior portion of the urostyle. The hypochordal cartilage pushes upwards, crushing the notochord and fuses with the dorsal arcualia, and the perichordal tube by this time becomes cartilaginous. The whole fused mass becomes osseous and replaced by a mass of bone-marrow.

The urostyle of *Bombinator* has no double articulation with the 9th vertebra as in the other two cases. The 9th vertebra is an opisthocœlous one. The hypochordal cartilage is only present in the urostyle region as GOETTE (1875) described.

In *Xenopus lævis* the urostyle is fused with the 9th vertebra. What RIDWOOD (1897) regarded as a hypochordal cartilage extending from the atlas up to the end of the urostyle is not homologous with the hypochordal of other cases as RIDWOOD thought. It has already been proved that this is a part of the perichordal tube. RIDWOOD has described another cartilaginous structure below his so-called hypochord in the urostyle region, which he calls the cartilaginous ridge of the hypochord. This ridge is the true hypochord. As he could not trace the ultimate fate of his so-called hypochordal structure, he attempted to homologise it with the lower part of the urostyle, but the present writer has traced it up to the adult form and found that what RIDWOOD calls hypochordal proper is nothing but a part of the perichordal tube which ultimately degenerates. The real hypochord (RIDWOOD's so-called ridge of the hypochord) persists till it fuses in the anterior portion of the urostyle with the dorsal arcualia crushing the notochord entirely; the rest forms the ventral part of the posterior portion of the urostyle.

The sclerotomic arches, as has already been stated, form corresponding to the caudal half of each myotome and corresponding to the cranial half there is the spinal nerve ganglion. The secondary segmentation displaces the position of the intervertebral structure, so that the arch generally bends caudally and within this curvature the spinal ganglion of the next cranial half segment of the myotome comes. The position of the primary segment was from one intermyotomal region to the next intermyotomal region. The secondary segmentation changes their position and a vertebral segment is from the middle portion of one myotome to the middle portion of the next myotome. The arches grow not only upwards but also extend their bases. With their growth, the arches envelop the spinal cord completely and there forms a dorsal projection which develops where the basidorsal of one side meets the basidorsal of the other. These neural spines slant cranio-caudally owing to the fact that the arch itself is a slanting structure.

The neural arches also extend dorsolaterally connecting one arch with the extension of the next arch. These extensions of two consecutive arch elements are continuous procartilaginous rods slanting craniocaudally which we have termed dorsointer-vertebrals. Connective tissue cells get in to divide these rods into two. In the cases of

Rana temporaria and *Bufo melanostictus* it divides into two in a horizontal line at the middle of the rod. In the cases of *Bombinator igneus* and *Xenopus laevis*, the connective tissue cells divide the rod into two in a curved line at the middle of the rod. The dorsal portion (because the rod is slanting craniocaudally) adheres to the anterior neural arch as post-zygapophysis, and the ventral portion articulates with the front end of the next neural arch as pre-zygapophysis. The strand of connective tissue cells which entered inside the procartilaginous dorsointervertebral bodies have split up into two with a cavity inside as synovial cavity.

The cartilaginous basidorsal becomes osseous and the bone is then hollowed out by marrow cavities. It is interesting to note that the marrow cavity starts from the dorsal portion of the arch and then spreads downwards towards the bases, although it is the base which first becomes ossified.

In *Bufo melanostictus*, after the appearance of the cartilaginous basidorsal there forms an incipient suture by a change of character of some of the cartilaginous cells. In a transverse section these sutures are quite distinct. The arrangement of the cells of this suture is quite different in either the basal portion of the basidorsal or the dorsal arch portion. The arrangement and character of the cartilaginous cells of the base of the basidorsal and the side of the arch is just the same. With the approach of the osseous condition this suture becomes invisible because everything is evacuated by a mass of bone-marrow. This suture reminds us of the neuro-central suture of reptiles. It is tempting if rather fanciful to regard the presence of this incipient suture as evidence that *Bufo* was the highest form amongst Anura, for although the reptiles have had an independent evolution side by side with the Anura, both have developed from Urodele-like amphibian ancestors through adaptation to land life.

Neither the presence of this suture in the case of *Bufo* nor the formation of bone-marrow in all cases of Anura examined, has been recorded by any previous worker.

The development of the 1st (atlas) vertebra is just the same in Anura as that of Urodela. There is an intercalated arch between the occipital arch proper and the atlas (1st) arch proper. This piece of arch is divided into two by the immigration of connective tissue cells in slanting manner craniocaudally. Thus are formed balls towards the occipital region and the sockets towards the atlas region. This intercalated arch is not completed dorsally. As the arch has two bases, so there are formed the two balls and the two sockets. Between the two bases of the arch we get the intervertebral element which divides in a vertical plane by the connective tissue cells. As the bases of this intercalary arch approach each other squeezing the notochord, the balls of the occipital region or, in other words, the occipital condyles are not far from each other, the interspace is formed by the division of the intervertebral region. It should be mentioned that, notwithstanding GOETTE'S statement (1875), there is no *intravertebral* cartilage in the case of Anura. The sockets articulate with the atlas arch proper. Thus the atlas vertebra has more than one pair of dorsal arcualia. The Anuran atlas vertebra however has no laterally deflected anterior portions like those of the Urodelan atlas. In *Rana temporaria*

the first spinal nerve which can be found between the atlas arch proper and the occipital arch proper, degenerates before metamorphosis is completed. So PETER (1895) could not see it, but in *Bufo melanostictus* this nerve persists during complete metamorphosis. As *Bufo melanostictus* hurries through the larval stages, tadpoles being comparatively much smaller than *Rana temporaria*, PETER could detect this nerve in the case of *Bufo*.

GOETTE stated that the transverse processes and the ribs (1875) were formed from two distinct centres of chondrifications. The recent worker EMELIANOFF (1925), who worked on *Rana esculenta* and *Pelobates fuscus*, says that the transverse process of Anura cannot be regarded as a dorsally shifted basiventral outgrowth as has been suggested in the case of the ventral fork of the diapophysis of Urodela. The transverse process of Anura is only a simple outgrowth of the upper arch.

RIDEWOOD (1896), who worked on *Xenopus*, says that the 2nd, 3rd and 4th vertebræ have no diapophyses, but only movable ribs which later become confluent with the neural arch and form the rigid so-called transverse processes of the adult.

According to the present writer, in the cases of *Rana temporaria* and *Bufo melanostictus* the transverse processes are directly formed from the dorsal arcualia, and there are cartilaginous pieces at their free ends. The situation of the transverse processes is between the supra and the infra vertebral arteries. Although the cartilaginous pieces at the free ends of the transverse processes resemble ribs, and are almost certainly homologous with them, there is no suture between them and the transverse processes (diapophyses). In the case of *Bombinator igneus*, GOETTE (1875) is right in stating that the diapophysis is formed directly from dorsal arcualia and in the 2nd, 3rd and 4th vertebræ there are ribs articulating with the free ends of the transverse processes. There is a peculiarity in this case that the 1st arch of the urostyle has a pair of transverse processes. RIDWOOD (1896) is mistaken when he says that in *Xenopus* the 2nd, 3rd and 4th vertebræ have no diapophyses. As a matter of fact, there are small diapophyses quite distinct from the ribs, and even the suture for the articulation could be easily detected. RIDWOOD could not detect this simply because he did not cut sections but only had whole mounts of the larval stages, and he himself has admitted that his enquiry makes no pretence to completeness.

In our paper on Urodela (1930) we stated that in this group the rib is formed as an aggregation of cells in the septum dividing two myotomes, and subsequently grows inwards, and joins a rib-bearer (diapophysis) developed from the side of the neural arches. In Anura it is obvious that the same thing occurs, but owing to the narrowness of the dorsal portions of the myotomes, the lower portions of which fuse to form the oblique muscles of the abdomen, the mesenchymatous rib is from the beginning formed close to the distal end of the rib-bearer, and this is especially marked in the case in *Rana* and *Bufo*.

Summary.

1. The notochord in all cases, which primarily has a uniform diameter throughout, undergoes moniliform differentiation. Dilatations are confined to the middle portion of the myotome, while the constrictions occur in the intermyotomal regions except in the tail, where the notochord has a uniform diameter till its degeneration.

2. The occurrence of two layers in the sheath of the notochord is universal in *Anura*, the inner being cuticular and the outer fibrillar.

3. GOETTE's so-called outer cartilaginous sheath is nothing but the cartilaginous perichordal tube.

4. After the liberation of the sclerotomic cells from the ventromedian corner of each somite, some of them become fibrous and aggregate round the notochordal sheath in the middle of the myotome forming the perichordal rings.

5. These rings extend in a cranial and caudal direction, forming a very thin layer round the notochord in the intermyotomal spaces, which makes up a complete tube—the perichordal tube.

6. The perichordal tube follows the moniliform structure of the notochord. The dorsal arcualia are formed from aggregations of cells on the dorsolateral sides of the notochord in the intermyotomal (vertebral) regions where the perichordal tube is very thin.

7. After the sclerotomic dorsal arcualia have become cartilaginous, the part of the perichordal tube at the base of the cartilaginous basidorsal becomes chondrified. The cartilaginous cells of the tube are absolutely different from the cartilaginous cells of the dorsal arcualia.

8. Next, the mid-dorsal, as well as the ventral portion, continuous with the fibrous perichordal parts of the vertebral centrum of the trunk, as well as of the urostyle region, become cartilaginous in the cases of *Rana temporaria* and *Bufo melanostictus*.

9. In *Bombinator igneus* it is only the mid-dorsal part of the perichordal tube which becomes cartilaginous.

10. In *Xenopus laevis* the mid-dorsal portion and a part of the ventral portion of the perichordal tube forming a rectangular structure become cartilaginous. This is a structure continued along the whole of the vertebral column up to the end of the urostyle. The rectangular cartilage constituting the ventral part of the perichordal tube of *Xenopus* degenerates, as does the whole of the notochord and its sheaths.

11. The bases of the right and the left basidorsals do not join together dorsally above the notochord, except at the two extreme ends of each vertebral region, contrary to the views of EMELIANOFF.

12. The anterior portion of the urostyle of *Rana temporaria* is not formed from paired cartilages ventral to the notochord as GADOW has stated.

13. There is no indication of the presence of an intravertebral cartilage in *Bombinator*, such as GOETTE describes. From his figure it seems that he mistook

some cells of the perichordal tube of the urostyle region for notochordal cells when the hypochordal cartilage has crushed the notochord.

14. In *Bombinator* and *Xenopus*, where there are distinct ribs in the larval stage, these fuse in the adult stage with the diapophyses.

15. The intervertebral cartilage, which arises from the intervertebral portion of the perichordal tube, ultimately crushes the notochord entirely in the cases of *Rana temporaria* and *Bufo melanostictus*. In the case of *Rana* it takes a long time, even more than two years, to crush the notochord entirely in the intervertebral region.

16. The connective tissue cells grow into the intervertebral cartilage slanting either inwards and backwards in the case of procoelous vertebræ, or in the case of opisthocœlous vertebræ, inwards and forwards, forming in this way crescentic shells of cells. These shells split into two layers in the line of the crescent forming the synovial cavity for the articulation of the vertebræ.

17. Thus we get the division of the intervertebral cartilage into ball and socket. In the case of procoelous vertebra the convexity of the arc is posterior and *vice versa* in the case of opisthocœlous vertebra. These cells ultimately become cartilaginous, forming a layer at the edges of the ball and the socket.

18. The cartilage cells of the basidorsal are evacuated by bone-marrow. Evacuation starts at an early stage, at the time of metamorphosis in the cases of Frog, Toad and *Bombinator*, and at a much earlier stage in *Xenopus*.

19. Between the occipital arch and the atlas arch proper there is an intercalated arch which divides into two. Each of the two laterally deflected basidorsals of this arch divides into anterior and posterior portions, the result being that two balls and two sockets are formed where anterior and posterior portions are in contact.

20. The socket portions of the intercalated arch fuse with the atlas arch proper. Thus it may be said that the atlas vertebra has more than one pair of dorsal arcualia. As a result, the atlas vertebra looks bigger than any other vertebra.

21. The anterior portions of the urostyles of *Rana temporaria* and *Bufo melanostictus* have deflected arcualia, which become divided like the intercalated arches; so that instead of having one condyle like other vertebræ, there are two condyles formed from the bases of the arches. The two condyles go to the 9th vertebra and the two sockets to the anterior portion of the urostyle. Between the two joints the intervertebral cartilage divides in a vertical plane.

22. In the adult stage of *Bufo melanostictus* the centrum proper is full of bone-marrow, which replaces the notochord.

23. In each of the four cases, viz., *Rana*, *Bufo*, *Bombinator* and *Xenopus*, the transverse processes of the 9th vertebra articulate on the side of the ilium a little below its tip—contrary to the views of BALFOUR, MARSHALL and GADOW.

24. In *Rana temporaria* the first spinal nerve can be detected in the region of the intercalated arch between the occipital arch and the atlas arch proper, but it degenerates before complete metamorphosis, and thus PETER could not see it in the adult stage.

25. The ventral ridge of the so-called hypochordal in the case of *Xenopus* is the structure homologous to the hypochordal of the other forms.

26. There are diapophyses of the 2nd, 3rd and 4th vertebræ in the case of *Xenopus*, although very small, contrary to the statement of RIDWOOD. With these the ribs become fused in a later stage of development.

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EXPLANATION OF PLATES.

All the figures are microphotographs from actual section except the sketches of the adult vertebral columns.

EXPLANATION OF LETTERING ON PLATES.

ao., aorta. *at.ar.*, atlas arch. *bd.*, basidorsal. *bd. 9*, basidorsal of the 9th vertebra. *bd.m.*, median union of the bases of the basidorsals. *bl.v.*, blood vessel. *c.c.*, cartilage cell. *c.c.t.*, connective tissue growing in to form articulation. *c.con.*, cartilage developed from ingrowing connective tissue. *cons.nch.*, constriction of the notochord. *cp.*, cup. *d.arc.II*, second dorsal arcualia. *d.c.t.ar.*, dorsal connective tissue arch. *d.pch.t.*, dorsal part of the perichordal tube. *dil.nch.*, dilation of the notochord. *dp.*, diapophysis. *d.r.*, dorsal ridge. *f.bv.*, fibrous basiventral. *f.n.ar.*, fibrous neural arch. *hy.*, hypochord. *i.c.t.*, ingrowing connective tissue. *iv.c.*, intervertebral cartilage. *iv.pch.t.*, intervertebral part of perichordal tube. *ma.*, marrow. *m.v.pch.t.*, midventral part of the perichordal tube. *n.ar.*, neural arch. *n.c.*, neural cord. *n.sp.I*, nerve spinalis I. *nch.*, notochord. *nchsh.*, notochordal sheath. *oc.ar.*, occipital arch. *oc.con.*, occipital condyle. *pch.l.*, perichordal layer. *pch.t.*, perichordal tube. *pr.z.*, pre-zygapophysis. *pt.z.*, post-zygapophysis. *r.*, rib. *s.*, suture. *s.c.*, spinal cord. *s.g.*, spinal nerve ganglion. *sy.c.*, synovial cavity. *ur.*, urostyle. *v.c.t.ar.*, ventral connective tissue arch. *v.con.*, vertebral condyle. *v.pch.t.*, ventral part of the perichordal tube.

PLATE 14.

FIG. 1.—Sagittal section through the occipital and the atlas regions of 20 mm. stage of the tadpole of *Rana temporaria*.

FIG. 2.—Parafrontal section through the trunk vertebræ of the tadpole of *Rana temporaria* at 22 mm. stage.

FIG. 3.—Transverse section through the end of a vertebral region of a tadpole of *Rana temporaria* at 25 mm. stage.

FIG. 4.—Transverse section through the middle of a trunk vertebra of a tadpole of *Rana temporaria* at 25 mm. stage.

FIG. 5.—Transverse section through the anterior portion of the urostyle region of a tadpole of *Rana temporaria* at 32 mm. stage.

FIG. 6.—Sagittal section through the urostyle and tail regions of a metamorphosing tadpole of *Rana temporaria* at 20 mm. stage.

PLATE 15.

FIG. 7.—Transverse section of the tail of a metamorphosing tadpole of *Rana temporaria* at 12 mm. stage.

FIG. 8.—Frontal section through the trunk vertebræ of young *Rana temporaria* at 12 mm. stage after metamorphosis.

FIG. 9.—Frontal section through the trunk vertebra of young *Rana temporaria* at 30 mm. stage after metamorphosis (about 2 years old).

FIG. 10.—Transverse section through the anterior portion of the urostyle of *Rana temporaria* at 30 mm. stage after metamorphosis.

FIG. 11.—Frontal section through the centrum of a trunk vertebra of a young *Rana temporaria* at 50 mm. stage (probably about three years old).

PLATE 16.

FIG. 12.—Frontal section through the trunk vertebræ of *Bufo melanostictus* at 9 mm. stage just after metamorphosis.

FIG. 13.—Sagittal section through the occipital and the atlas regions of a young *Bufo melanostictus* at 12 mm. stage after metamorphosis.

FIG. 14.—Transverse section of a trunk vertebra of a young *Bufo melanostictus* at 15 mm. stage after metamorphosis.

FIG. 15.—Frontal section through the centra of two vertebræ of a young *Bufo melanostictus* at 27 mm. stage after metamorphosis (probably two years old).

FIG. 16.—Transverse section through the trunk vertebra of a tadpole of *Bombinator igneus* at 12 mm. stage.

FIG. 17.—Transverse section of a trunk vertebra of a tadpole of *Xenopus laevis* at 31 mm. stage.

PLATE 17.

FIG. 18.—Transverse section through the occipital and the atlas vertebræ of a tadpole of *Xenopus laevis* at 52 mm. stage just before metamorphosis.

FIG. 19.—Transverse section of a trunk vertebra of *Xenopus laevis* at 52 mm. stage before complete metamorphosis.

FIG. 20.—Transverse section through the intervertebral portion of the trunk region of *Xenopus laevis* at 52 mm. stage just before metamorphosis.

FIG. 21.—Transverse section of the anterior portion of the urostyle of *Xenopus laevis* at 52 mm. stage before complete metamorphosis.

FIG. 22.—Transverse section through the tail region of *Xenopus laevis* at 52 mm. stage just before metamorphosis.

PLATE 18.

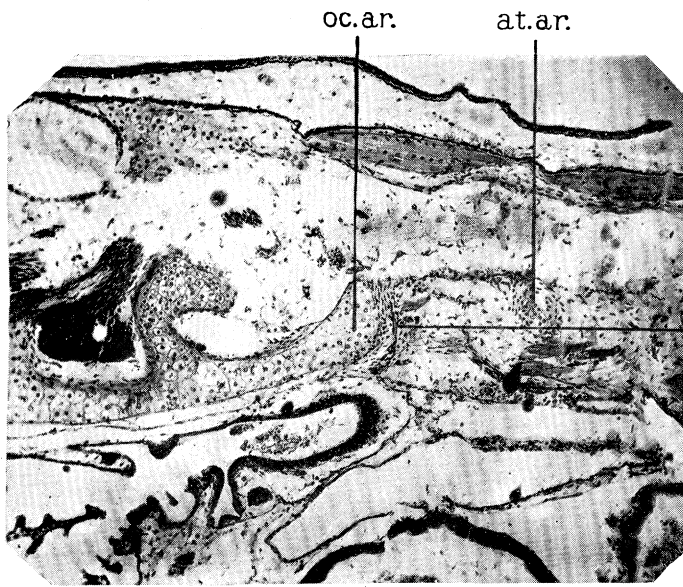
FIG. 23.—Transverse section of a trunk vertebra of *Xenopus laevis* at 40 mm. stage during metamorphosis.

FIG. 24.—Transverse section of a trunk vertebra of *Xenopus laevis* at 32 mm. stage after metamorphosis.

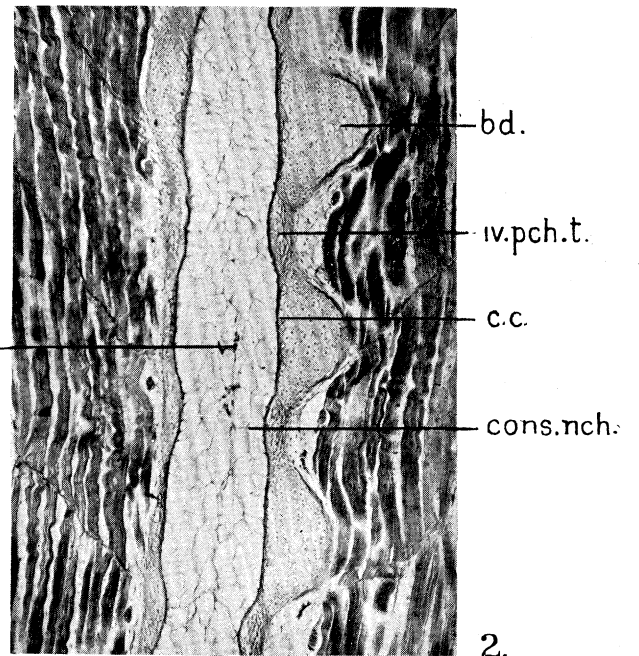
FIG. 25.—Transverse section through trunk vertebra and the rib of *Xenopus laevis* at 32 mm. stage after metamorphosis.

FIG. 26.—Transverse section through the intervertebral region of *Xenopus laevis* at 32 mm. stage after metamorphosis.

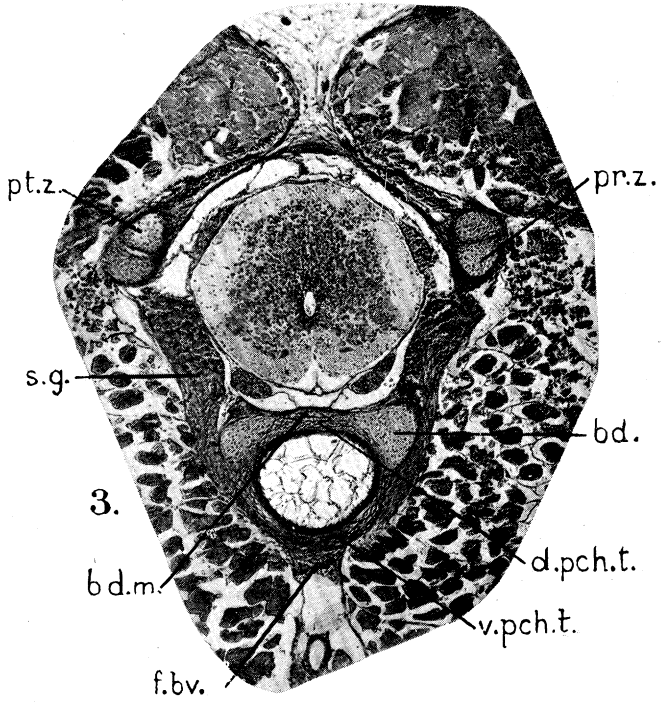
FIG. 27.—Transverse section through the anterior portion of the urostyle of *Xenopus laevis* at 32 mm. stage after metamorphosis.



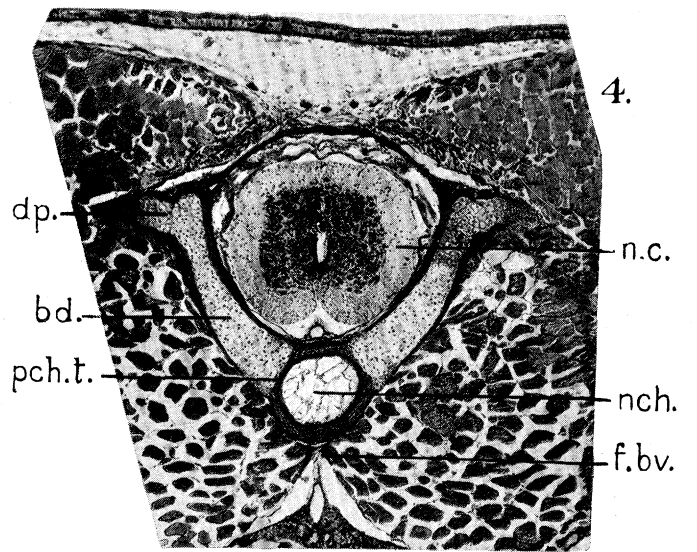
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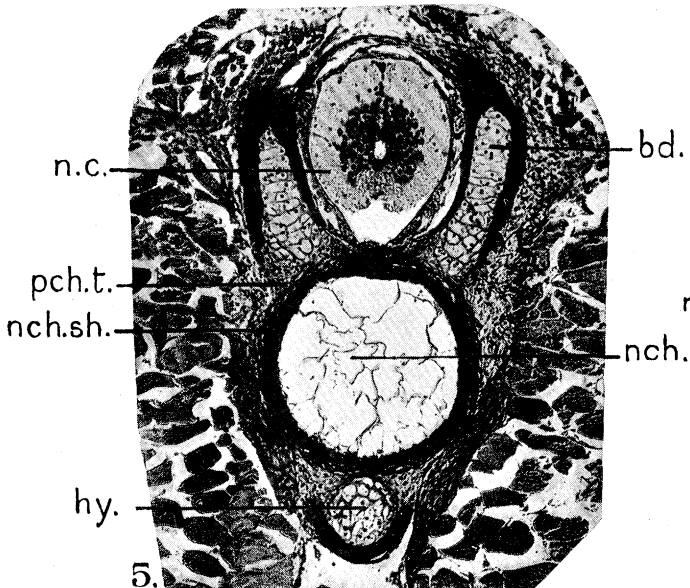
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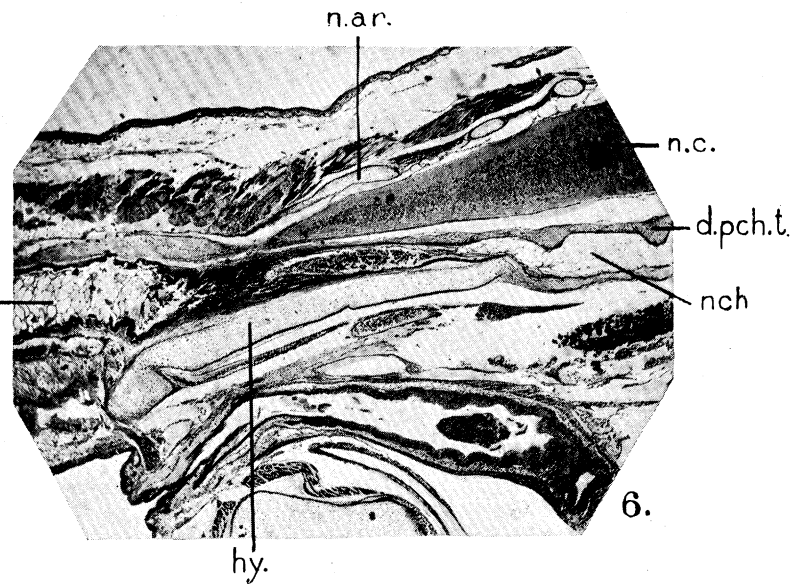
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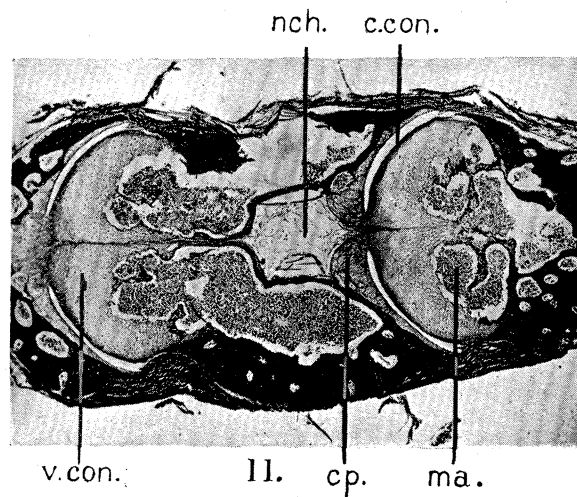
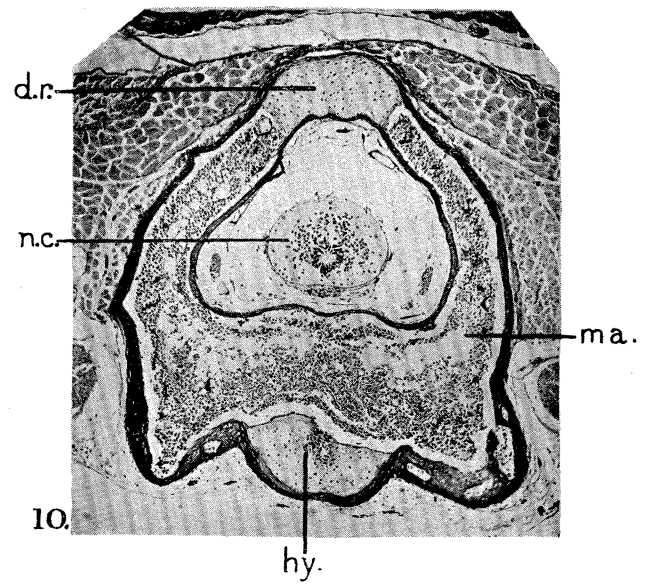
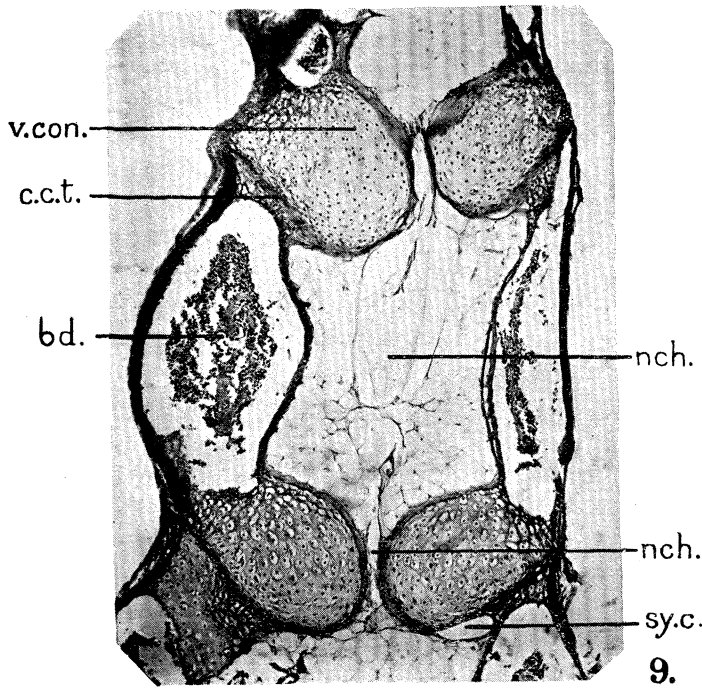
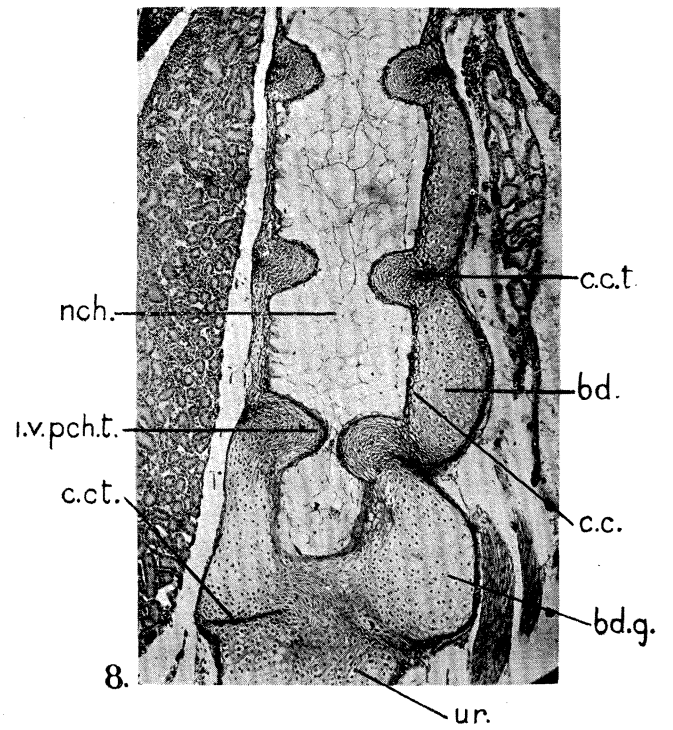
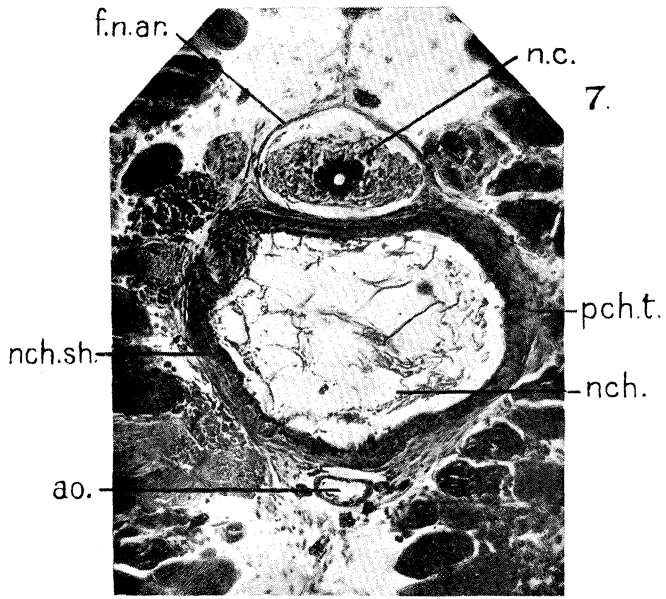
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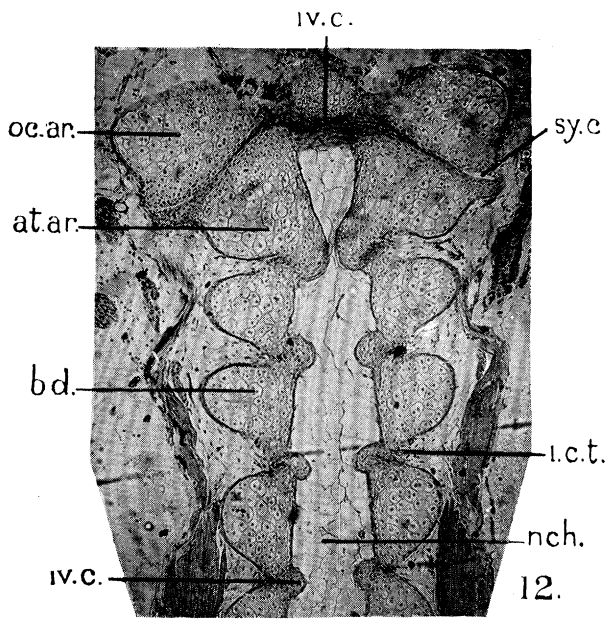


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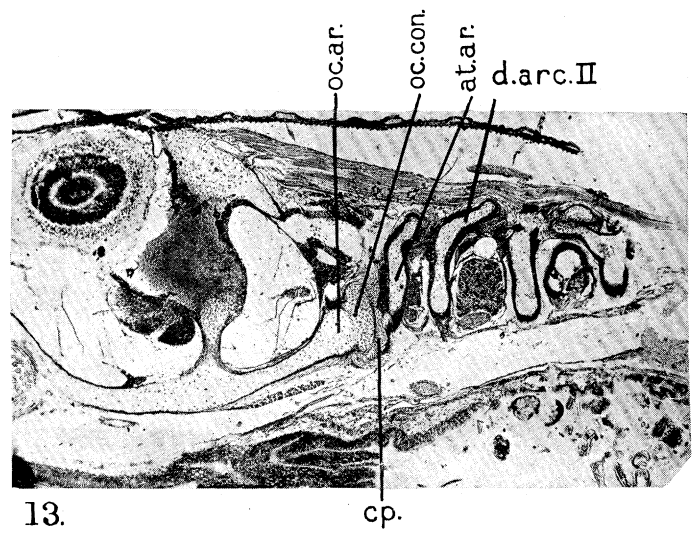


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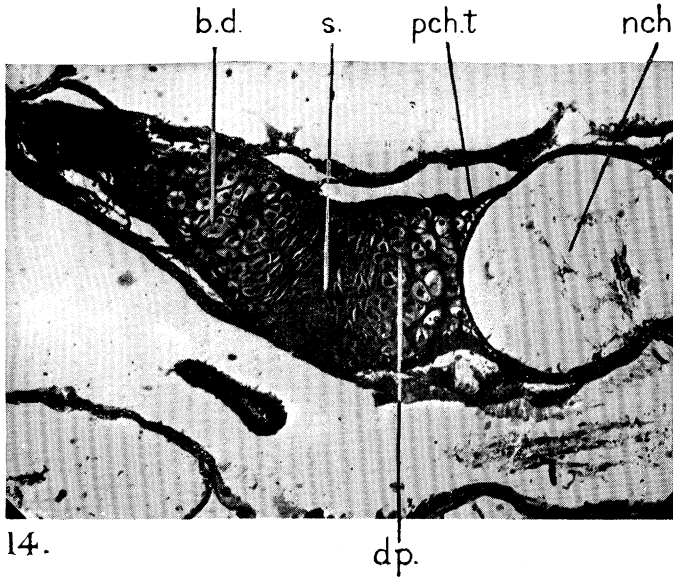




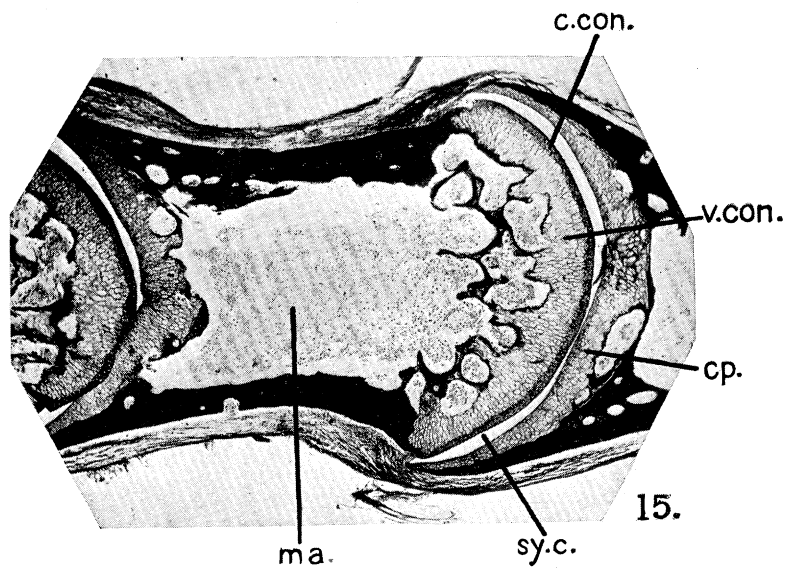
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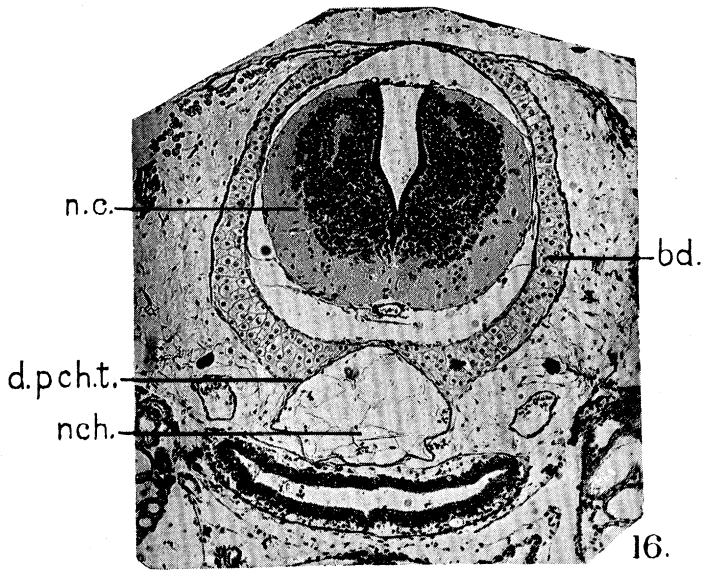
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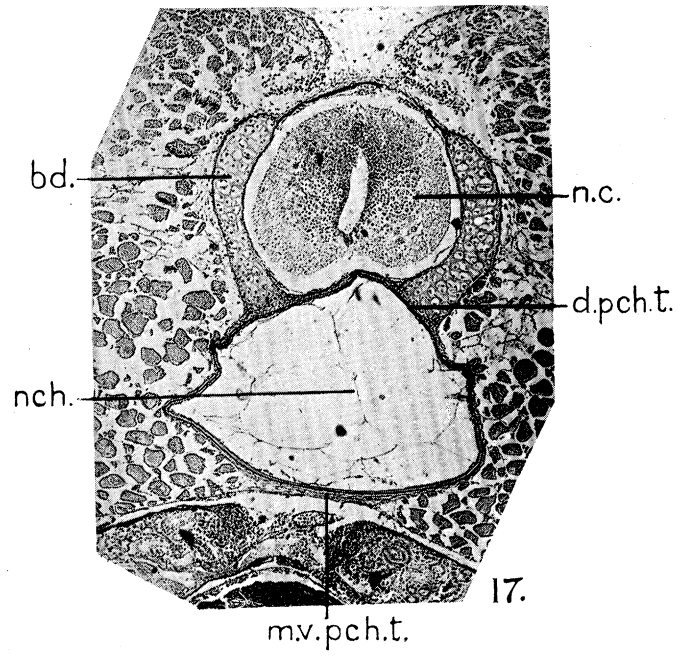
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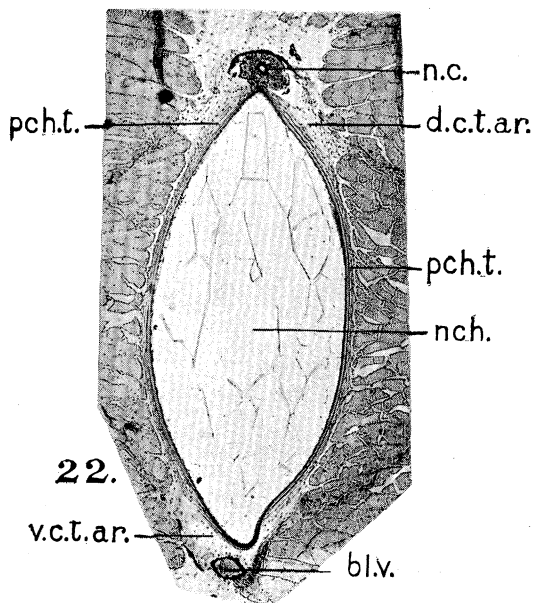
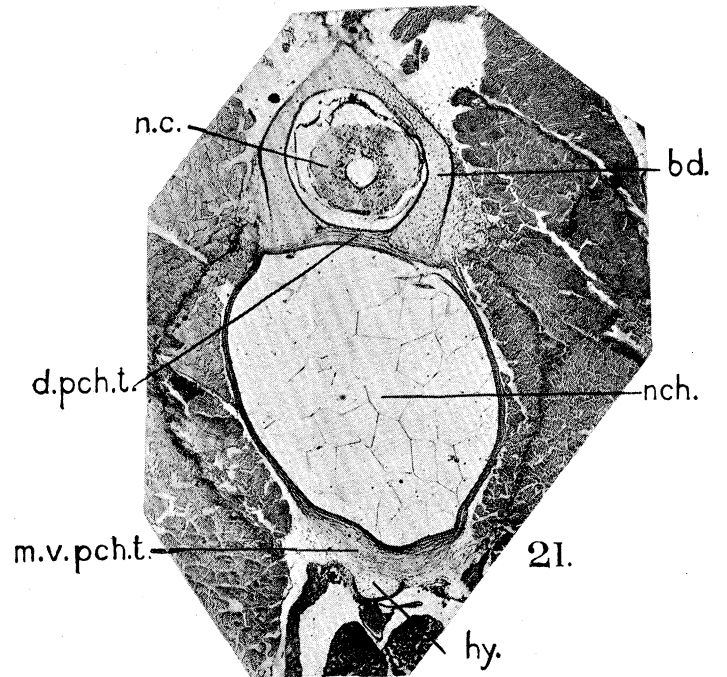
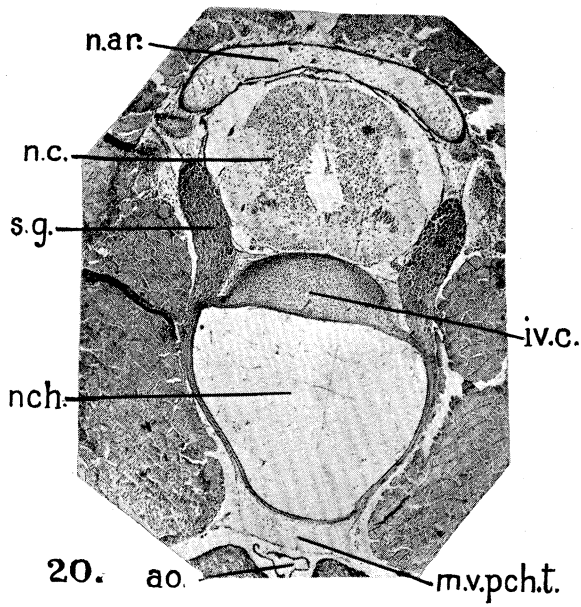
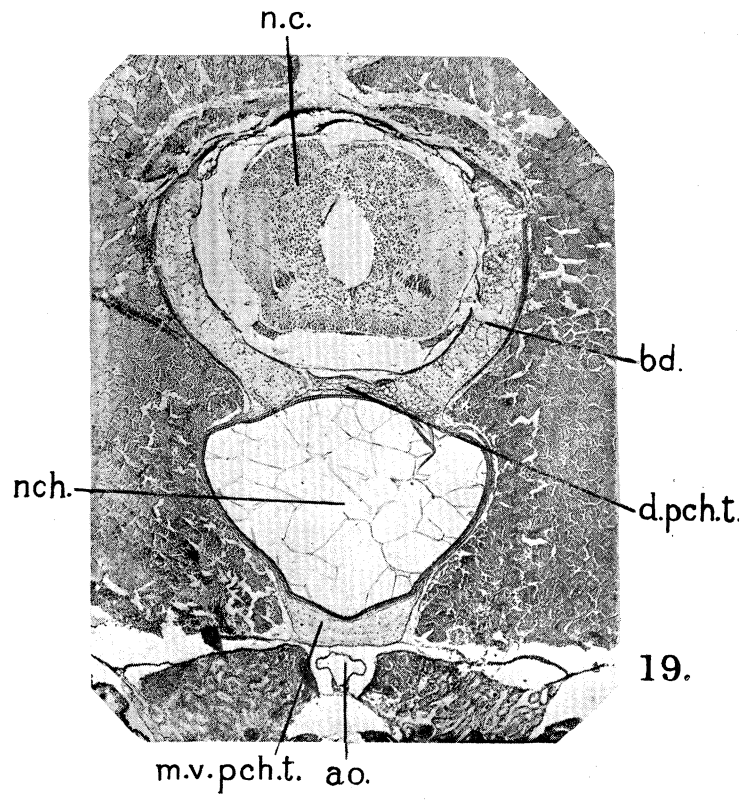
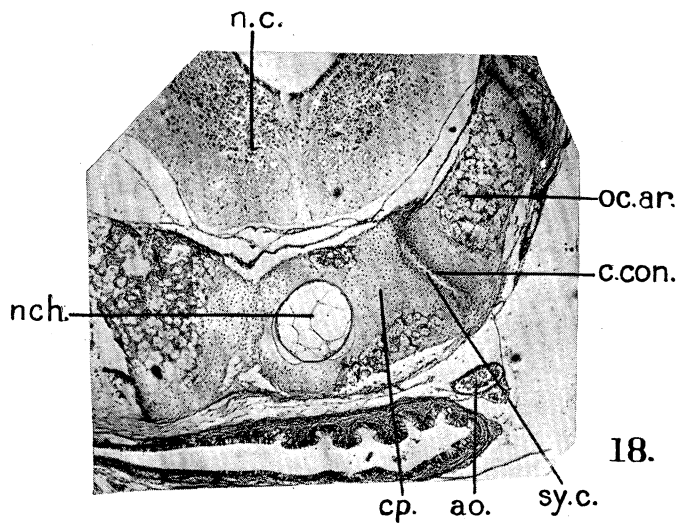
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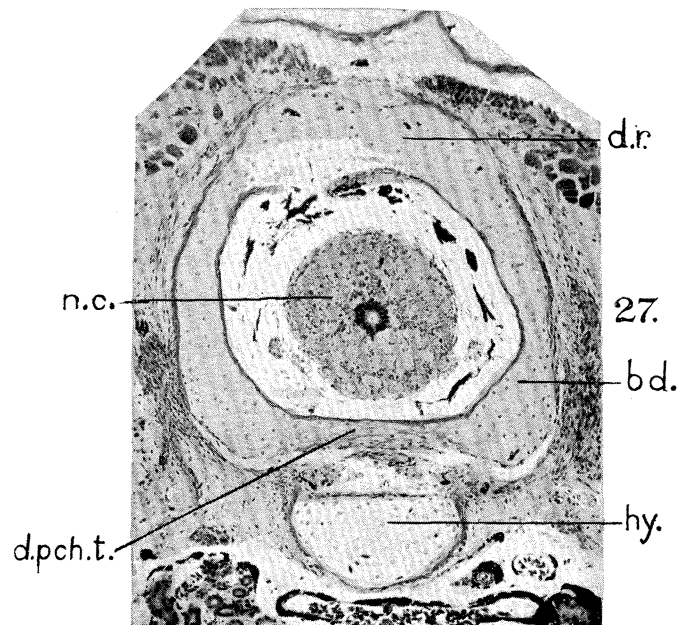
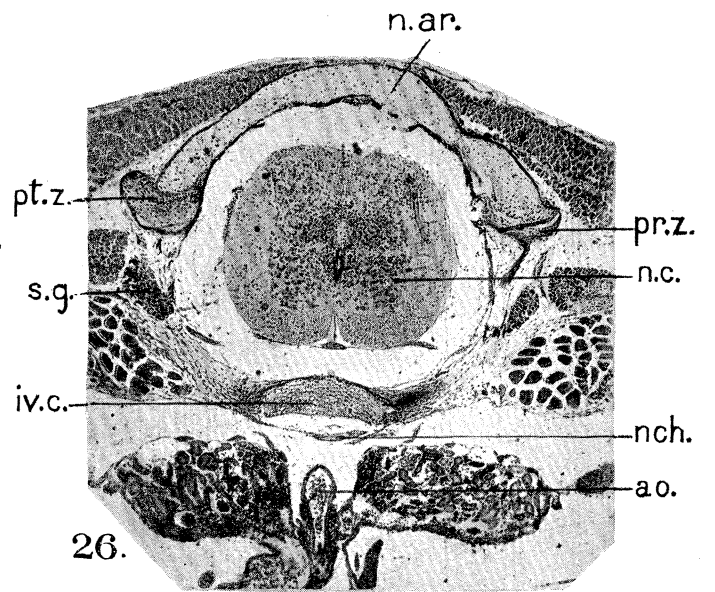
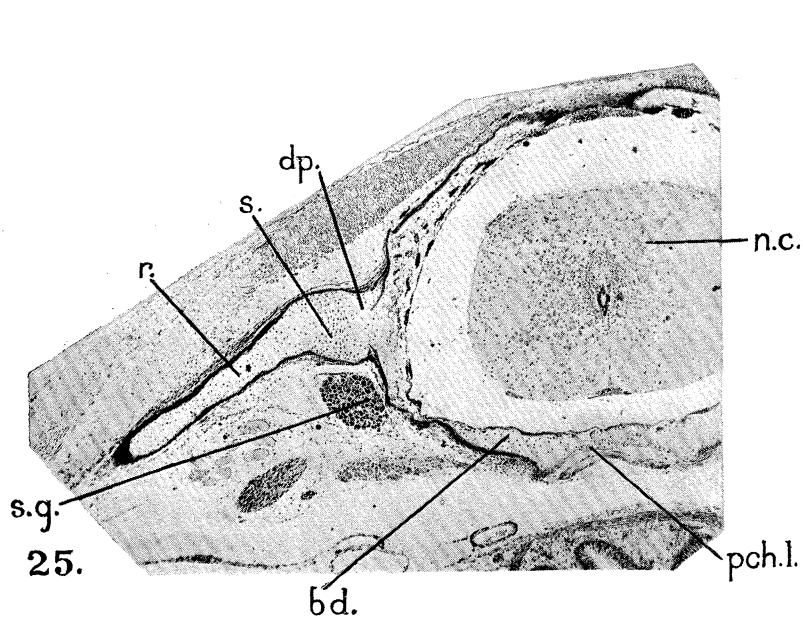
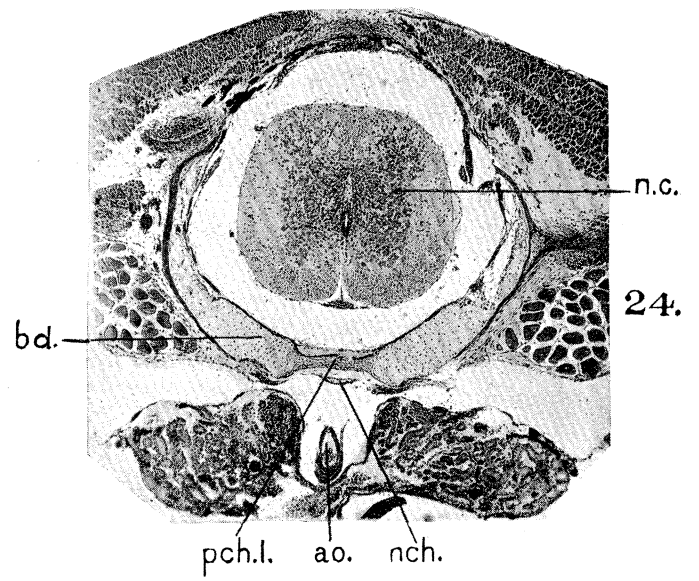
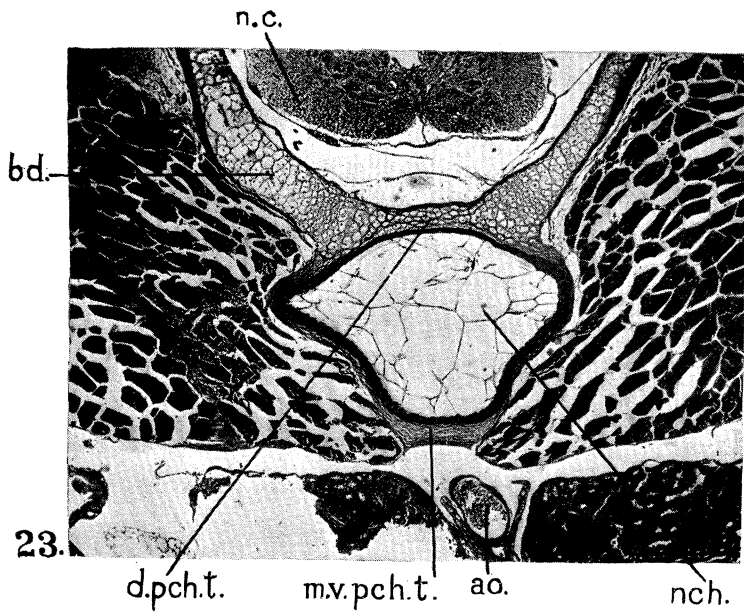


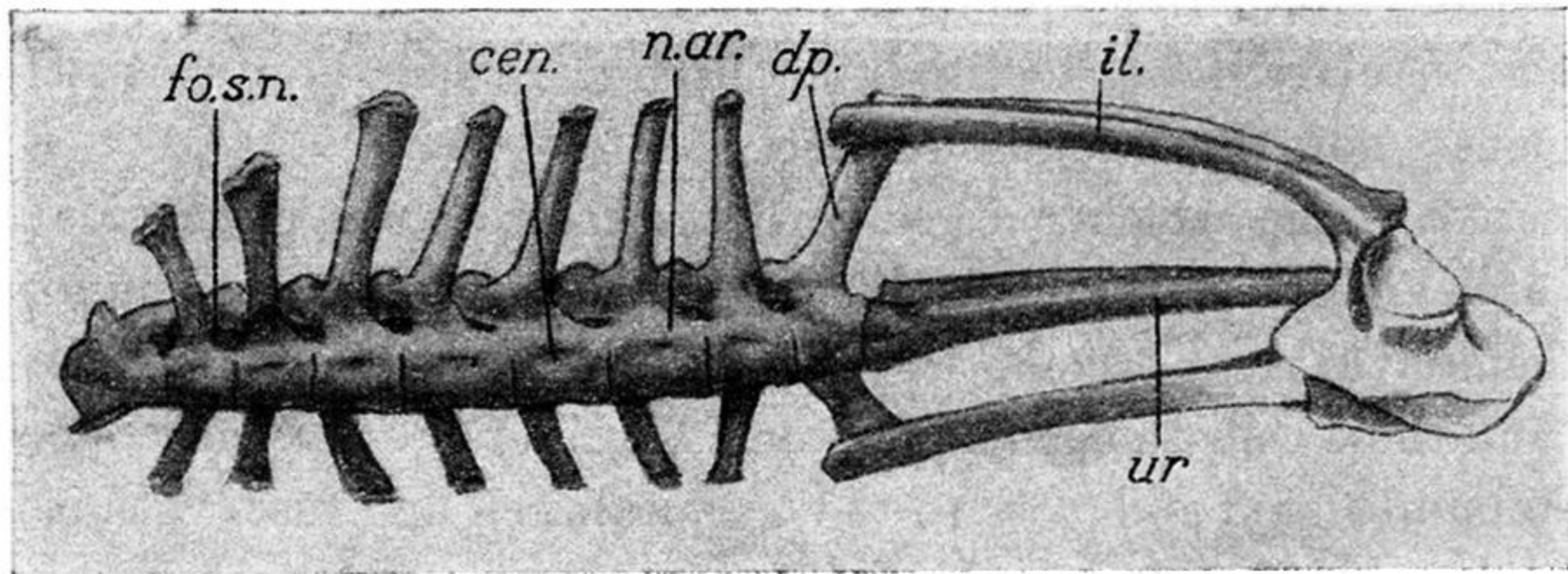
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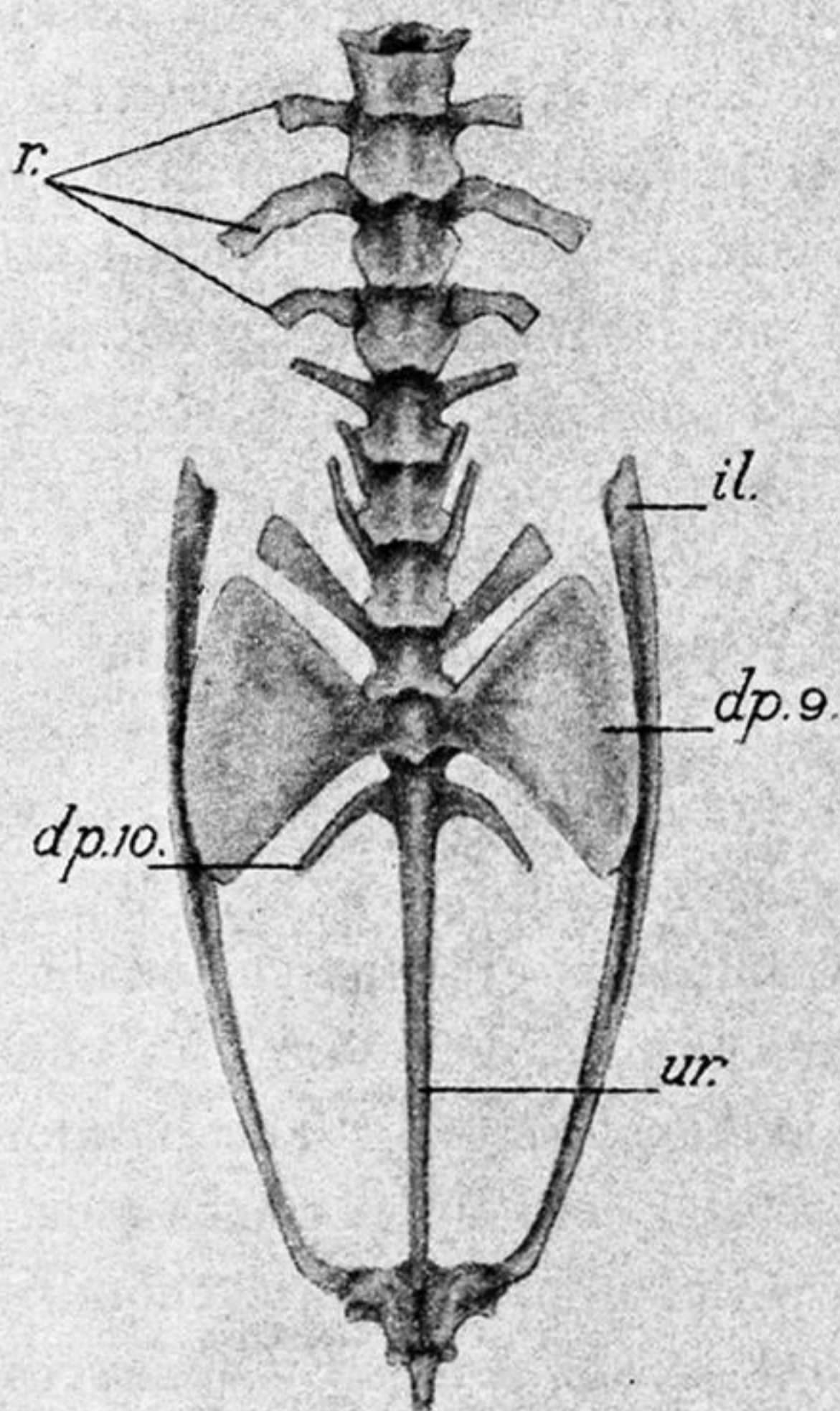
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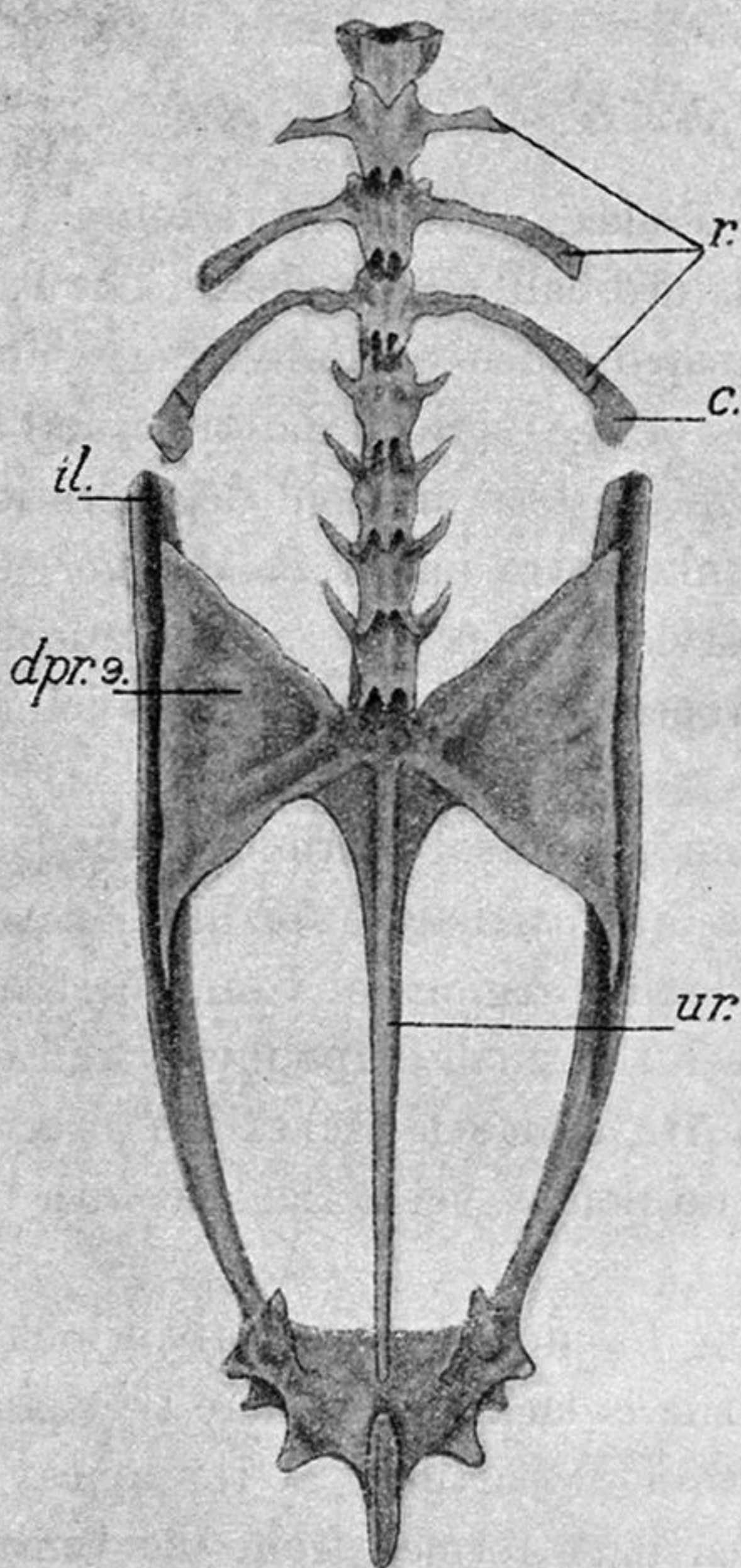




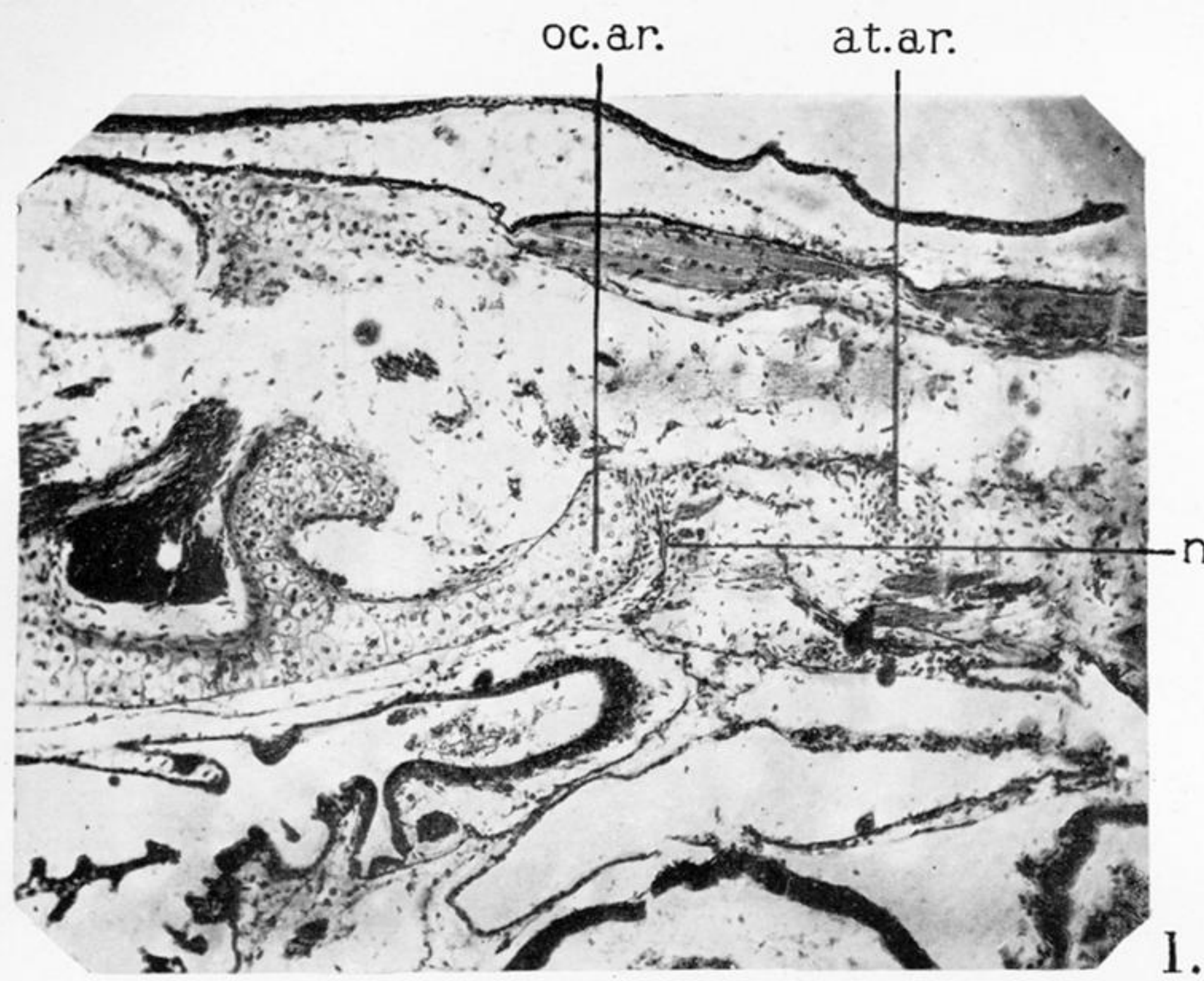
TEXT-FIG. 1.—Lateral view of the entire vertebral column of an adult *Rana temporaria*. *dp.*, diapophysis; *cen.*, centrum; *fo.s.n.*, foramen for a spinal nerve; *il.*, ilium; *n.ar.*, neural arch; *ur.*, urostyle.



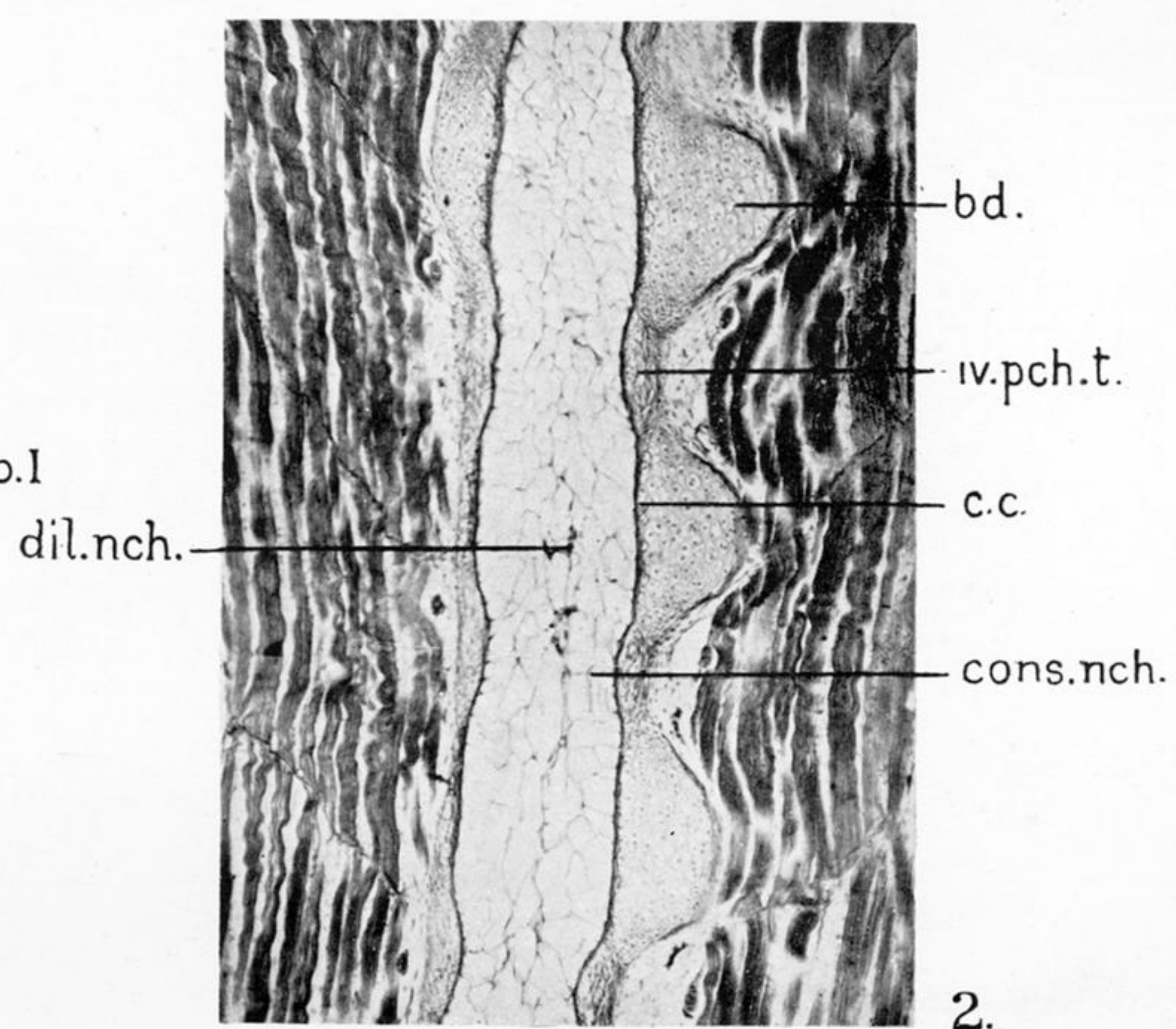
TEXT-FIG. 2.—Vertebral column of adult *Bombinator igneus* seen from dorsal side. *d.p.9.*, expanded diapophysis with which the ilium articulates ; *d.p.10.*, diapophysis of the 10th vertebra fused with the urostyle ; *il.*, ilium ; *r.*, ribs fused with the diapophyses of the 2nd, 3rd and 4th vertebræ.



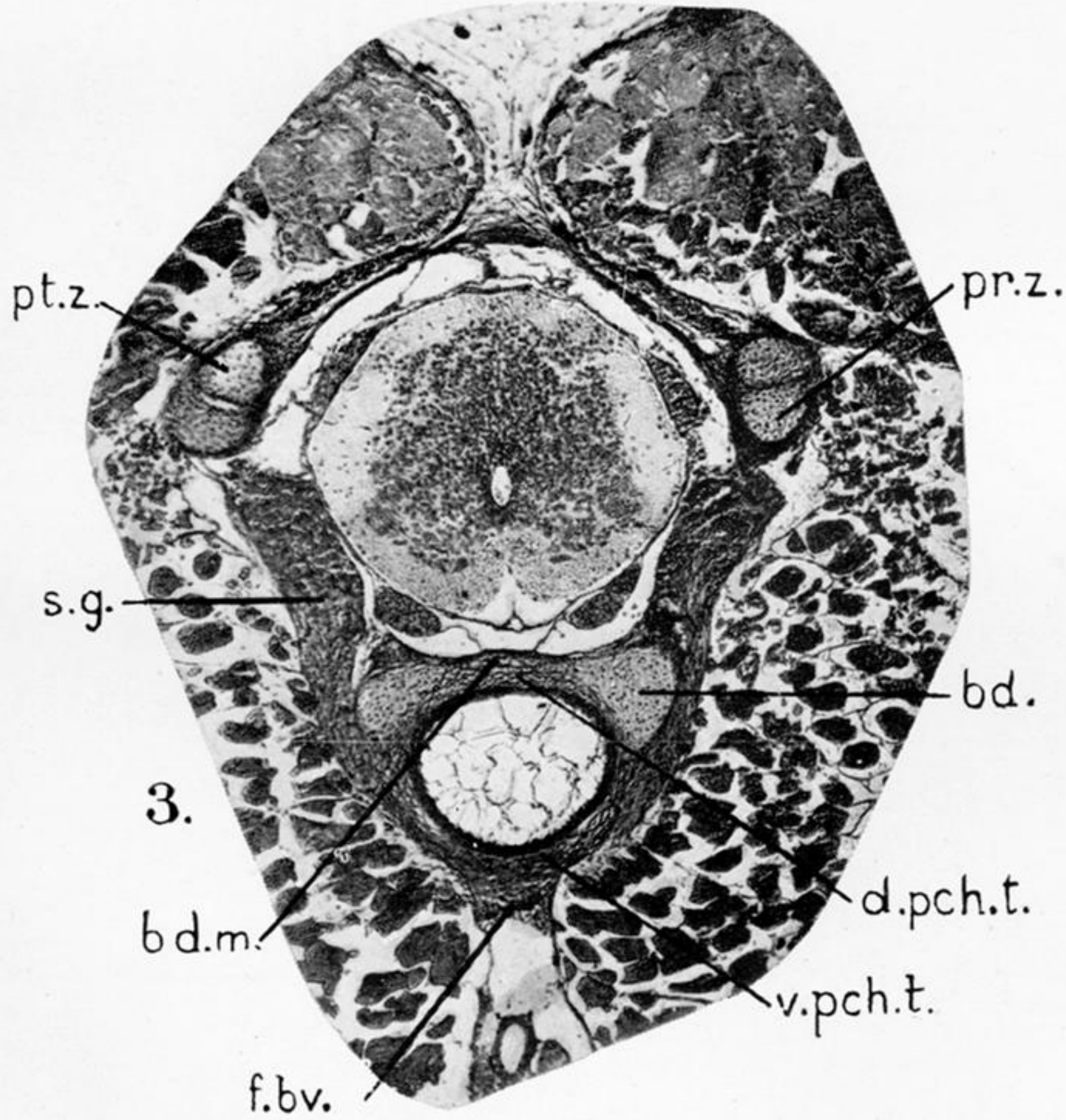
TEXT-FIG. 3.—Vertebral column of adult *Xenopus laevis* seen from the dorsal side. *C.*, Cartilage at the end of the 4th rib; *dpr.9.*, diapophysis of the 9th vertebra with which the ilium articulates; this vertebra is fused with the urostyle; *il.*, ilium; *r.*, ribs attached to diapophyses of the 2nd, 3rd and 4th vertebræ.



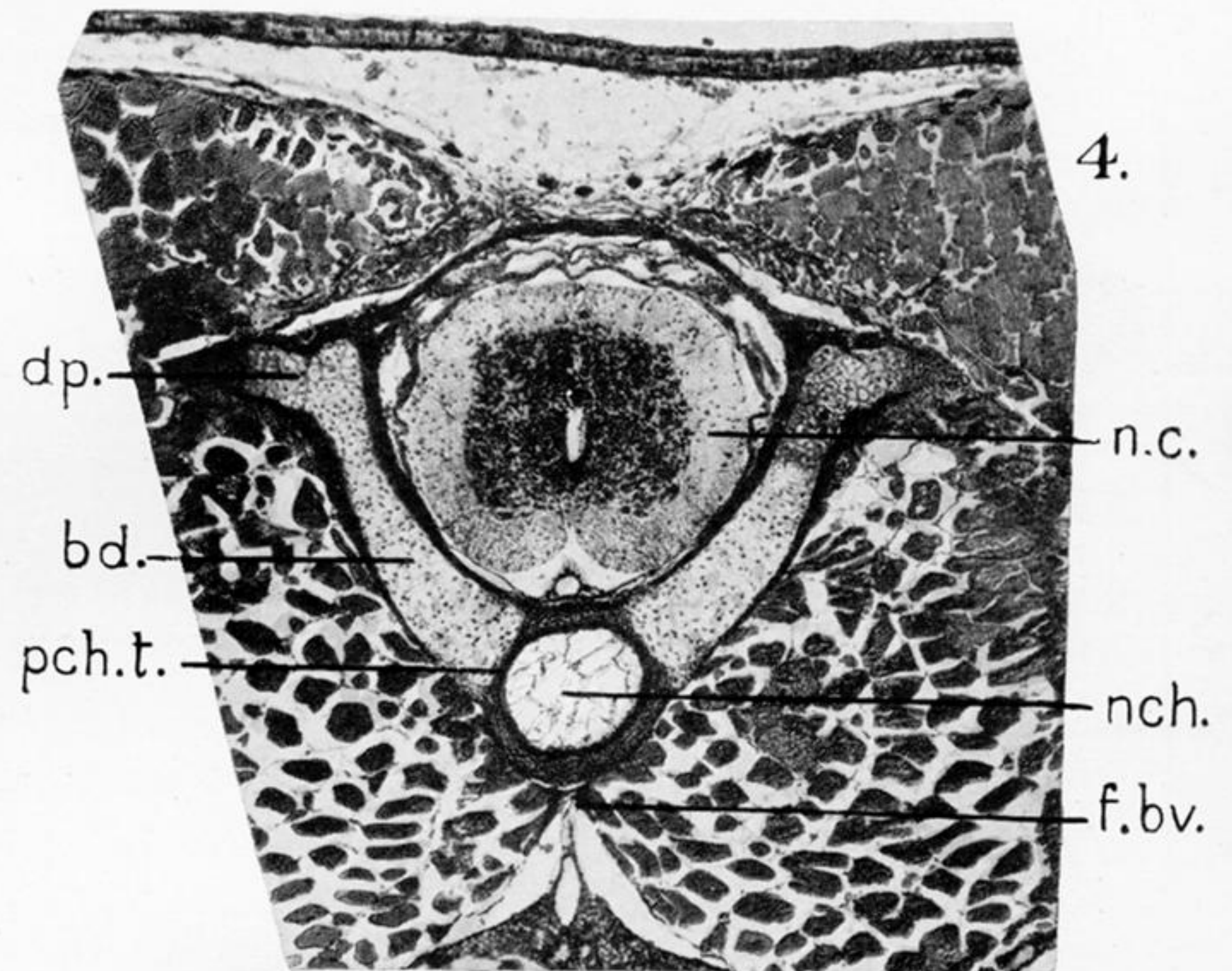
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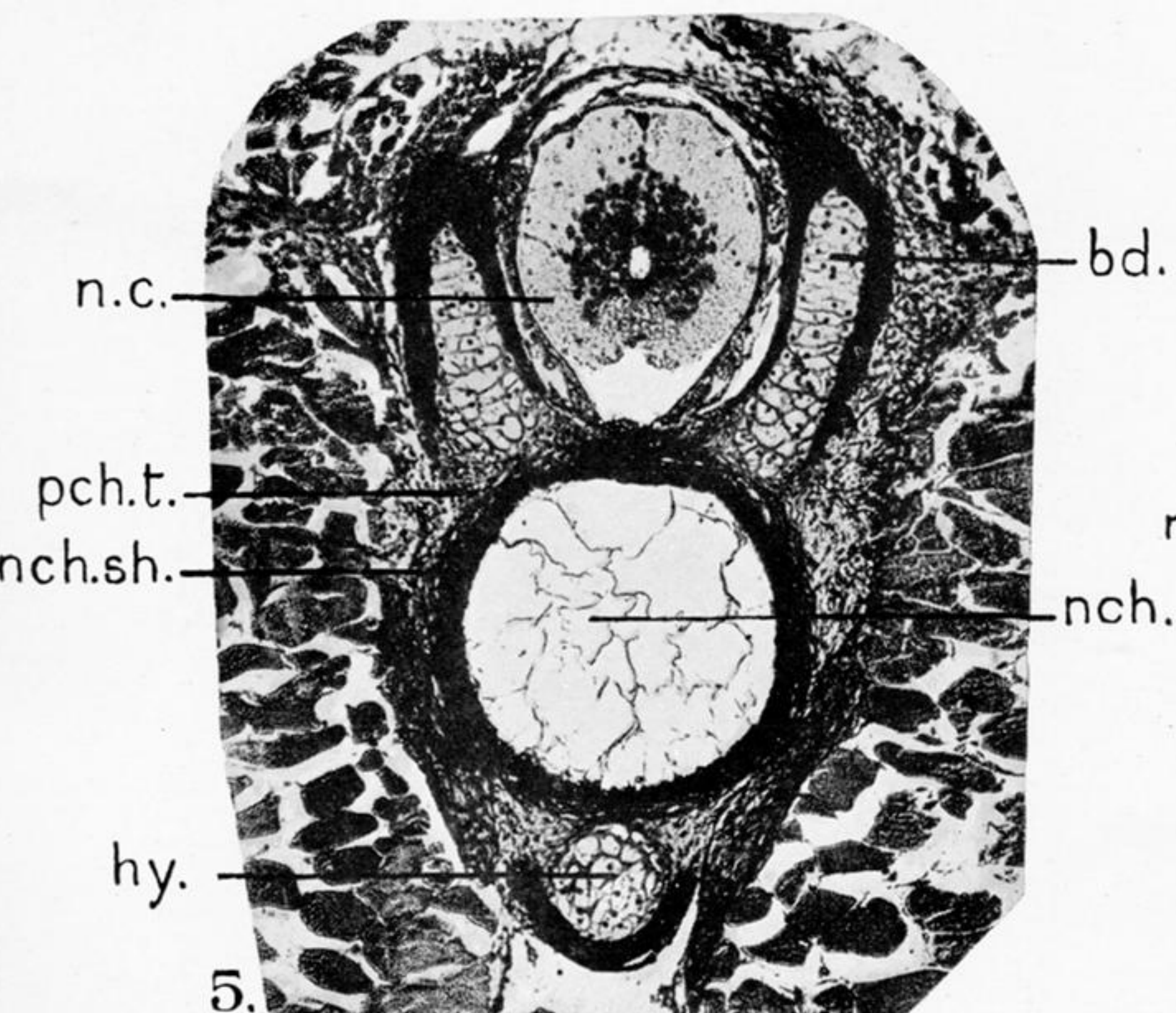
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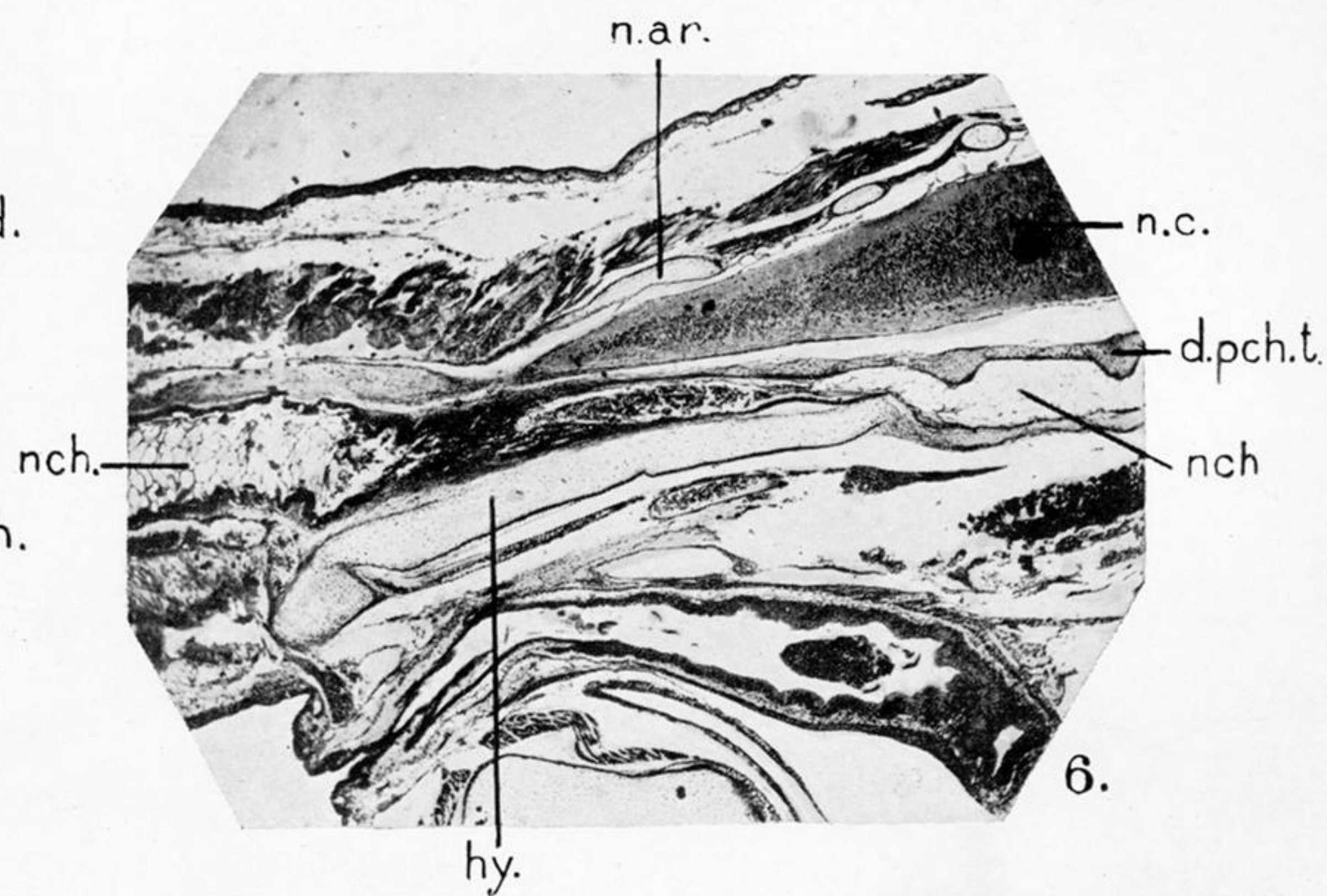
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6.

PLATE 14.

FIG. 1.—Sagittal section through the occipital and the atlas regions of 20 mm. stage of the tadpole of *Rana temporaria*.

FIG. 2.—Parafrontal section through the trunk vertebrae of the tadpole of *Rana temporaria* at 22 mm. stage.

FIG. 3.—Transverse section through the end of a vertebral region of a tadpole of *Rana temporaria* at 25 mm. stage.

FIG. 4.—Transverse section through the middle of a trunk vertebra of a tadpole of *Rana temporaria* at 25 mm. stage.

FIG. 5.—Transverse section through the anterior portion of the urostyle region of a tadpole of *Rana temporaria* at 32 mm. stage.

FIG. 6.—Sagittal section through the urostyle and tail regions of a metamorphosing tadpole of *Rana temporaria* at 20 mm. stage.

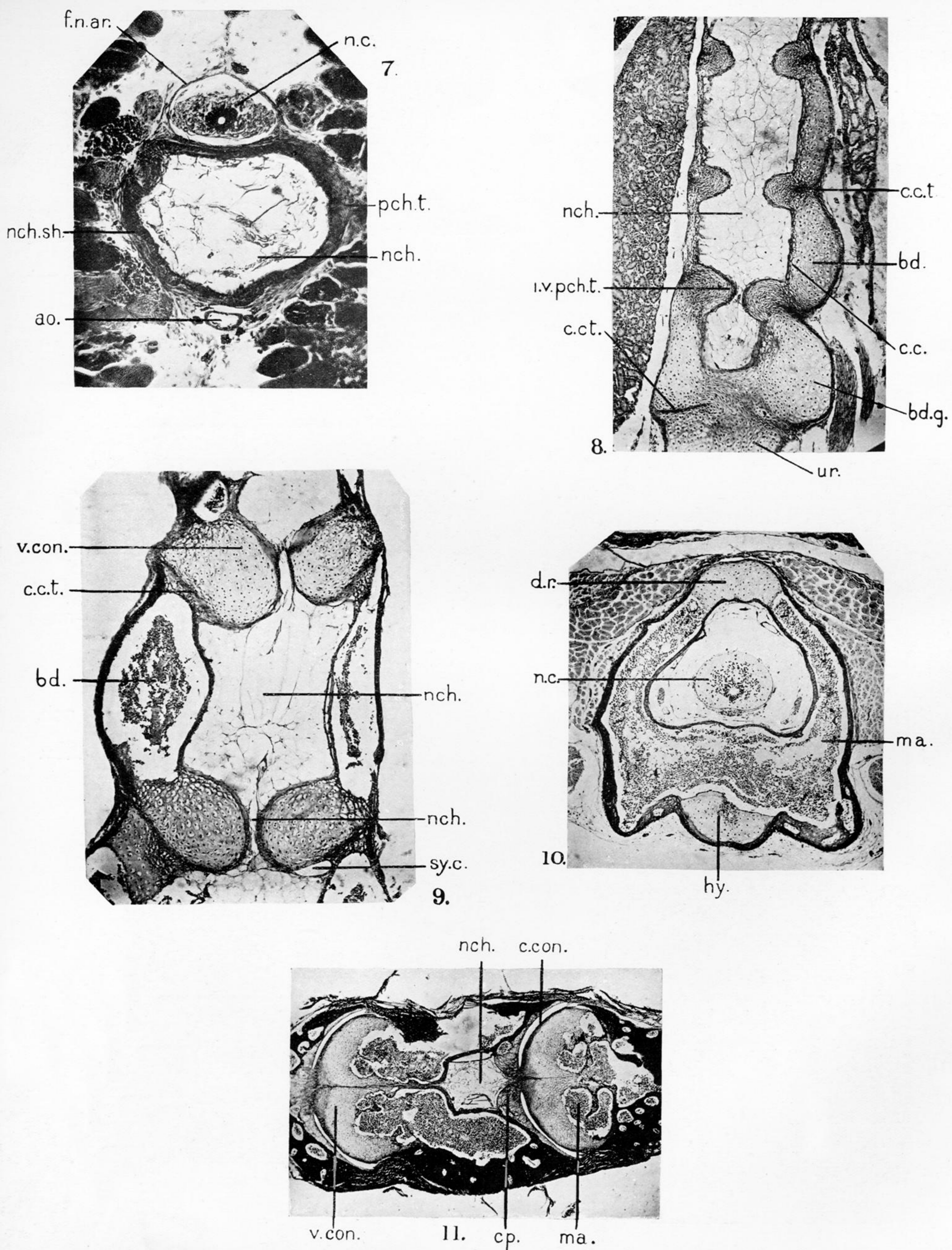


PLATE 15.

FIG. 7.—Transverse section of the tail of a metamorphosing tadpole of *Rana temporaria* at 12 mm. stage.

FIG. 8.—Frontal section through the trunk vertebræ of young *Rana temporaria* at 12 mm. stage after metamorphosis.

FIG. 9.—Frontal section through the trunk vertebra of young *Rana temporaria* at 30 mm. stage after metamorphosis (about 2 years old).

FIG. 10.—Transverse section through the anterior portion of the urostyle of *Rana temporaria* at 30 mm. stage after metamorphosis.

FIG. 11.—Frontal section through the centrum of a trunk vertebra of a young *Rana temporaria* at 50 mm. stage (probably about three years old).

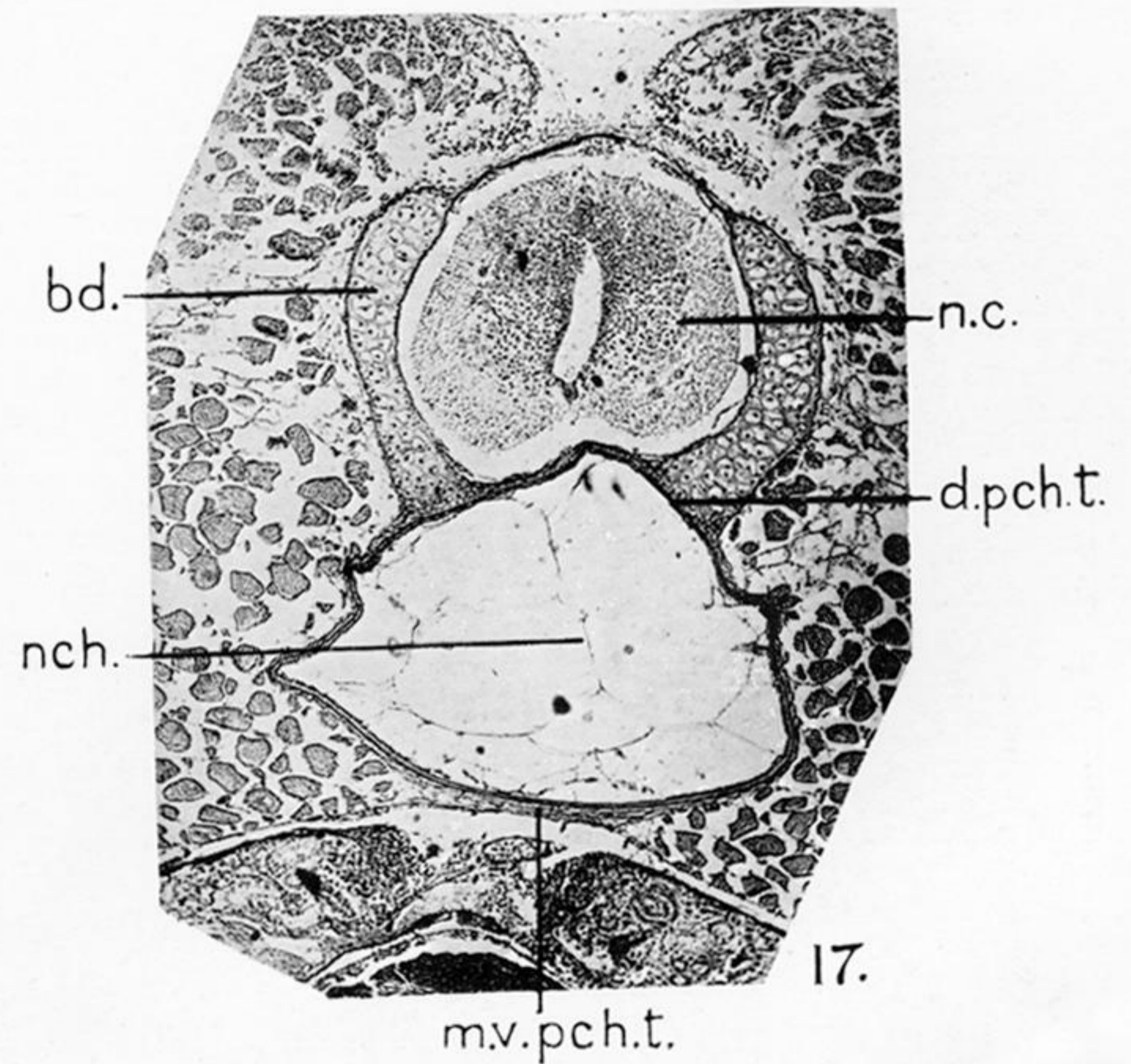
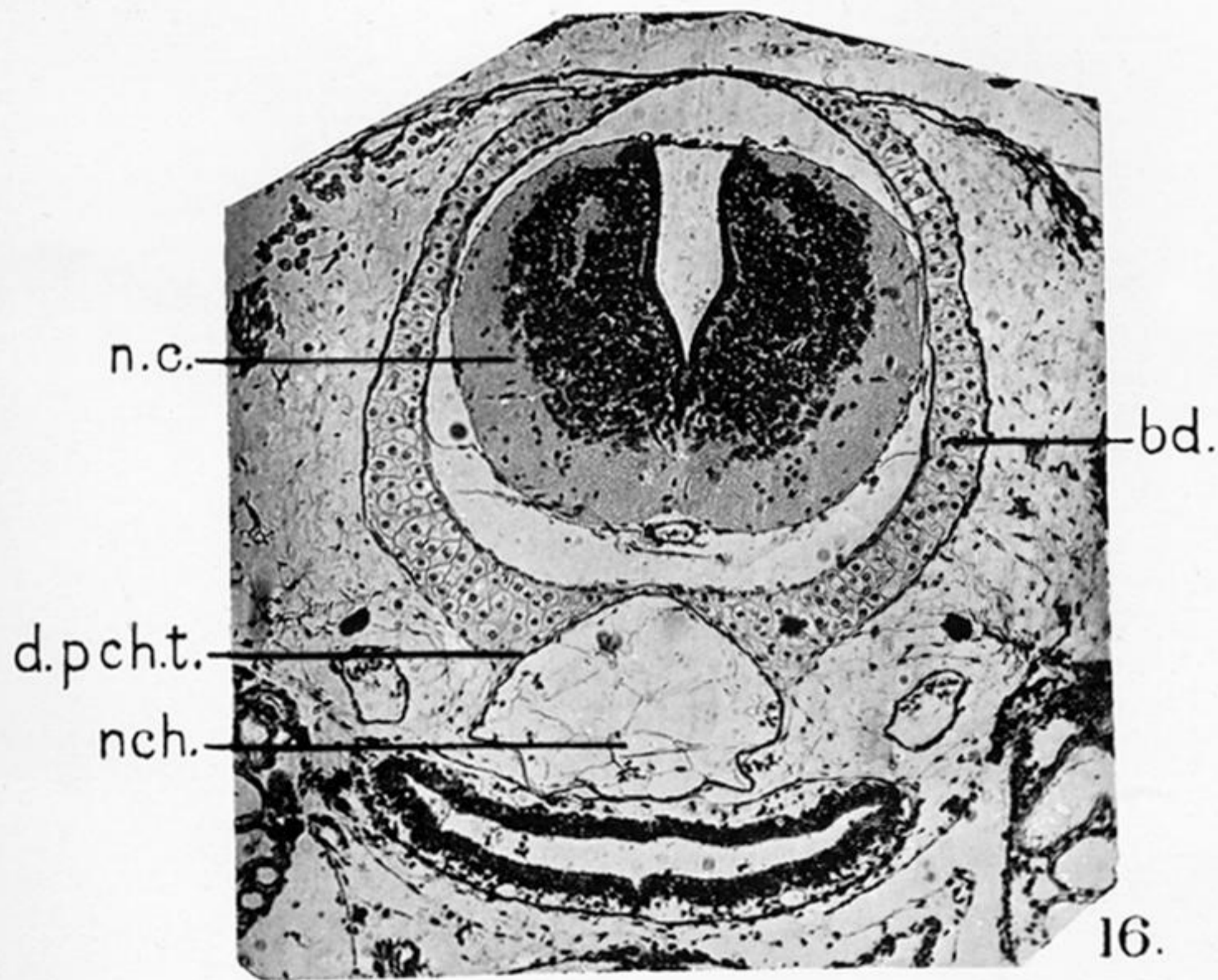
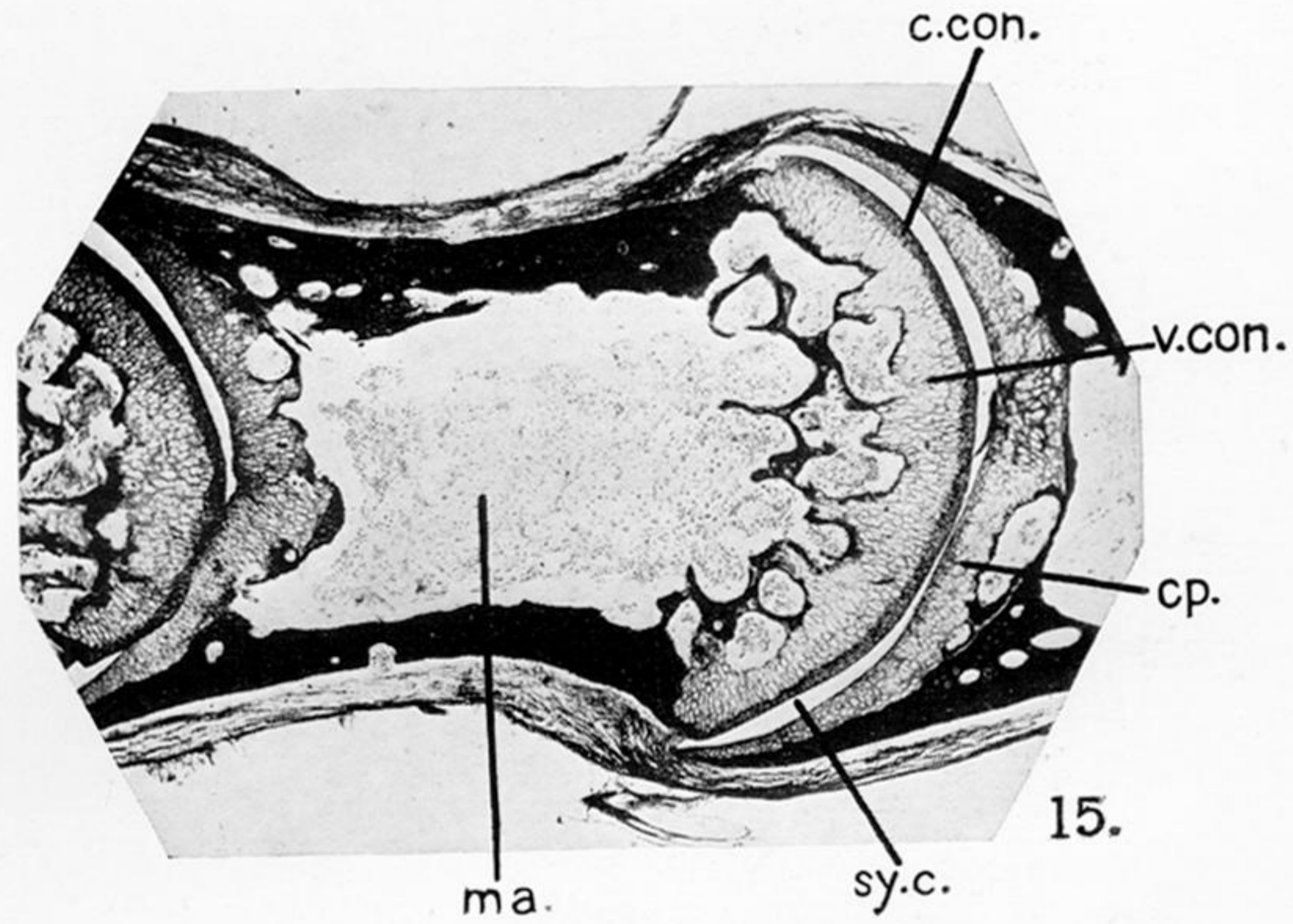
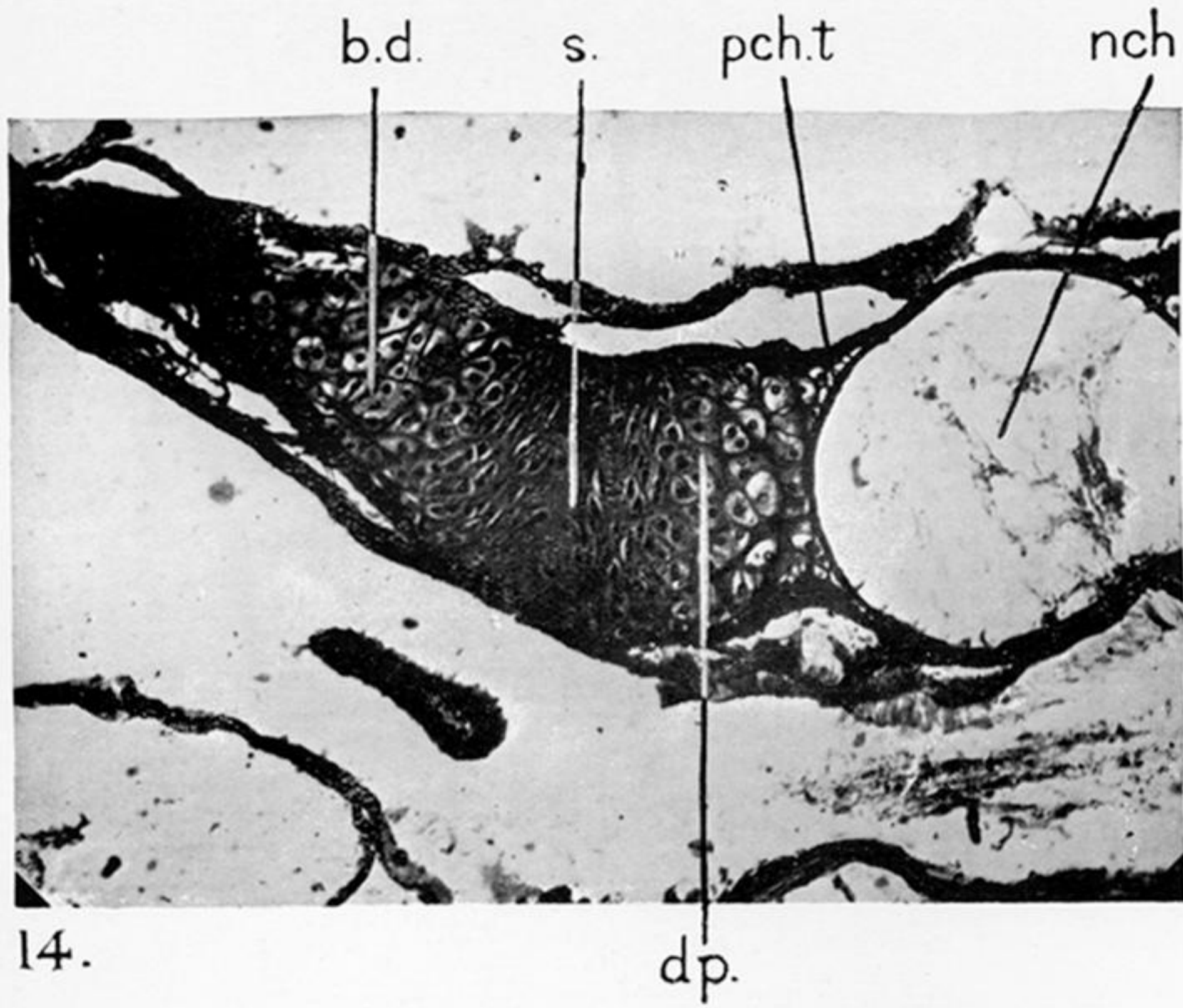
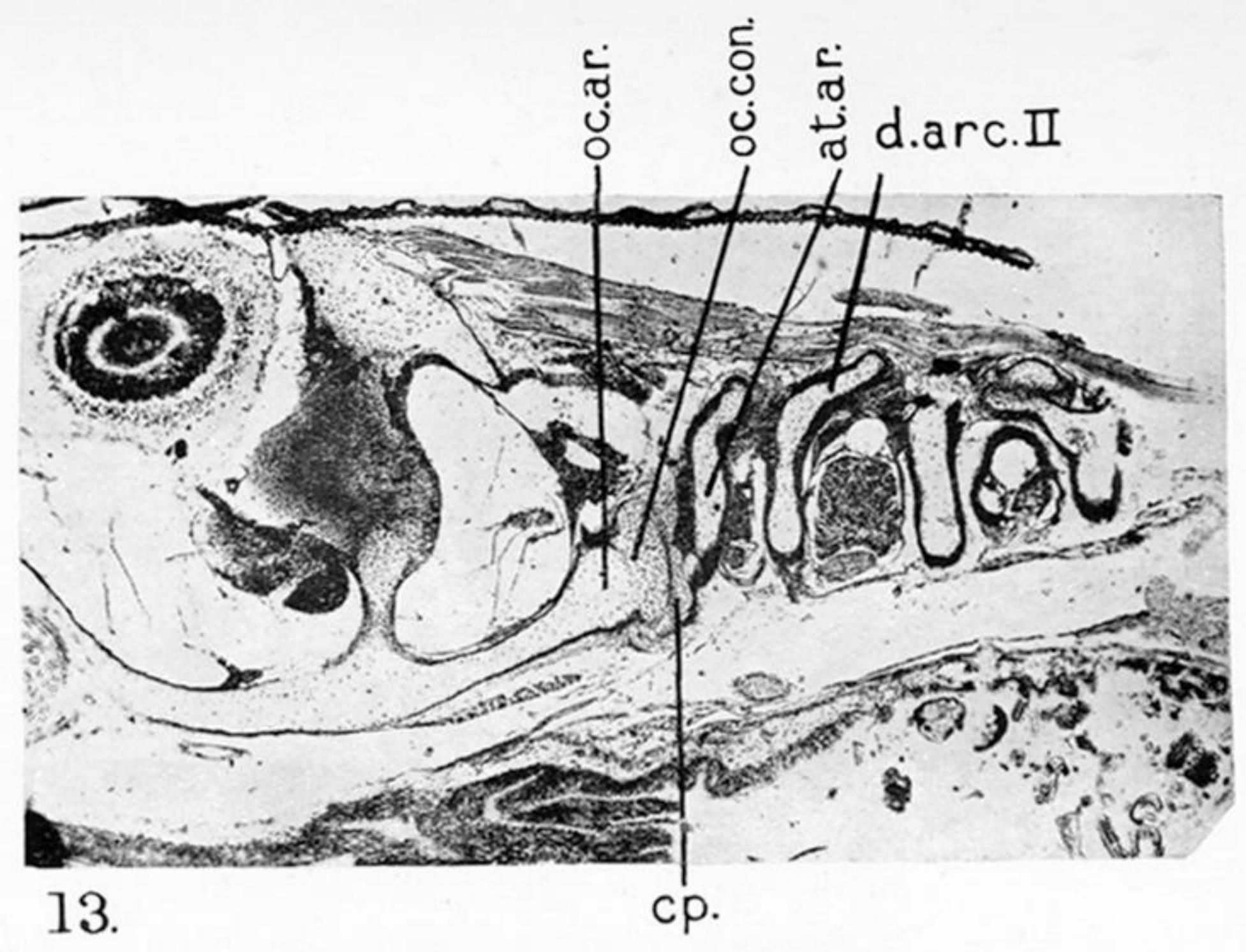
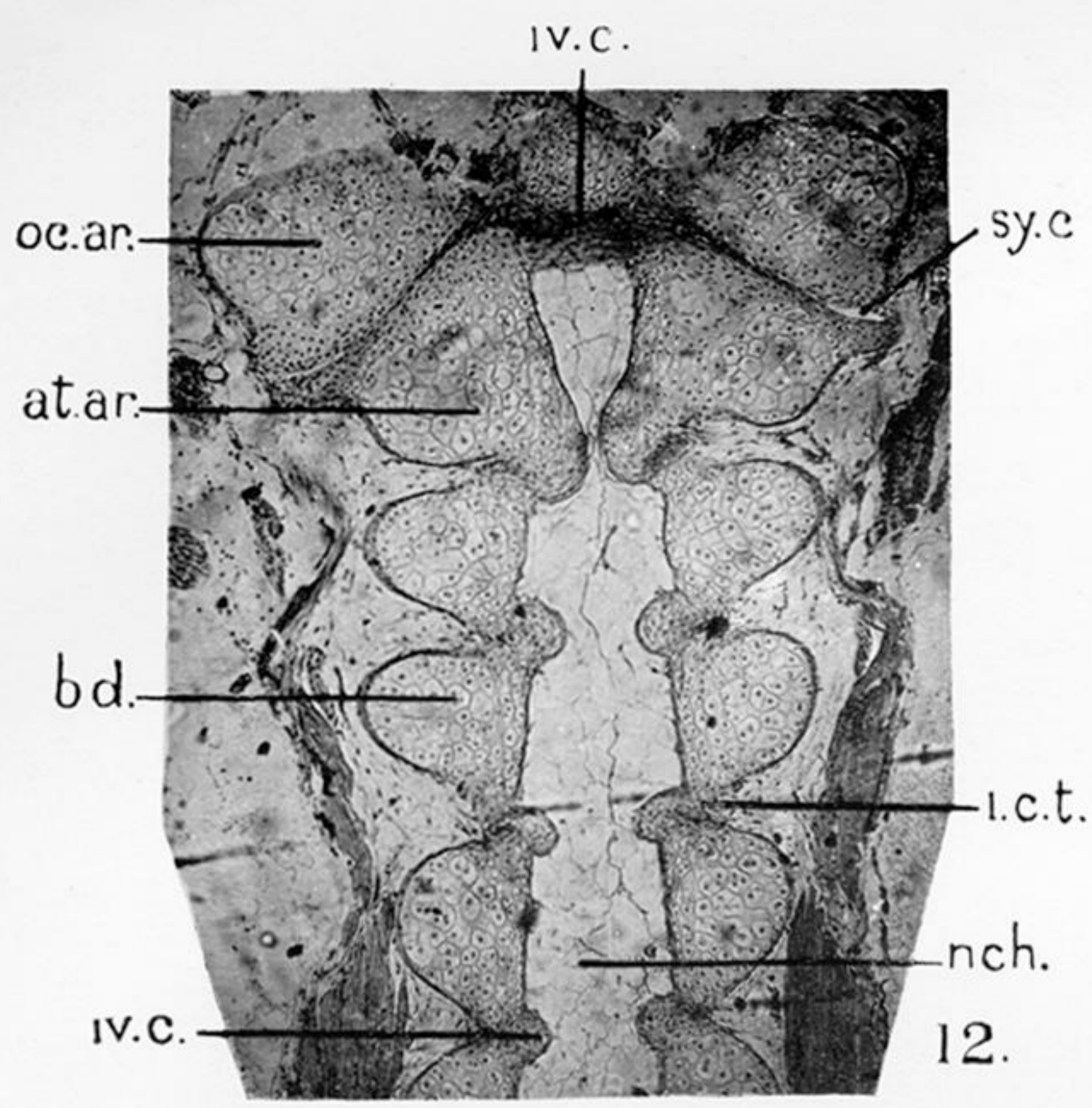


PLATE 16.

FIG. 12.—Frontal section through the trunk vertebrae of *Bufo melanostictus* at 9 mm. stage just after metamorphosis.

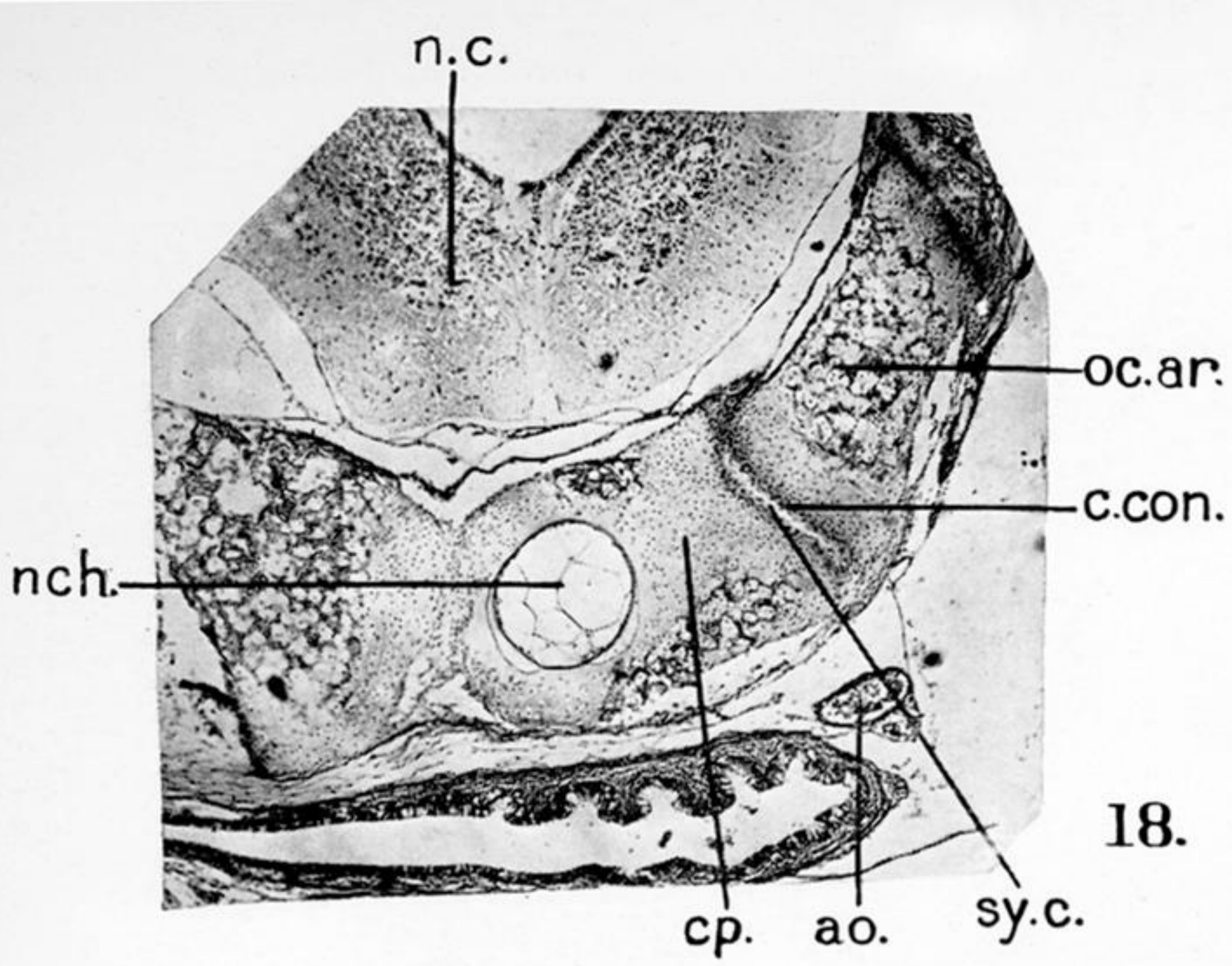
FIG. 13.—Sagittal section through the occipital and the atlas regions of a young *Bufo melanostictus* at 12 mm. stage after metamorphosis.

FIG. 14.—Transverse section of a trunk vertebra of a young *Bufo melanostictus* at 15 mm. stage after metamorphosis.

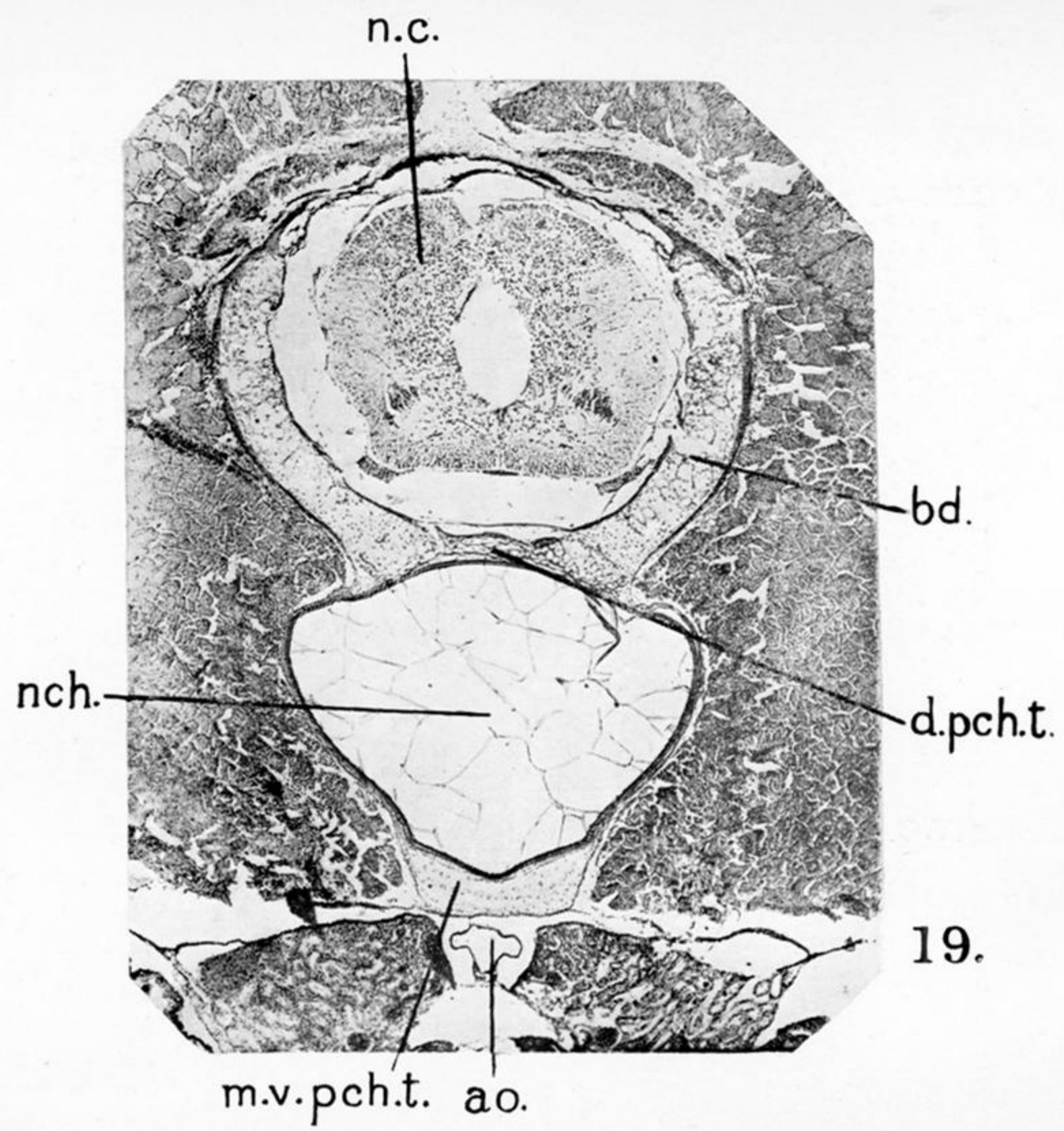
FIG. 15.—Frontal section through the centra of two vertebrae of a young *Bufo melanostictus* at 27 mm. stage after metamorphosis (probably two years old).

FIG. 16.—Transverse section through the trunk vertebra of a tadpole of *Bombinator igneus* at 12 mm. stage.

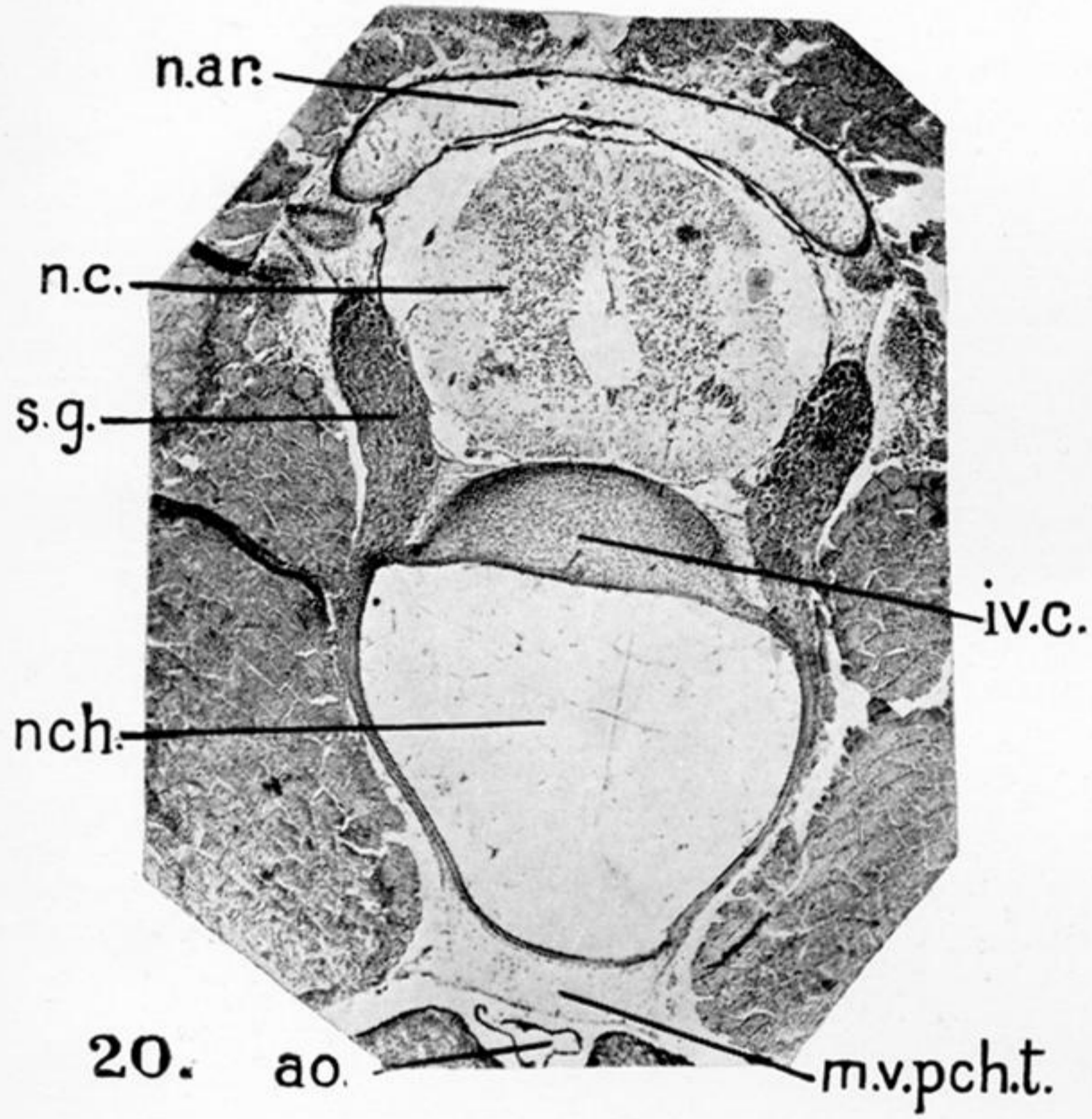
FIG. 17.—Transverse section of a trunk vertebra of a tadpole of *Xenopus laevis* at 31 mm. stage.



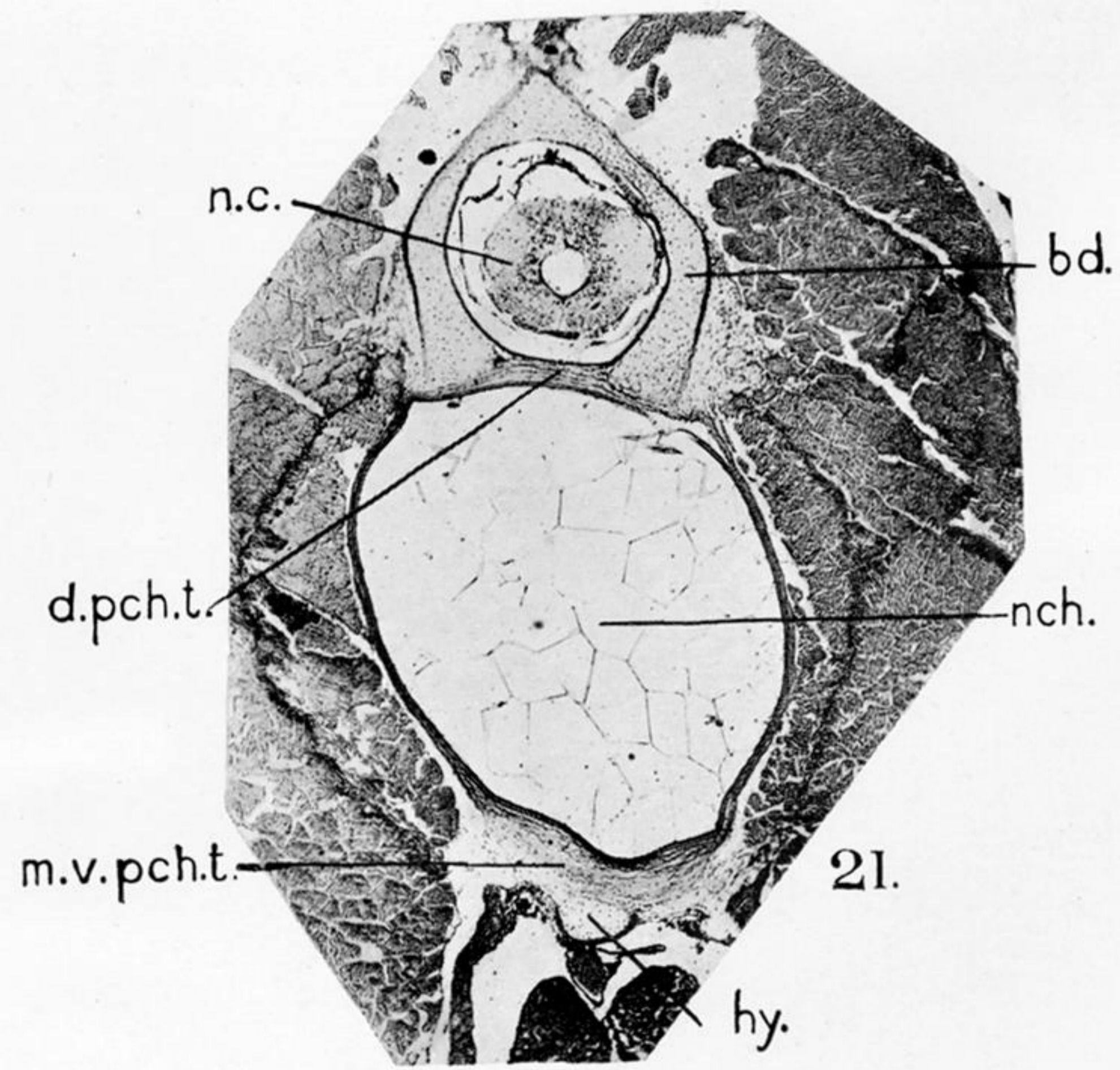
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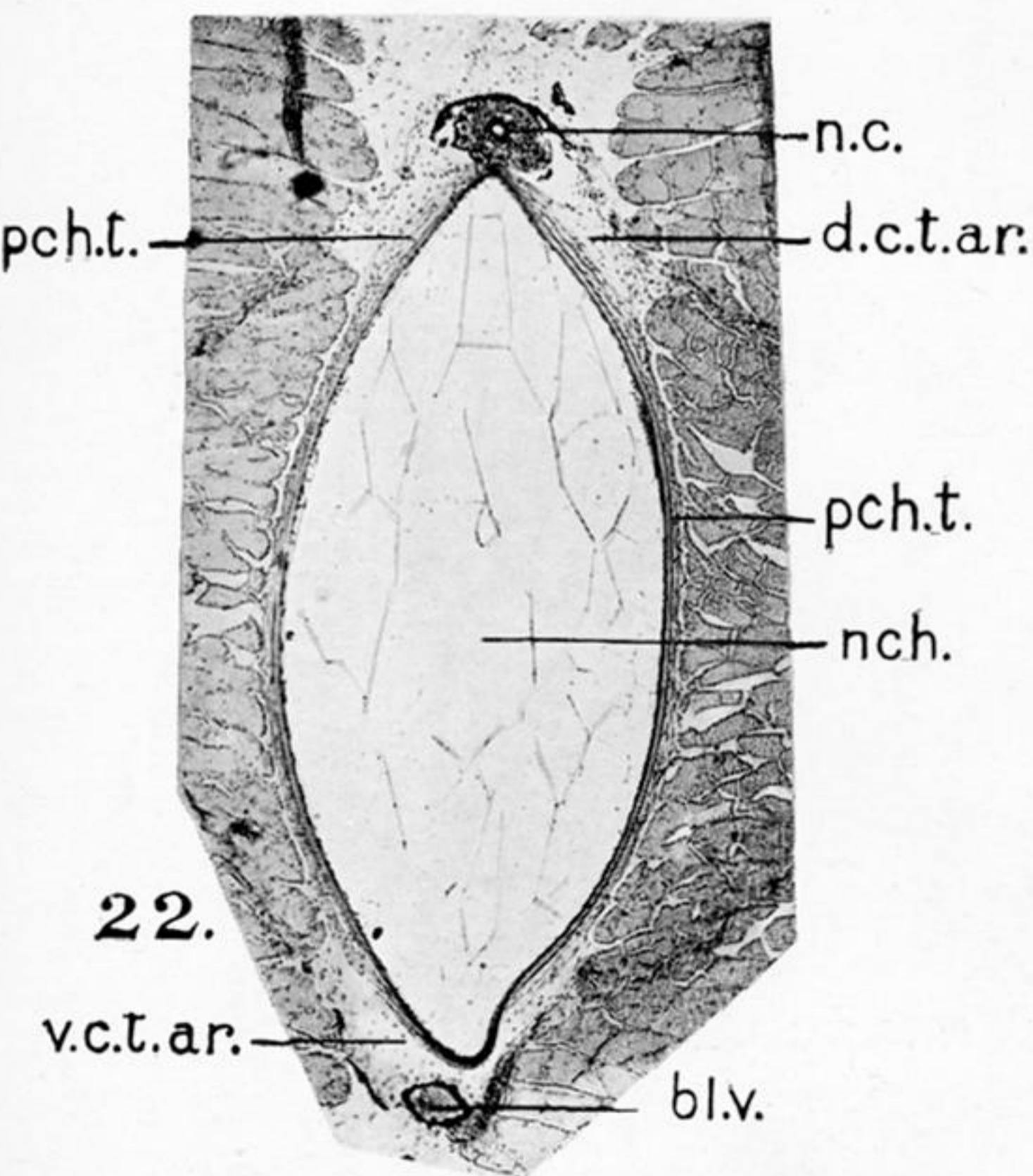
19.



20.



21.



22.

PLATE 17.

FIG. 18.—Transverse section through the occipital and the atlas vertebrae of a tadpole of *Xenopus laevis* at 52 mm. stage just before metamorphosis.

FIG. 19.—Transverse section of a trunk vertebra of *Xenopus laevis* at 52 mm. stage before complete metamorphosis.

FIG. 20.—Transverse section through the intervertebral portion of the trunk region of *Xenopus laevis* at 52 mm. stage just before metamorphosis.

FIG. 21.—Transverse section of the anterior portion of the urostyle of *Xenopus laevis* at 52 mm. stage before complete metamorphosis.

FIG. 22.—Transverse section through the tail region of *Xenopus laevis* at 52 mm. stage just before metamorphosis.

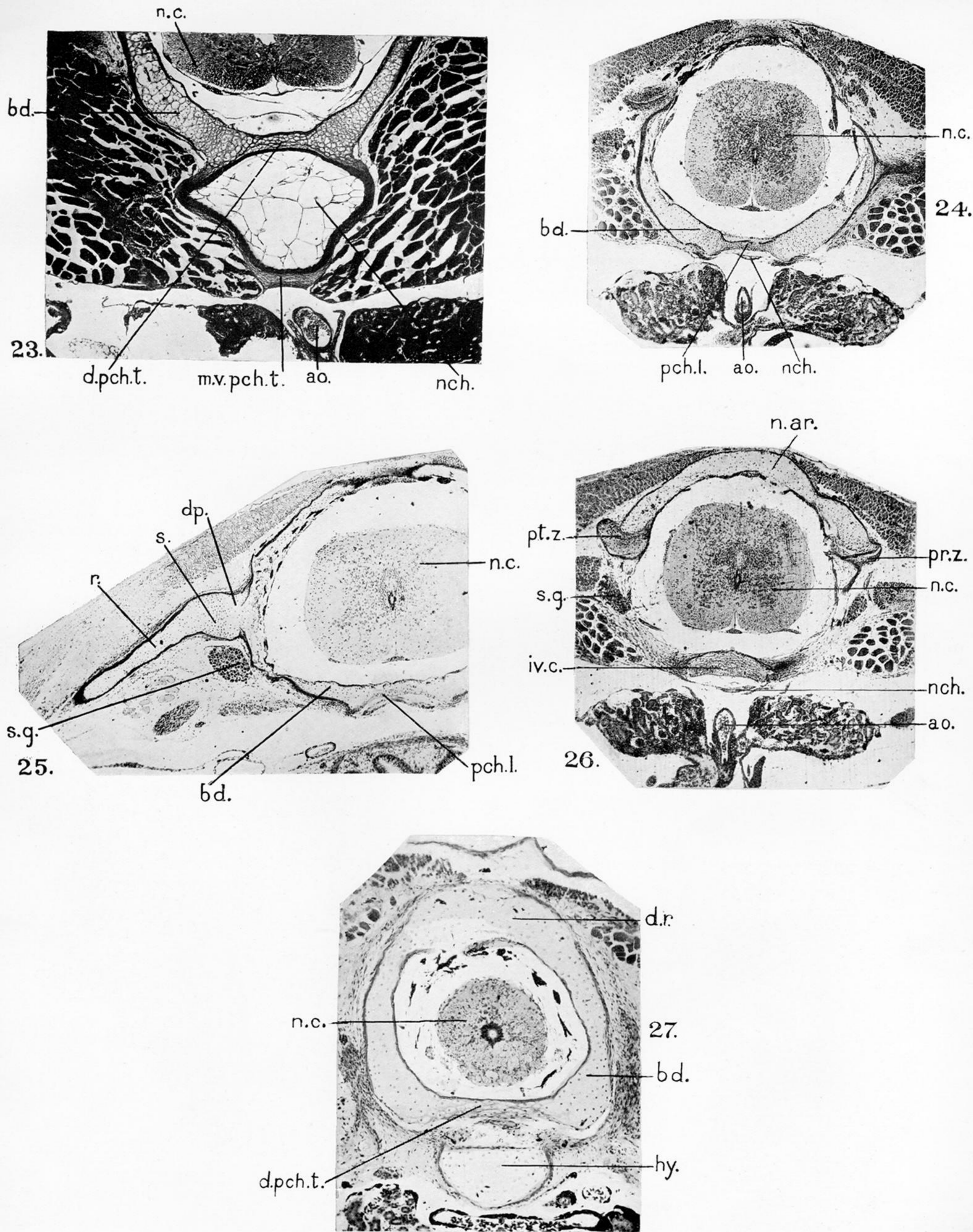


PLATE 18.

FIG. 23.—Transverse section of a trunk vertebra of *Xenopus laevis* at 40 mm. stage during metamorphosis.

FIG. 24.—Transverse section of a trunk vertebra of *Xenopus laevis* at 32 mm. stage after metamorphosis.

FIG. 25.—Transverse section through trunk vertebra and the rib of *Xenopus laevis* at 32 mm. stage after metamorphosis.

FIG. 26.—Transverse section through the intervertebral region of *Xenopus laevis* at 32 mm. stage after metamorphosis.

FIG. 27.—Transverse section through the anterior portion of the urostyle of *Xenopus laevis* at 32 mm. stage after metamorphosis.