

# Developmental and Adult Morphology of the Vertebral Column in the Plethodontid Salamander *Eurycea bislineata*, with Comments on Vertebral Evolution in the Amphibia

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**ABSTRACT** The first description of vertebral development in a plethodontid salamander is presented. *Eurycea bislineata* has larvae that hatch at a rather early stage of development. Somites and the notochord appear early. Somitic differentiation is slight, and no distinct sclerotome can be found. As a result, there is no clear primary segmentation of the skeletogenous tissue. No evidence of a sclerocoelae can be found. The amount of sclerotomal cells surrounding the notochord is very low, relative to other tetrapods. Yet discrete perichordal rings of cells do form, in nearly midsegmental positions, and these give rise to the intervertebral cartilages. Osteogenesis of the centra is initiated prior to hatching and is coincidental with ossification of the neural arch. There is no sign of a neurocentral suture. The centrum forms as a thin shell of bone directly from sclerotomal cells. The notochord is a prominent feature of the vertebral column throughout life, retaining its integrity until late in life when some disintegration occurs locally. The notochord is filled with cartilage midvertebrally in late larval stages, and some additional cartilage forms later in life. The intervertebral cartilage enlarges greatly in late larval life. An opisthocoelous joint forms in this cartilage, apparently as a result of differential changes in the cells of the perichordal ring rather than by an invasion of cells from an external source. The intervertebral cartilage is a dominant structural and highly important functional feature of the adult vertebra. In metamorphosed individuals it may become extensively mineralized, and it consists of many different structural kinds of cartilage.

The cranio-vertebral joint seems to form in a single segment, contrary to the condition reported by some early investigators. It is complex, and consists of articulations between the odontoid process of the atlas and the occipital arch, as well as between the occipital condyles and atlantal cotyles. The notochord plays a dominant role in the early development of the odontoid, but then changes radically and is absent in the adult process.

The anterior trunk region seems to be much more conservative than posterior parts of the column. The patterns of nerve routes and nature of development of the ribs and rib bearers differs greatly from conditions elsewhere in the column. The rib patterns are similar to presumed ancestral conditions. Rib development on the sacral and caudosacral vertebrae is in some ways more similar to that of the anterior vertebrae than of the central trunk vertebrae.

Quantitative aspects of variation in the vertebrae of adult salamanders are presented. There is more regional variation and less site variation than would be expected from literature reports.

Evolutionary aspects of the origin of the cranio-vertebral joint, transverse process and ribs, patterns of segmentation, and centrum development are considered in the light of the new information on *Eurycea*. There is no evidence that more than one vertebra is involved developmentally or evolutionarily in the cranio-vertebral joint. The most generalized condition of rib bearers in living salamanders is one in which the dorsal and ventral bearers are in cartilaginous continuity during development. There are many variations on this theme in living species. It is inappropriate to speak of a resegmentation of the sclerotome in *Eurycea*, even though the adult vertebra is a transsegmental structure, because there is no primary segmentation of the scanty sclerotome. The important feature found in vertebral development in all tetrapods is the perichordal tube and its subsequent differentiation. Questions concerning precise homologies of the salamander vertebral centrum with those of other vertebrates cannot be answered by data from development sequences with currently used criteria of homology. On the other hand, it appears that all centra, regardless of subdivision, are homologous in all tetrapods.

Since publication of the valuable critical review of vertebral development in tetrapods by Williams ('59), the study of vertebral evolution has entered a new analytical phase (Panchen, '67; Parrington, '67; Schaeffer, '67; Thomson and Vaughn, '68; Thomson and Bossy, '70; Andrews and Westoll, '70a,b; Wake, '70). Our attention has focused on the living amphibians, which differ in several important features of vertebral structure from other tetrapods. We believe that the pattern of vertebral development and features of adult morphology have many implications for interpretations of vertebral structure in fossils. Further, because fossil vertebrae are relatively abundant, these studies have direct relevance to analysis of phylogenetic relationships.

Among the more valuable studies of vertebral development in amphibians prior to the review of Williams ('59) are those of Marcus and Blume ('26), Marcus ('37) and Ramaswami ('42) on caecilians, Gadov (1896), de Gaay Fortman ('18), Mookerjee ('30), Teege ('57) and Schmalhausen ('57, '58) on salamanders, and Mookerjee ('31) and Mookerjee and Das ('39) on frogs. Some relatively recent accounts of salamander vertebral development (Mauger, '62; Schmalhausen, '68) properly belong to the pre-1959 period. An important analysis that deals mainly with ontogenic variation has recently been published by Worthington ('71). All of these workers studied only one or a few species. No information has been presented for the majority of major groups of the Amphibia, and there is no comprehensive review.

Within the Order Caudata, no account of vertebral development is available for any member of the family Plethodontidae. This highly derived group includes more than two-thirds of the living species of salamanders. We have studied the vertebral column of the Two-lined Salamander, *Eurycea bislineata*, in order to present a relatively complete account of vertebral morphogenesis in a plethodontid. This study includes an examination of the general pattern of development, as well as a consideration of regional differentiation and aspects of ontogenetic variation. Special attention is given to early stages of morphogenesis, the nature of the cranio-vertebral and intervertebral joints, and

the development of ribs. The data and analysis of their evolutionary implications presented here hopefully will provide a base for investigations of functional and evolutionary morphology in plethodontid salamanders.

#### MATERIALS AND METHODS

Specimens were fixed in neutral formalin and embedded in either paraffin or parlodion. The paraffin sections were cut at 10  $\mu$  and sectioned in frontal, longitudinal and transverse planes. The parlodion sections, of the larger specimens, were cut at 15 to 20  $\mu$ . Azan and Van Gieson stains were routinely employed. In order to demonstrate special features, such as fibers in cartilage, Sirius Red was occasionally used in preference to Van Gieson. Von Kossa's stain was used to demonstrate the process of mineralization. The sectioned material ranged from small, entire embryos to large, mature adults. Specimens of the following snout-vent length were prepared: 3.0 mm (3 specimens), 3.2 mm, 4.0 mm, 5.0 mm (2 specimens), 6.9 mm, 7.2 mm, 7.4 mm (3 specimens), 7.5 mm, 10.2 mm, 11.4 mm, 12.0 mm, 13.0 mm, 15.5 mm, 16.9 mm, 17.2 mm, 17.4 mm, 20.5 mm, 21.8 mm, 22.0 mm, 27.0 mm, 29.0 mm, 30.0 mm, 36.0 mm, 37.3 mm, 41.0 mm, 41.6 mm, and 43.4 mm.

Specimens cleared in potassium hydroxide and trypsin solutions and stained with alizarin red were available in the following sizes: 7.8 mm, 8.0 mm, 8.8 mm, 9.0 mm, 10.0 mm, 10.3 mm, 11.0 mm, 11.5 mm, 12.0 mm, 14.0 mm (2 specimens), 14.1 mm, 15.0 mm, (2 specimens), 17.0 mm, 18.8 mm, 20.7 mm, 22.3 mm, 37.3 mm, 37.4 mm, 38.2 mm, 39.1 mm, 39.5 mm, 39.8 mm, 40.2 mm, 42.5 mm, and 44.1 mm snout-vent length.

Nearly one hundred disarticulated skeletons from animals which ranged in size from 36 to 46 mm in snout-vent length were also available. Measurements of the vertebrae of some of these were made with a dial reading vernier calipers under a binocular dissecting microscope. Presentation of data and the analysis follows the procedures of Worthington and Wake ('72).

In addition to the above material of *Eurycea bislineata*, representatives of all other families of salamanders were examined as cleared and stained as well as skel-

etal preparations, and sectioned materials of larvae and adults from the following families were studied for comparative purposes: Hynobiidae, Proteidae, Sirenidae, Salamandridae, Ambystomatidae, Amphiumidae and Plethodontidae.

#### VERTEBRAL DEVELOPMENT

The general pattern of vertebral development in *Eurycea*, as described below, is based mainly on observations of the trunk region. Special topics are considered separately.

Normal tables of development for plethodontid salamanders have not been constructed, and it is not possible to do so with our material. Accordingly we have attempted to compare stages of *Eurycea* with those in the Harrison stages of *Ambystoma maculatum*, illustrated by Leavitt (Rugh, '62). Development is not precisely comparable in these two species. *Eurycea*, like most other plethodontids, lays large, yolky eggs, and the large amount of yolk imposes constraints. *Eurycea* hatches at quite a different level of development than does *Ambystoma*. In our material we have found that the earliest embryos available, collected from the field, have a full complement of trunk somites, but that these individuals, in terms of apparent cellular differentiation, are not as advanced as individuals of *Ambystoma* with approximately equal numbers of somites. Therefore, in our analysis we have used distance from the snout to posterior margin of the vent as a standard measurement and have assumed a high correlation with the level of development. Based on features of external morphology, our smallest specimens (3 mm) correspond roughly with a Harrison stage 30 in *Ambystoma maculatum*, and to stages B and C of Mookerjee ('30). Our 5 mm embryos correspond fairly closely to Harrison stages 36 and 37, our 6.9 and 7.5 mm animals are about equivalent to Harrison stage 40, and our 7.4 mm material is roughly equivalent with Harrison stage 46. We feel that attempts to correlate stages between species, especially across familial borders, is not very productive, particularly as far as the later states (Harrison 25 and beyond) are concerned. Brandon ('61) has demonstrated that within *Ambystoma* alone significant differences occur on the species level in the time of

appearance of several features used in production of normal tables.

#### Prevertebral stages

**3 mm stage.** The embryo is curved over the large yolk mass. The trunk region is differentiated into nerve cord, notochord and somites, and large numbers of yolk granules occur in all cell types (fig. 10, 13). The notochord and notochordal sheath are well differentiated, but the sheath is extremely thin (fig. 13). Cellular detail within the notochord is greatly obscured by the massive number of yolk granules, but the nuclei appear to be arranged more or less peripherally within the sheath. Morphologically these cells appear to be about stage d ("pile of coins" arrangement) of Mookerjee, et al. ('53), characteristic of Harrison stage 23 in *Ambystoma* and *Triturus*. The number of cells is not so great and the amount of yolk and size of individual yolk granules is much greater in *Eurycea* than in these genera. Boundaries of the somites are clearly defined, but the somitic cells are relatively undifferentiated and contain many large yolk granules. The developing septa are directed anteromedially and they may join the notochordal sheath at the lower level of the somite mass. No discrete sclerotome can be seen, but a few scattered cells surround the notochordal sheath. These cells do not appear to be segmentally arranged. The myotomal parts of the somite are now highly organized, and the nuclei of the promuscle cells tend to be arranged around the periphery of each somite. An occasional myocoel is seen, but usually it is suggested only by the arrangement of the cells. Segmental ganglia cannot be seen.

**4 mm stage.** The embryo remains curved around the yolk mass and the differentiating embryonic cells contain yolk granules. Details of somite arrangement are now well established and the pro-muscle cells are elongate (fig. 11). Intrasomatic differentiation has proceeded, and the nuclei of the myoblasts are largely concentrated towards the anterior and posterior limits of each developing myotome. The yolk granules are most common at the ends of the somites so that each developing muscle block has a relatively clear area in the center and denser areas at

each end. In frontal section at the level of the notochord the septa extend sharply lateroposteriorly. The topography of the septa produces some overlap of successive somites and results in a triangular recess at the anterior limit of each somite. Midway between the ends of the somites (intra-segmentally) is a rounded depression in the medial wall of the myotome. This depression and the intersegmental recess run dorsoventrally for most of the depth of each somite.

The notochord is somewhat larger than at the 3 mm stage but has essentially the same structure with large amounts of yolk. The notochord terminates anteriorly, below the developing brain in the region of the auditory placodes.

The sclerotome consists of a number of scattered cells, with rounded nuclei, which are closely applied to the inner border of the myotomes. The sclerotomic tissue of adjacent somites is not segmented, but forms an essentially continuous layer along the medial border of the developing somites. There is no evidence of a sclerocoel, nor is it possible to distinguish areas of differential cell density corresponding to cranial or caudal portions for a given somite. There is, however, some segmental differentiation of the sclerotome, with the mesenchyme forming aggregations in the intersegmental recesses. The cells between the segments are topographically more closely associated with the posterior rather than anterior somite. These intersegmental aggregates are the first prevertebral cells. Clusters of cells behind these prevertebral cells eventually form the segmental ganglia.

*5 mm stage.* Little change is noted from the 4 mm stage. The notochord retains large amounts of yolk. Differentiation of the somites and the sclerotome has advanced somewhat; the more posterior somites are in the process of differentiation whereas the anterior somites and segmental ganglia are distinct. The cells of the ganglionic rudiments lie in a depression in the medial wall of each somite, immediately posterior to the intersegmental recesses, in a position corresponding to the cranial portion of each segment.

The sclerotomic layer, though very thin, is clearly defined, especially anteriorly

(fig. 12). It is not possible, however, to find evidence of segmentation or differences in cell density relating to somitic patterns. The cells have been derived from the medial margins of the somites but the intersomitic septa fail to divide the layer.

#### *Vertebral stages*

Embryos of *E. bislineata* hatch when they measure between 6.5–7.5 mm in standard length. Hatching results in an acceleration of the developmental process and during the immediate post-hatching period, size cannot be absolutely correlated with developmental stage. In our material, one embryo removed from the egg measured 7.5 mm but was at the same level of differentiation as a larva of 6.9 mm. Two embryos at 7.4 mm were more advanced than either of the others. Embryos in this general size range are grouped into two developmental states (6.9–7.5; 7.4) in the discussion below.

*6.9–7.5 mm stage.* Vertebral elements first appear in embryos at this stage of development. The notochord remains filled with yolk particles, and in some areas indications of vacuolated notochordal cells can be seen. The notochordal sheath surrounding the cells of the notochordal epithelium is readily visible. The sheath is surrounded by perichordal mesenchyme cells which are somewhat elongate and tend to have their longitudinal axes around the notochord. These cells around the notochord constitute the perichordal tube of other authors (see Mookerjee, '31; Williams, '59). The "tube" in *Eurycea* is not a continuous layer but a loose assemblage of cells lying close to the notochordal sheath. Near the middle of each segment the perichordal cells increase in number and become organized into two or three layers around the notochord. These indistinct areas are the perichordal rings and mark the position of the future articulation of successive vertebrae. The remainder of the perichordal tube (i.e., between successive perichordal rings) is concerned with the production of the vertebral centrum. The perichordal tube is generally not more than one or two cells in thickness, and the cells are very flattened. Plethodontid salamanders differ consider-

ably from more primitive salamanders, and especially caecilians and amniotes, in this regard.

The cells occupying the intersegmental recesses form broadly based columns which rest directly on the notochordal sheath. The columns extend dorsally and posteriorly to enclose the lower one-half to two-thirds of the neural tube. The outer cells of the columns are elongate while the inner cells are rounded. The rounded cells within the base of each column produce the cartilaginous rudiment of the neural arch base. This rudiment is the basidorsal of Gadow (1896). This term implies that the structures are derived from discrete, paired anlagen (dorsal arcualia) which correspond to the upper part of the caudal sclerotome half of each somite. However, in view of the fact that no division of the sclerotome occurs in *Eurycea*, the terminology of Gadow is inapplicable. We prefer the term neural pedicel rudiment for these early vertebral structures.

The segmental ganglia are well differentiated and lie immediately posterior to each neural pedicel rudiment. The ventral ramus of each spinal nerve is clearly visible as it curves ventrolaterally from the segmental ganglion.

Muscle fibers of the somites are well developed and contain a few yolk inclusions. The cells are elongated, slender and clearly striated.

*7.4 mm stage.* The notochord is characterized by a further reduction in the amount of yolk and an elaboration and enlargement of the "vacuolated" cells (figs. 14, 15).

The most significant feature of this stage is the initiation of osteogenesis by the perichordal cells. The centrum ossifies as a cylinder, with no indication of laterally paired elements such as those described by Schmalhausen ('68) for *Ranodon*. Bone first appears in the perichordal tube, midway between successive perichordal rings, and around the base of the neural pedicel rudiment (fig. 17). In this respect *Eurycea* differs from *Ranodon*, where ossification of the rudiment is initiated dorsally, then extends ventrally to join the centrum (Schmalhausen, '68). The developing cylinder of bone increases in diameter to partially enclose the cells of the

perichordal ring. A gap exists between the bony centra of adjacent vertebrae in the region of the perichordal ring (fig. 1). This is the region of the prospective intervertebral joint.

While it is the cells lying immediately against the notochord that give rise to the bony centrum for most of its length, it is the cells farthest from the notochord which produce bony tissue in the region of the perichordal ring (see also Schmalhausen, '68). In the region between successive rings, the amount of perichordal tissue is so small that virtually all of it is involved in bone production. In the region of the rings, however, cell proliferation has occurred, and it is only the outermost cells that are involved in the production of bone.

The cells which form the perichordal rings are well differentiated and elongated, with attenuated nuclei (fig. 14). In some cases a single cell may extend about one-third of the way around the notochord. As the organism grows, a restructuring of the vertebra takes place, with growth of the notochord within the centrum occurring. In order to accommodate for notochordal growth, the inner margins of the centrum must be partially resorbed. This function may be performed by perichordal ring cells which migrate toward the neural pedicel rudiment, between the bony centrum and the notochord. In older individuals, such cells extend further inward than they do in this stage.

The general pattern of centrum development in *Eurycea* is similar to that described in hynobiids by Schmalhausen ('57, '68), but there are important differences. In addition to those cited above, the neural pedicel rudiments lie directly on the notochordal sheath in *Eurycea* (figs. 16, 17). There are no underlying perichordal cells, such as occur in hynobiids (this has been a subject of controversy in the literature and should be systematically re-examined; see Mookerjee, '30, and Wake, '70).

The inner cells of the neural pedicel rudiments are now cartilaginous. The chondrocytes rest on the notochordal sheath at the base of each ascending column of cells, and are bounded by elongated cells which are oriented dorsoven-

trally (fig. 16). There is some evidence of fiber production by these elongate cells, which extend posterodorsally, beyond the main cells of the rudiment, over the dorsal aspect of the spinal cord. The dorso-lateral and dorsal component of each neural pedicel rudiment thus consists of a pro-skeletogenous blastema.

In the most advanced individual of this stage a procartilaginous, rod-like rudiment of the first rib lies in the intermyotomal septum opposite the second vertebra, but no rudiment of the rib-bearer is evident.

The segmental ganglia are well developed (fig. 17). In the anterior parts of the trunk they lie almost immediately dorsal to each perichordal ring, but over most of the trunk they are located in front of the rings and just behind each neural pedicel rudiment.

Yolk within the muscle cells is further reduced (fig. 17). The neural pedicel rudiments are closely associated with the inter-

myotomal septa, which usually envelop the pedicel, or lie just anterior to it.

The trunk vertebrae at this stage consist of a series of thin osseous cylinders which are expanded at each end around the perichordal rings (fig. 1). The latter are in the process of differentiating, but as yet no cartilage has formed. It is possible that at least some of the most peripheral cells of the ring (i.e., those that are close to the junction of the bony centrum and the notochordal sheath) may be incorporated into the bone of the centrum, as has been reported by Schmalhausen ('57, '68). Over most of the length of the vertebra (at least for two-thirds of the total length) the bone of the centrum is closely applied to the notochordal sheath with no intervening cells, and only in the vicinity of the perichordal rings do the two layers become separated. Bone completely surrounds the base of the column-like neural pedicel rudiment, firmly anchoring it to

#### Abbreviations

a.c, atlantal cotyle	n.s, notochordal sheath
at, atlas	occ.a, occipital arch
b, bone	occ.co, occipital condyle
b.pl, brachial plexus	occ.fa, occipital facet
b.r.od.c, bony remnant of odontoid cartilage	od, odontoid process of the atlas
c, cartilage	od.c, cartilage of the odontoid process
c.b, cartilage bridge joining dorsal and ventral rib bearers	od.co, articular condyle of the odontoid process
c.c, columnar cartilage	os, ossifying area
c.d.r.b, cartilaginous core of dorsal rib bearer	ot.c, otic capsule
ce, centrum	p, ventral rib bearer (parapophysis)
c.m, cartilaginous mass which gives rise to dorsal and ventral rib bearers	pocc, preoccipital arch
cr.se, crista sellaris	po.z, postzygapophysis
d.b, developing bone	pp, palatopterygoid
d.r.b, dorsal rib bearer	p.p.m, presumptive plane of movement in intervertebral cartilage
d.r.h, dorsal rib head	p.r, perichordal ring
d.r.r, dorsal rib head rudiment	pr.z, prezygapophysis
d.v.r, dorsal vertebral roof	r, rib
e.n.p.r, eroded neural pedicel rudiment	r.b, rib blastema
f, line of fibroblasts representing presumptive dorsal head of rib	r.hyp, ramus hypoglossus
f.c, fibrocartilaginous joint	s, cells of the sclerotome
f.n.s, fragmented notochordal sheath	s.b, somitic border
g, ganglion	s.c, cells separating occipital arch from notochord
h.c, hyaline cartilage	sp.n 1-6, spinal nerves one through six
h.t, haemopoietic tissue	sq, squamosal bone
hy, rudiment of hyperapophysis	st, bony strut attaching ventral rib bearer to the centrum
hyp.c, hypochordal commissure	su, suspensorium
i.m, intermyotomal septum	tra, trabecula
i.r, intersegmental recess	T 1-4, trunk somites one through four
l.j, lower jaw	t.vert, trunk vertebra
m, cells of the myotome	V 1-4, trunk vertebrae one through four
M3 + M2, fused metotic somites three and two	v.a, vertebral artery
n, notochord	v.n.c, vertebral notochordal cartilage
n.c, notochordal cartilage near intervertebral joint	v.c, intervertebral cartilage
n.c.s, nerve cord support	v.r.b, ventral rib bearer
n.e, notochordal epithelium	v.r.f, ventral root foramen
n.p.r, neural pedicel rudiment	v.r.h, ventral rib head
	y, yolk granules

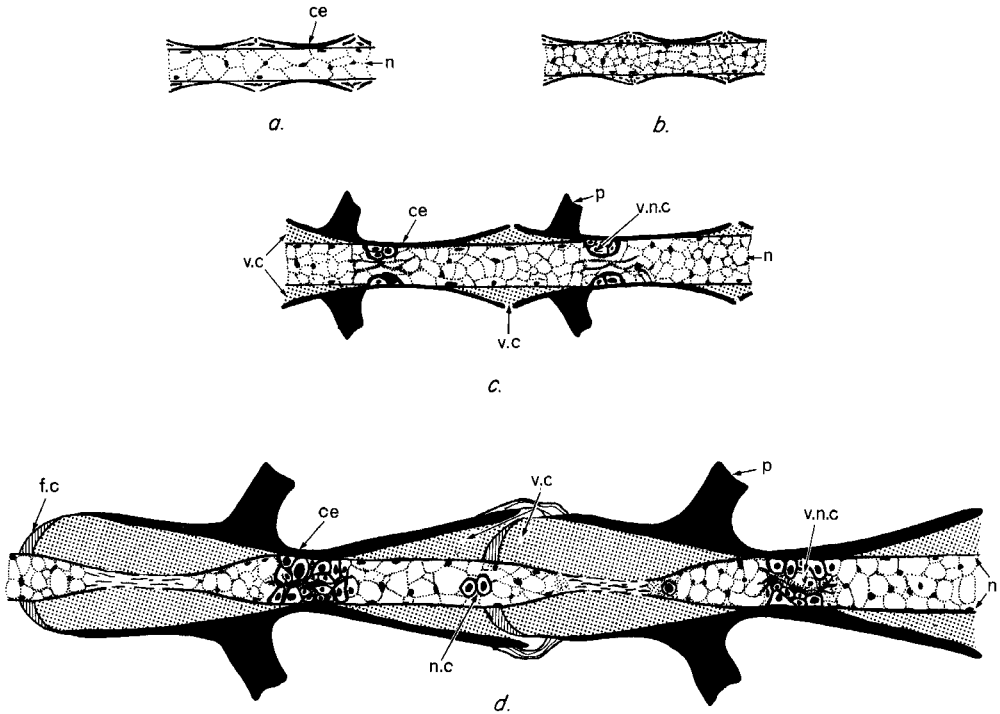


Fig. 1 Diagrammatic representations of frontal sections through the centra of *Eurycea bislineata* at various stages of development. a, 7.4 mm stage; b, 10.2 mm stage; c, 15.5–17.4 mm stage; d, adult stage. Anterior to the left. See text for explanation.

the developing centrum, and retaining the intimate contact of the rudiment and the notochord (fig. 17). Accordingly, no neuro-central suture is present in *Eurycea*, and the bony vertebra develops as a single entity.

*10.2–11.5 mm stage.* Animals in this size range are free-living larvae, and considerable change in vertebral structure occurs. Significant features are the formation of the vertebral roof, the appearance of zygapophyses, and the initial stages of rib and transverse process development. Developmental rate of the vertebral column is no more correlated with size than in earlier states (see above).

Differentiation of notochordal and muscle cells is essentially complete, and yolk particles are rarely encountered. The notochordal cells are large and vacuolated, usually with small nuclei (fig. 18). Cells of the notochordal epithelium lie along the inner margins of the rather thick notochordal sheath. The notochord is constricted midvertebrally, at the level of the neu-

ral pedicel rudiments, and slightly dilated in the intervertebral region.

The centrum is two or three times as thick as the notochordal sheath (fig. 18). The gap between adjacent centra, so obvious in the smaller individuals, has virtually disappeared. The centra of adjacent vertebrae are in close proximity, and are joined by a thin, narrow band of dense fibrous connective tissue.

Each perichordal ring is a rather spindle-shaped structure, with the notochord extending through the axis. At either end of the ring the cells have become chondroblasts. In the center of the ring the cells are more flattened, but are also in the process of differentiation, and in the largest individuals they are chondrocytes. Just anterior to the midpoint of each perichordal ring, the cells retain the form of fibroblasts. There is no indication that these are differentiating, although they lie in the region that eventually becomes the intervertebral joint. The joint region is not quite symmetrical since the cells dorsal to

the notochord are slightly anterior to those below it. The dorsal lip of the centrum is also anterior to the ventral lip, and the gap separating adjacent bony centra is oriented obliquely in a vertical plane.

The perichordal ring tends to increase in size, and is composed of from five to six concentric layers of cells (fig. 18). As these cells undergo chondrogenesis, restructuring of the bony centrum accommodates the expanding intervertebral cartilage.

Development of the neural arch is proceeding rapidly. The neural pedicel rudiments are cartilaginous rods which curve dorsally around the nerve cord. As the top of the cord is reached they extend posterodorsally and meet in the midline. The area of contact is thickened and enlarged, extending anteriorly and posteriorly (fig. 24). This cartilaginous structure has been called the supradorsal by Mookerjee ('30), who said that it formed as a separate element in *Triturus*. Teege ('57) also indicated the presence of a separate center of chondrification in *Ambystoma mexicanum* and Mauger ('62) found it in *Pleurodeles waltl*. These authors have shown a dorsal element joined by areas of lower cellular density to the neural pedicel rudiments on either side. One of our specimens (10.2 mm) has incomplete neural arches on the atlas and first three trunk vertebrae. Another individual (10.3 mm) has a complete neural arch on all trunk vertebrae and the atlas. On the first trunk vertebra there are some cartilage cells where the neural pedicel rudiments join above the nerve cord. The cells are few in number and do not form a separate piece of tissue. Cells of the neural pedicel rudiments as well as those mobilized from surrounding area of mesenchyme cooperate in the early stages of the formation of the roof of the vertebra. The "supradorsal" does not develop from a distinct mesenchymatous block or as a separate center of chondrification in *Eurycea*. The dorsal cartilaginous roof gives rise to a posterolateral process on either side and also has a middorsal posterior extension. Transverse sections through the region where the three processes separate give the impression of three distinct pieces of cartilage joined by less well differentiated cells. This arrangement might

have been mistaken by earlier authors for the independent origin of the "supradorsal."

Ossification of the neural arch is initiated in the area surrounding the base of each neural pedicel rudiment. Bone develops anteriorly and posteriorly to form a low sheet attached to the dorsolateral margins of the centrum. This sheet spreads dorsally in front of the neural pedicel rudiment until it reaches a point just slightly below the top of the nerve cord. Behind the neural pedicel rudiments of the mid-trunk vertebrae are the dorsal and ventral spinal nerve roots and spinal ganglia and there is little bone (figs. 2, 21). However, in more advanced individuals the sheet of bone forms a narrow bridge which grows dorsally to join the backward sloping neural pedicel rudiment just posterior to each ganglion.

The progression of ossification has been verified by the examination of cleared and stained individuals (10.2–11.5 mm) which bracket in size the sectioned material. In the smaller individuals all of the neural pedicel rudiments fail to meet dorsally and ossification surrounding the pedicel rudiments extends only to the level of the center of the nerve cord. In the larger specimens the neural arches are complete on most of the trunk vertebrae. However, the top of the arch remains cartilaginous on the third trunk vertebra, and the pedicel rudiments fail to meet on the first two trunk vertebrae, and the atlas. The nerve cord is enlarged anteriorly to form the brain stem, and neural arch completion is delayed because of the increased amounts of bone deposition required on these anterior vertebrae.

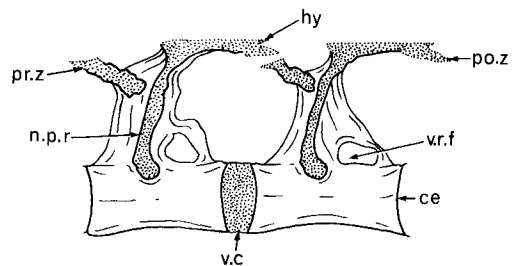


Fig. 2 Lateral view of two vertebrae of a cleared and stained specimen of *Eurycea bislineata*, 10.2 mm standard length. Cartilage stippled. Anterior to the left. See text for explanation.



Zygapophyseal formation differs from that described by Mookerjee ('30) for *Triturus*. He reported that during early development an aggregation of cells, the "dorsointervertebral," lay on either side of the nerve cord between adjacent vertebrae. Later this was invaded by a sheet of fibroblasts which grew in a horizontal plane into the new cartilaginous block and divided it into ventral (prezygapophysis) and dorsal (postzygapophysis) parts. Teege ('57) was unable to confirm these observations and our data are also in conflict with those of Mookerjee (see also comments of Smit, '53). The earliest indications of any zygapophyseal element in *Eurycea* is a small cartilaginous rod lying parallel to the nerve cord. From its earliest appearance this rod has a bony connection to the neural pedicel rudiment behind it, but is not connected to the arch in front. This rod gives rise to the prezygapophysis. The postzygapophysis arises from a process which grows posteriorly from the thickened dorsal cartilage produced by the posterodorsal growth of each neural pedicel rudiment. This small process of procartilage cells extends posteriorly in the 10.2 mm stage and comes to lie just above the anterior end of the developing prezygapophysis. At this stage pre- and postzygapophyses are clearly separated and there is no evidence of their origin from a common blastema (fig. 22). In the 11.3 mm individual zygapophyseal development has proceeded much further and the pre- and postzygapophyses are both well formed and are enclosed in bone. They are located high on the neural arch, at the level of the top of the nerve cord. A synovial capsule has formed, with a space and small amounts of fibrocartilage separating the cartilaginous cores of the two processes. The prezygapophyses are joined by bone to the neural pedicel rudiment, but the cartilaginous parts of these two elements are not continuous at any stage.

In cleared and stained specimens (10.2 mm) small amounts of stain in the intermyotomal septa mark the sites of the first two pairs of ribs, but no other indications of either ribs or rib bearers are present. In two cleared and stained specimens (10.0 mm, 11.0 mm) two pairs of ribs and their ventral bearers are ossified. There

is an unossified gap between rib and bearer. Only parts of the ventral bearer of the third rib are ossified. In the smallest sectioned individual (10.2 mm) rudiments of the first three pairs of ribs are found and traces of the rib-bearers are present for the first two. Only the first pair of ribs has begun to ossify (fig. 19).

The development of the ribs and their rib-bearers is initiated independently. The rib rudiments grow medially and the bearers laterally until they meet. The ventral rib bearers of the first two trunk vertebrae are more advanced in development than the dorsal bearers. The ventral rib bearer is a small rod of cartilage located at the level of the junction of the neural pedicel rudiment and the centrum. This rod is oriented so that its medial tip is lower than its lateral end. It is connected to the ventrolateral margin of the centrum by a cartilaginous process which is entirely ossified at the centrum. This strut is the *Basalstumpfe* of Göppert (1896), *Centrapophyse* of Keller ('46), *Zentrapophysis* of Teege ('57) and the basiventral of Gadow (1896) and Mookerjee ('30). A dorsal process of the cartilaginous rod extends along the side of the neural pedicel rudiment to a point just above the level of the center of the nerve cord. There it joins the slightly enlarged cartilaginous rudiment of the bearer of the dorsal rib head. The vertebral artery and vein lie between the dorsal process and the strut which attaches the lower rib bearer rudiment to the centrum. The dorsal head of the rib is only partially developed and does not reach the dorsal rib bearer rudiment. The ventral head of the rib is at a more advanced state and is joined to its rib bearer by fibrocartilage (fig. 19). The ventral rib bearer is slightly anterior to the dorsal one and its ventral bony strut extends almost to the anterior end of the centrum.

Rib bearers are only slightly developed on the third trunk vertebra but the rudiment of the ventral rib head is well differentiated. Procartilaginous blastema of the ribs of the fourth and fifth trunk vertebrae is present, but no evidence of rib bearers is seen (fig. 21).

On the larger individuals (10.3 mm) rib rudiments are present on the first seven trunk vertebrae, but rib bearer rudiments are present only on the first five.

The ribs clearly develop prior to the rib bearers, and in a distal position.

The first two vertebrae resemble those of the smaller individuals. In each, dorsal and ventral rib bearer rudiments are joined by a continuous rod of cartilage lying along the neural pedicel rudiment. The ventral rib bearer is a little anterior to the dorsal. Both are represented by enlargements at either end of the rod. Upper and lower heads of the first two ribs are developed and both have fibrocartilage connections with their respective rib bearers. The cartilaginous bearers, the connections between them, and the major portion of each rib are surrounded by bone. A bony strut extends from the ventral rib bearer, below the vertebral artery and vein, to attach to the ventrolateral side of the centrum near its anterior end.

The third vertebra has a different arrangement. The dorsal and ventral rib bearer rudiments are completely separated and the ventral one is located far anteriorly. In addition, the ventral bearer is no longer attached to the neural arch, except by a small amount of bone which extends to a point just anterior to the neural pedicel rudiment. The ventral rib bearer is below the vertebral artery and vein and is attached by bone to the centrum. The dorsal rib bearer is a separate aggregation of cartilage cells that lies high on the neural arch, opposite the neural pedicel rudiment. It is connected to the slender cartilaginous dorsal rib head by an area of elongated cells. These seem to be differentiating into chondroblasts.

The fourth vertebra resembles the third, but all elements are less well developed. The ventral bearer rudiment, joined to the anteroventral surface of the centrum, is poorly differentiated. The attachment, which is far anterior to the base of the neural pedicel rudiment, is by means of a short, dense aggregation of fibroblasts. There is no trace of a connection between the developing ventral bearer and the walls of the neural arch. The only sign of the developing dorsal rib bearer is a small aggregation of elongate cells lying against the dorsolateral margins of the neural arch, just opposite the neural pedicel rudiment. No dorsal rib head is

present, but the ventral head is in contact with the ventral bearer rudiment.

Dense fibers and fibroblasts connect the ventral bearer of the fifth vertebra with the centrum, but the attachment is less extensive than on the fourth. The ventral rib head is in contact with the small, cartilaginous rudiment of the ventral bearer. No sign of the dorsal rib head or dorsal rib bearer is seen.

No dorsal or ventral rib bearer rudiments are present on the sixth or seventh vertebrae, but both have rib rudiments in the form of small cartilaginous rods. Even these are absent in more posterior vertebrae.

*12.0 mm stage.* The notochord, still comprised mainly of vacuolated cells, is being somewhat constricted by the enlarging intervertebral cartilages (fig. 29). The spindle-shaped intervertebral cartilages are well formed and consist of about six to seven layers of chondroblasts wrapped around the notochord. Cartilage formation is active. Undifferentiated fibroblasts lie on the outer margins of the cartilage. Some of these are oriented anteromedially into the cartilage. This is the area of the intervertebral joint. These fibroblasts have not migrated into the cartilage, as Mookerjee ('30) reports, but are undifferentiated cells of the perichordal ring.

The vertebral roof is now complete on all but the last few caudal vertebrae. In cleared and stained material the ventral heads and bearers of the first three ribs are ossified. Dorsal heads of the first two ribs are ossified at their distal extremity, and parts of their bearers, from which they are well separated, are also ossified. In sectioned material dorsal and ventral rib bearers of the first two trunk vertebrae are cartilaginous with an osseous outer covering. They are joined by a piece of cartilage (figs. 25-28). The bearers are separated on succeeding vertebrae, as far as the sacrum, and the ventral bearer is well in front of the dorsal one. Dorsal and ventral rib heads and well developed ribs are present on the first three trunk vertebrae, and the heads extend to the bearers; the cartilage of the heads and bearers is continuous. On the fourth vertebra, the part of the dorsal head nearest the rib is cartilaginous and is covered by bone, but

more distally, in the direction of its bearer, it is blastemal. On the fifth trunk vertebra the connection between the small, procartilaginous dorsal head and its bearer is a scanty line of enlarged, elongated cells. On the sixth vertebra the dorsal rib bearer is blastemal. The dorsal head is represented by two procartilaginous cells lying against the rib, and a line of elongated cells extends to the rudimentary bearer. Ventral rib bearers are connected to the centrum by a mass of fibers on the sixth through tenth trunk vertebrae, and are absent on the remaining trunk vertebrae. Dorsal rib bearer rudiments are barely evident behind the eighth trunk vertebra. Small ribs, consisting of a row of cartilage cells covered by a thin bony layer, lie in the intermyotomal septa opposite the eleventh through thirteenth trunk vertebrae, but the last rib (opposite the fourteenth trunk vertebra) is blastemal. It is clear that ribs develop first, in a distal position, and that the bearers, first the ventral and then the dorsal, develop later. The connection between the ventral rib head and its bearer precedes attachment of the bearer to the centrum on the more posterior trunk vertebrae. The attachment of the dorsal head to its bearer occurs late, as a result of growth of the head from the rib toward the bearer. The sacral ribs have not established contact with the ilia. The sacral rib bearers are well developed and in cartilaginous continuity with each other and with the rib heads. Both bearers lie in approximately the same plane, opposite the neural pedicel rudiment. The ventral bearer is attached to the neural arch rather than to the centrum.

*13.0–15.0 mm stage.* The bony centrum is thick, especially at the ends, where it expands around the intervertebral cartilages, and near the midpoint. Pairs of small, posteroventrally directed processes (basapophyses) arise from the ventrolateral margins of the first five to six vertebrae. As the centrum grows, the notochord becomes progressively constricted within the enlarging intervertebral cartilage.

Zygapophyses are well developed and have synovial cavities. The cartilage of the postzygapophyses is not continuous with that of the dorsal vertebral roof. The posterior part of the prezygapophysial carti-

lage has been invaded by blood vessels, and the cartilage in this area is gradually being replaced by haemopoietic tissue.

The neural arch is complete on all vertebrae and has almost gained adult proportions. Earlier, ossification of the central part of the neural canal was completed. At the 12 mm stage a thin layer of bone covers the inner margins of the cylindrical neural pedicel rudiments and the undersurface of the dorsal cartilaginous mass (figs. 27, 28). Now this bony layer has been eroded at the level of the center of the nerve cord, and ossification of the neural pedicel rudiment is proceeding in both dorsal and ventral directions (fig. 30). In some of the more anterior vertebrae, only the dorsal and ventral tips of the rudiment remain. The result of this process of vertebral canal enlargement is to effect a separation of a mid-dorsal cartilaginous area, which is enclosed between inner and outer bony layers and is being eroded at its lateral margins (cf. Mookerjee, '30). The ventral parts of the neural pedicel rudiment remain cartilaginous, but they are being steadily eroded dorsomedially.

All of the ribs are present in at least a blastemal condition and most are well developed. On the first trunk vertebra, the ventral rib bearer is joined to the centrum by an anteriorly directed bony strut. The bearer is also attached to the neural arch by a second, dorsomedially oriented bony process, which extends to a point just anterior to the site of the neural pedicel rudiment. The bearer is a small, bony covered block of cartilage into which small blood vessels extend. Erosion of the cartilage is proceeding. The dorsal rib bearer is no longer connected to the ventral bearer, but lies far dorsally and posteriorly, just opposite the neural pedicel rudiment. The first pair of ribs have well developed dorsal and ventral heads that are joined to their respective bearers by regions of fibrocartilage. Distally, the first two pairs of ribs are greatly expanded and serve as the origin of the serratus muscles.

A strip of cartilage, one cell thick, extends along one side of the second vertebra, connecting the rib bearers. The ventral rib bearer is more firmly attached to the neural arch than to the centrum, but

retains a slender bony attachment to the latter. The ribs are not as well developed as those of the first vertebra.

The cartilaginous ventral rib bearer of the third vertebra has lost its attachment to the neural arch and is loosely attached to anterior parts of the centrum. The vertebral artery and vein pass above the anteriorly located ventral bearer. The position of the dorsal rib bearer is similar to that of the first two vertebrae. The rib heads are less well developed than those of the first two pairs of ribs. The slender, cartilage filled heads join to form the short, slender ribs.

The remainder of the trunk vertebrae resemble the third in the arrangement of rib bearers and heads. More posteriorly the ribs and bearers become shorter and less well developed. The dorsal rib head of the fifth vertebra is procartilaginous, and the corresponding region on the seventh vertebra is occupied by a line of fibroblasts. Dorsal rib heads are present on the seventh, eighth, and ninth vertebrae but do not contact the remainder of the rib. The dorsal head is absent on the tenth vertebra. By the eleventh vertebra even the dorsal rib bearer is reduced in size and is procartilaginous. Traces of the dorsal bearer are present, however, on all trunk vertebrae.

Ventral rib heads are present, at least in cartilaginous form, on all but the last trunk vertebra, where there is a procartilaginous blastema. The ventral rib bearer is well developed until the eleventh vertebra. It is present as a procartilaginous structure on the next one or two vertebrae, but is always more advanced than the dorsal bearer. On the two vertebrae preceding the sacral, the ventral bearer increases in size and again becomes cartilaginous.

The sacral vertebra has a well developed ventral rib bearer which is a cartilaginous block covered with bone. The ventral bearer is attached firmly to both the centrum and the neural arch. The dorsal rib bearer is well separated from the ventral one, and is a well developed, cartilaginous block encased in bone. The large rib head is also bony with a cartilage core. Masses of collagenous fibers attach the short sacral ribs to the ilia.

In cleared and stained material ossifica-

tion of the ribs may be observed. A range of conditions is found with the earliest developmental stages in a 15 mm animal. All ribs are represented but dorsal heads are present only on the first four trunk and on the sacral rib. In the next stage (14 mm) dorsal heads are present on the first six trunk vertebrae and a small ossification attached neither to the rib or bearer is present on the seventh. In another individual (15 mm) dorsal heads are present on the first ten vertebrae, and in the most advanced larva (14 mm) dorsal heads are present on the first thirteen vertebrae.

*15.5–17.4 mm stage.* A well developed notochord is present, but increased size of the intervertebral cartilage has led to constriction of the notochord between vertebrae. Cartilage is present within the notochord at the midpoint of each centrum (figs. 1, 31). On the more anterior vertebrae, a ring of large, rounded chondroblasts lies within the notochordal sheath, just opposite the neural pedicel. This ring is a single cell layer thick and two or three cells broad. In some vertebrae, capsules are forming around the cartilage cells, which grow medially and compress the inner notochordal cells. The notochordal sheath is intact, and the chondroblasts seem to have been derived from notochordal cells. Much controversy exists concerning the origin of these cells, and early workers (see Gadow, 1896; '33) concluded that they were derived from cells which migrate through the notochordal sheath. We have seen no indication of such migration, nor did Mookerjee ('30), Mauger ('62), and Lawson ('66).

Bone of the centrum has increased greatly in thickness since the 13.0 mm stage, and the posteriorly directed basapophyses are well developed in the atlas. The bone is especially thickened where the neural arch joins the centrum. On the more posterior vertebrae, the midpoint of the centra becomes progressively smaller in diameter relative to the intervertebral region.

The bony neural arch is well developed, with mid-dorsal crests present on the more anterior vertebrae. The neural pedicel rudiments are greatly eroded and replaced by a thin, semicircular layer of bone. Near the base of each arch a bony core remains that may be hollow, filled with scattered

cells, or have a fragment of cartilage next to the notochordal sheath. On the first two vertebrae the basal region is almost entirely bony, but on the more posterior vertebrae more cartilage remains. Parts of the neural pedicel rudiment, near the mid-dorsal cartilaginous block, remain as fibrous cylinders filled with cartilage.

Zygapophyses have larger facets but smaller cartilaginous cores than at the 13.0 mm stage. The cartilage of the post-zygapophyses and the posterior neural arch roof is no longer continuous.

A distinct strand of fibrous tissue lies along the ventrolateral margin of the nerve cord. This strand arises near the center of the atlas and extends along the length of the neural canal. The inner margin of the neural arch of the atlas is thickened behind the neural pedicel rudiment and immediately posterior and dorsal to the origin of the first spinal nerve. This bony thickening extends into the neural canal, contacting the longitudinal strand of fibers (fig. 36). These paired nerve cord supporters are present in all of the trunk vertebrae as well as in the atlas. In the first trunk vertebra the process arises immediately behind the nutritive foramen, directly below the dorsal rib bearer. In the second trunk vertebra the process is below the dorsal rib head bearer, at the dorsoposterior margin of a foramen that accommodates a blood vessel and the anterior rootlet of the third spinal nerve. On the third trunk and succeeding vertebrae, the process lies below the dorsal rib head bearer and above the ventral root of the spinal nerve. The process lies between the foramina for the dorsal and ventral roots (and the segmental blood vessels). These supports for the nerve cord have not previously been described in salamanders.

The pattern of rib head bearers and ribs has not changed significantly since the 13.0 mm stage. The inner margins of both the dorsal and ventral bearers are being eroded, and the cartilage at their bases replaced with a thin bony layer (fig. 36). On the first two trunk vertebrae the cartilage core of the ventral bearer is attached to the neural arch and lies above the vertebral artery and vein; on the succeeding vertebrae the core attaches to the centrum and the

blood vessels lie dorsal to it. No connection now occurs between the dorsal and ventral rib bearers on the first two trunk vertebrae.

*21.8–27.0 mm stage.* Metamorphosis occurs at this stage, but the vertebral column is little changed from the 17.4 mm stage. The notochord is a well developed rod, constricted slightly where it passes through the intervertebral cartilage and at the midpoint of each vertebra. The notochordal sheath is complete. Well developed notochordal cartilage has largely displaced the inner notochordal cells. As development proceeds, cells in the center of the notochord also become involved in chondrogenesis.

The intervertebral cartilages are now large and well developed. Each cartilage is spindle shaped, with the notochord extending through the long axis. The cartilage extends toward the center of the vertebrae, within the bony centrum but outside the notochordal sheath. It terminates at a point just short of the limits of the notochordal cartilage. The short axis of each intervertebral cartilage lies at the junction between adjacent vertebral centra, and the cartilage is firmly attached to the bony centrum of the more posterior vertebra by a ring of densely packed fibers. This tissue extends into the cartilage for a short distance. From this point a narrow arc of fibrocartilage extends anteriorly to meet the notochordal sheath. This is the joint region, derived from undifferentiated cells which have occupied this area since early stages of development. The main part of the intervertebral cartilage, which forms the articular condyle, lies posteriorly, as a thick ring of hyaline cartilage with few cells and large quantities of matrix. This cartilage merges laterally with flattened fibroblasts and posteriorly with columns of chondrocytes. Another area of tightly packed columns of chondrocytes lies in front of the arc of fibrocartilage and is associated with the centrum of the adjacent, anterior vertebra. Thus, the main portion of each intervertebral cartilage is attached to the more posterior vertebra, producing an articulation that is functionally opisthocoelous (Wake, '63, '65, '70).

The bony centrum is well developed

with thickened walls. The anterior and posterior margins of the centrum are thin, and fibrous connective tissue joins adjacent vertebrae at these points. The neural arch has a thick wall. Only the base of the neural pedicel rudiment remains. It is a small semicircular plug of bone or mineralized cartilage within a fibrous sheath (the original perichondrium) lying against the notochordal sheath just opposite the notochordal cartilage. The block of cartilage which previously formed the roof of the neural arch has largely been replaced by bone and haemopoietic tissue. Ossification of the block is initiated anteriorly and proceeds until only a few cartilage cells remain at the extreme posterior end of each neural arch.

The cartilaginous cores of the pre- and postzygapophyses have been replaced by haemopoietic tissue.

The rib bearers and ribs have increased in size. The neural canal has enlarged and internal remodeling has occurred. As a result, cartilage of the dorsal rib bearer has been eroded medially. Haemopoietic tissue is found in the cavity of the bearer. The cartilage of the ventral rib bearer is also being eroded medially and new bony struts attach the bearer to the centrum. Occasionally, a slender bony strut passes from the base of the bearer over the vertebral artery and vein to join the base of the neural arch.

*33.0–44.6 mm stage.* During the post-metamorphic period a number of changes take place in the vertebral column, especially in the intervertebral joint region. These are not closely correlated with age. A smaller individual may have a vertebral column that is considerably advanced in a developmental sense relative to a larger one.

The notochord persists throughout life (figs. 1, 38). Near the middle of each vertebra the notochord is a large, unstricted rod that has increased in size as the surrounding bony centrum has grown. At the anterior end of each centrum the notochord becomes progressively constricted. This constriction is greatest in the center of the hyaline cartilage ring portion of the intervertebral cartilage. At the 37.3 mm stage the notochordal sheath in this region becomes fragmented and

discontinuous for a length of about 100  $\mu$ . More anteriorly, at the intervertebral joint region, the notochord is unstricted and the sheath is complete. Hyaline cartilage with large quantities of matrix fills the notochord in the mid-centrum region. Remnants of notochordal cells, crushed by the inward growth of the notochordal cartilage, are found in the center of this cartilaginous core. Some of the notochordal cells in the region of the intervertebral cartilage form chondroblasts. Isolated capsules, surrounded by small amounts of matrix, appear within the notochord, both in front of and behind the area of notochordal constriction (fig. 1). These small nodules of intervertebral notochordal cartilage never fill this region of the chord, even in the largest individuals examined, and the scattered capsules usually do not join.

Thin extensions of the bony centrum grow anteriorly for a short distance, encircling parts of the intervertebral cartilage. The enlarged hyaline ring portion of the cartilage forms a condylar structure on the end of each centrum, but the intervertebral joint remains non-synovial throughout life. The fibrocartilage present in earlier stages forms a distinct region on the anterior face of each condyle (fig. 38). The cotylar (anterior) portion of each intervertebral cartilage is comprised of cells arranged in columns, and the articular surface is continuous with the fibrocartilage of the joint.

Mineralization of the intervertebral cartilage is actively proceeding, first in the columns of cells behind the condyle and then in more external parts of the condylar region. Finally, all but the central portions of the cartilage condyle are mineralized. However, these mineralized tissues do not ossify. This process is clearly seen in sections of mineralized tissue stained by the Von Kossa method, and in cleared and stained material. In macerated specimens, the condyles are stout and have a granular appearance. Only the outer margins of the cotylar cartilage mineralizes.

The bony centrum is fully developed and forms a thick layer of bone surrounding the notochord. At a point opposite the anterior and posterior ends of the intra-

vertebral notochordal cartilage, the bony centrum diverges around the tips of the intervertebral cartilage (figs. 1, 38). Thus, from its first appearance and throughout life the bony centrum maintains contact with the notochordal sheath in the mid-vertebral region.

The neural canal has increased considerably in diameter and the inner margins of the original neural arch have been extensively eroded and remodeled. On the anterior two trunk vertebrae this process has produced a discontinuity in the wall of the neural arch, near its base. This potential gap has been filled by a dense layer of bone which has grown medially from the base of the ventral rib head bearer. This tissue also has extended below the centrum to join the bone growing from the other side of the vertebra. As a result the ventral rib bearer is a massive structure, containing a cavity filled with haemopoietic tissue. Only the tip of the ventral bearer, near its articulation with the rib, is cartilaginous. Most of the original dorsal bearer has been eroded or replaced with a cylinder of bone filled with haemopoietic tissue (fig. 37). The articular surfaces of the ribs and rib bearers are areas of fibrocartilage resting on a cylinder of hyaline cartilage within an osseous tube. The ribs have extensive amounts of haemopoietic tissue within the bony cylinders. All ribs are cartilaginous at their distal tips.

The neural pedicel rudiment is absent except at its base, where vestiges remain. In each vertebra but the atlas a small fibrous cylinder containing two or three capsules of mineralized cartilage contacts the notochordal sheath, just opposite the anterior part of the intravertebral notochordal cartilage.

Nerve cord supports are well-developed bony struts that extend medially and slightly anteriorly into the neural canal (fig. 37).

No cartilage remains in the dorsal roof of the neural arch, and well developed, dorsomedial crests are present on the anterior part of each arch.

No cartilaginous core remains in the zygapophyses. The articular fibrocartilage rests on a thin layer of mineralized cartilage. In contrast to earlier states, no

haemopoietic spaces are seen in the zygapophyses of adults.

#### REGIONAL VARIATION IN DEVELOPMENT

##### *Cranio-vertebral joint*

*5-7.4 mm stage.* In early stages of development (up to the 6.9 mm stage) the notochord extends slightly beyond the anterior margin of the otic region. At the 7.4 mm stage the notochord terminates further anteriorly, at the level of the hypophysis. In this area the notochord is intimately associated with the developing parachordal elements. The parachordals join in front of the notochord to form a cartilaginous plate which in turn is connected by cartilaginous rods to the laterally located trabeculae. At its most anterior limit, the notochord blends with the cells of the developing parachordals.

The occipital arch develops between the otic capsule and the neural pedicel rudiment of the atlas. Its development resembles that of a typical neural pedicel rudiment and it forms as a column of mesenchyme cells resting on the notochord (fig. 15). The columns on either side of the nerve cord do not meet middorsally. A major difference between the occipital arch and a typical neural pedicel rudiment is the presence of a layer of flattened perichordal cells between the developing arch and the notochordal sheath (fig. 15). A preoccipital arch is present in the 6.9 mm stage as a loose mesenchymatous condensation just in front of the occipital arch rudiment. By the 7.4 mm stage this condensation has been incorporated into a low occipital crest, an undivided structure derived primarily from material of the occipital arch.

The mass of mesenchyme cells which differentiates to form the base of the occipital arch rudiment is continuous with that which forms the neural pedicel rudiment of the atlas. Later, the mesenchymatous connection between these arches becomes procartilaginous and forms an occipital condyle and an atlantal cotyle. There is no evidence of an intervening arch, or part of one. In the region of the notochord the two arches are close together, but they diverge dorsally.

10.2 mm stage. Anteriorly the notochord terminates in a block of cartilage below the point where trabecular projections meet in the ventral midline. This block of cartilage, formed by the fusion of the parachordals and the trabeculae, is the crista sellaris (fig. 3). Some scattered cartilage cells, remnants of the parachordals, lie in a bony recess on either side of the notochord. Behind the parachordals, the notochord is enclosed in a layer of bone, continuous with the parasphenoid. Immediately posterior to the developing stapes, the notochord separates from the parasphenoid and is covered ventrally by the cartilaginous hypochordal commissure, which is the posterior limit of the basicranial fenestra. Beyond the posterior limit of the parasphenoid the articular condyles of the odontoid appear. These are a pair of ventrolaterally directed cartilaginous masses on either side of the notochord. At this point the notochord is invested dorsally by a bony layer. The articular condyles lie closely apposed to small masses of fibrocartilage lining concave depressions in the medial aspect of the developing occipital pedicels. Although we have not been able to trace development of these two pairs of cartilages completely, it is likely that they arise from the same general blastema that gives rise to the atlantal cotyles and occipital condyles. In *Ambystoma* the atlantal cotyles and odontoid condyles develop from a single cartilaginous mass (Teege, '57; Worthington, '71), but we have not seen such a condition in *Eurycea*. Immediately posterior to this region the developing occipital arch curves ventrally to approach the notochord, but remains separated from it by a number of undifferentiated cells. Dorsally and anteriorly the occipital arch joins the otic capsule. Notochordal cartilage appears at a point slightly anterior to the base of the developing occipital arch, near the posterior ends of the odontoid condyles (figs. 3, 20). This cartilage extends posteriorly across the articulation between the occipital arch and the atlas, terminating at the base of the neural pedicel rudiment of the latter. At the anterior end of the notochordal cartilage, the bony covering of the notochord extends ventrally. The notochord is completely enclosed in bone at the level of the occipital condyle (fig. 23).

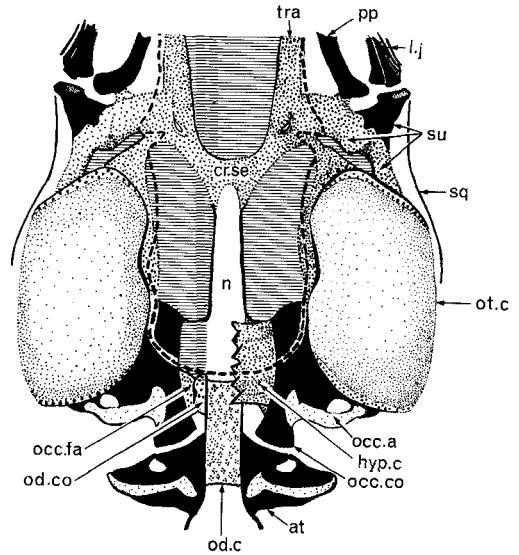


Fig. 3 Diagrammatic representation of a dorsal view of the posterior part of the skull and the craniovertebral joint of a larval *Eurycea bislineata*, 10.2 mm stage. Partially cut away. Bone is solid black, cartilage stippled, membrane lined. Parasphenoid bone on ventral surface of skull outlined by broken line.

This section of the notochord eventually forms the odontoid process (or tuberculum interglenoidium). Posteriorly the bone of the odontoid process becomes continuous dorsally and ventrally with the bone of the atlantal cotyle. The lateral margins of the notochordal sheath are in direct contact with cotylar cartilage which is continuous with cartilage of the neural pedicel rudiment of the atlas (fig. 23). The well differentiated occipital condyles and atlantal cotyles are separated by a posterolaterally directed cavity.

13.0 mm stage. The parachordals have almost disappeared, but a small crista sellaris remains. The anterior part of the notochord has been either reduced or lost, and it no longer extends to the crista. The notochordal remnant lies in a depression in the parasphenoid. This depression contains a few flattened cells which are covered dorsally by a delicate membrane. At the level of the hypochordal commissure the depression has disappeared.

The dorsolateral part of the hypochordal commissure is being eroded and ossified where it contacts the otic capsule. It is no longer in continuity with the carti-



lage of the occipital condyles or the ear capsule.

The odontoid process appears near the end of the hypochordal commissure. The odontoid condyles are completely covered by bone except for the distal articular surfaces. A bony layer surrounds the notochordal portion of the odontoid and all but the articular parts of the condyles. The occipital facets which receive the condyles are continuous anteriorly with the hypochordal commissures. Behind the facets the odontoid is a notochordal remnant surrounded by bone. Slightly posteriorly the bony layer covering the dorsal portion of the notochord is lost, exposing the notochordal sheath to the neural canal. This condition prevails until the base of the atlantal cotyles is reached, where the bony centrum is complete. At this point, cartilage of the atlantal cotyle borders the notochord, and a few cells extend ventrally between the bone layer and the notochordal sheath. The cartilage of the cotyle rests directly on the notochordal sheath, but it is separated from the cartilage of the small neural pedicel rudiment by a bony wall.

When the notochord first appears between the facets of the odontoid it is completely filled with cartilage. This tissue, entirely within the notochordal sheath, consists of few cells separated by large amounts of matrix. In contrast cartilage of the odontoid condyles and the hypochordal commissure is cell rich. The notochordal cartilage extends to a point slightly posterior to the site of the neural pedicel rudiment of the atlas.

*17.4 mm stage.* The crista sellaris is lost, and further reduction of the notochord anterior to the odontoid has occurred. The parasphenoid is thicker and the depression housing the remnant of the notochord is filling with bone.

The hypochordal commissure is well defined, despite further ossification of its mesotic area. More posteriorly the commissure is replaced by a sheet of fibers. Immediately anterior to this point the cartilage of the commissure is continuous dorsolaterally with that of the occipital facet. Except for the articular surface, cartilage of the odontoid condyles and occipital facets is completely surrounded by bone. The occipital facets are joined by a

band of fibers extending below the odontoid process (fig. 33). At its anterior end the odontoid process in cross section consists of the large odontoid condyles on either side, and a median piece of bone which contains the remnant of the notochord, here replaced by bone (figs. 33, 35). The notochordal sheath has eroded dorsally. At the level of the occipital condyle the odontoid process is a U-shaped piece of bone containing the eroding notochord (fig. 32). More posteriorly the bone of the odontoid becomes flattened and the arms of the U spread to join the bone surrounding the atlantal cotyles (fig. 34). At the level of the atlantal cotyles the bone of the odontoid process merges with that of the centrum. Here the intact notochord is bounded ventrally by bone of the centrum.

The cartilaginous atlantal cotyles lie directly against the notochordal sheath, in front of the neural pedicel rudiment. Small amounts of cartilage lie ventrally, between the notochordal sheath and the bony centrum of the atlas. The dorsal part of the notochord is uncovered by bone. The notochordal sheath is disrupted in this area and erosion of the notochordal cartilage is progressing as the region ossifies. Slightly posteriorly, the notochord is completely surrounded by bone, save for the minute areas of contact of the neural pedicel rudiments and the sheath.

*37.3 mm stage.* Degeneration of the anterior part of the notochord is complete. The hypochordal commissure remains, but the erosion of the dorsolateral parts of the cartilage and their replacement with bone is progressive. The bone which forms the odontoid process is roughly rectangular, with the odontoid condyles attached laterally. The process contains no trace of the notochord or its sheath. The odontoid condyles are now longer and thinner, and not inclined at such a sharp angle. The amount of cartilage is reduced and there is a corresponding increase in the bone surrounding it. The occipital facets now lie mainly below the odontoid condyles. Cartilage of the atlantal cotyles and occipital condyles has been invaded by blood vessels, and there is extensive production of bone and haemopoietic tissue. The notochord first appears in the atlas at the level of the foramen for the first spinal nerve, in front of the neural pedicel rudiment.

The notochord is not bounded dorsally by bone at this point, and the centrum is incomplete. Here dorsal parts of the notochordal sheath are absent and there is some conversion of the notochordal cartilage to bone. Posterior to this region, the centrum resembles that of other vertebrae.

#### *Anterior trunk region*

Goodrich ('11) demonstrated the presence of three metotic segments in the developing skull of *Ambystoma*, thus corroborating the earlier work of Platt (1898) on *Necturus*. The first metotic segment produces no muscle in *Ambystoma*. Myotomes of the second and third segments fuse. Portions of the promuscular tissue produced by the third metotic segment and the first two trunk segments migrate ventrally to form the hypobranchial musculature. Between the second and third segments, a transitory preoccipital arch occurs. The occipital arch forms between the third and fourth segments. The neural pedicel of the atlas lies at the posterior end of the fourth (the first trunk) segment. According to Goodrich, a transitory ventral root innervates the musculature of the third segment. This nerve persists in the adult in *Cryptobranchus* and some *Hynobius* (see Fox, '57, '65). It has variously been called the spino-occipital nerve (Fürbringer, 1897), the occipital nerve (zv) (Edgeworth, '23, '35) and the hypoglossal nerve (Fox, '57, '65).

In *Eurycea* (7.4 mm) rudiments of two blocks of muscle develop in this region. These fuse, and adults have a single muscle. Reorientation results in the attachment of two intermyotomal septa to the dorsal roof of the atlas by the 10.2 mm stage. No septa attach to the occipital arch. As a result, the first segment of the dorsal trunk musculature (derived from two and possibly three metotic segments) is much longer than the second. The first intermyotomal septum slopes anteriorly and the second, posteriorly, from the atlas. The third septum, between the second and third trunk somites, slopes posteriorly from the first trunk vertebra.

No spino-occipital or hypoglossal nerve penetrates the occipital arch in *Eurycea*. Further, no nerve is found between the occipital arch and the first spinal nerve

corresponding to the "suboccipitalis" reported by Hoffmann (1878, '02) and Froerip ('17).

The first spinal nerve of *Eurycea* leaves the nerve cord through a foramen located just behind the atlantal cotyles, but in front of the neural pedicel rudiment (figs. 4, 5). It is a ventral root only, but has a transitory ganglion appearing at the 6.9 mm stage and persisting until the 13.0 mm stage. This nerve has been called the first spinal nerve by Coghill ('02), Goodrich ('11), Francis ('34), Fox ('54, '57, '62) and many others. Mookerjee ('30) and De Beer ('37) used suboccipital or spinal nerve 1, Gadow (1896), Teege ('57) and Mauger ('62), suboccipital, and Albrecht (1880), proatlanticus. We consider it to be the first spinal nerve. Upon leaving the foramen, this nerve gives off a large dorsal ramus supplying the musculature derived from the metotic and first trunk segments. The long ventral ramus joins a ventral ramus of the second spinal nerve, forming the ramus hypobranchialis (cf. Fox, '59) (fig. 4). These observations are at variance with those of Bowers ('00), who reported that the first and second spinal nerves of *Eurycea* do not join. She found that the first spinal nerve has two ventral roots which pass through the "cranial cartilage." We are unable to interpret these remarks. Norris ('11) observed that in *Eurycea* the second but not the first spinal nerve has a ganglion, and the hypoglossal nerve is formed "chiefly, if not entirely from the first spinal nerve." This observation differs from ours.

The ventral root of the second spinal nerve issues through a large foramen in front of the neural pedicel rudiment of the first trunk vertebra. The foramen is larger than that of the small dorsal root of the same nerve. The dorsal root leaves the neural canal some distance posterior to the emergence of the ventral root, but in front of the neural pedicel rudiment (figs. 4, 5). A small ganglion lies above the ventral rib bearer in front of the rib. Both dorsal and ventral rami extend from the ganglion (fig. 4). The ventral ramus has several branches, the largest of which joins the ventral ramus of the first spinal nerve (see above). Another branch extends toward the shoulder (fig. 4).

The ventral root of the third spinal

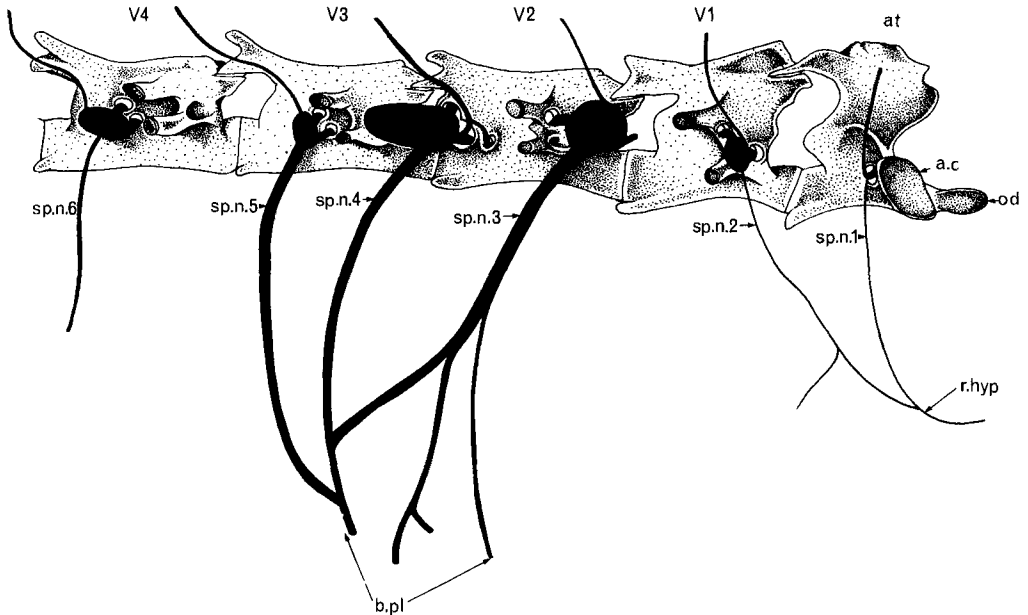


Fig. 4 Lateral view of the first five vertebrae of an adult *Eurycea bislineata* vertebral column, drawn from cleared and stained as well as sectioned material of several specimens. Anterior to the right.

nerve emerges directly above the articulation of the bony centra of the first and second trunk vertebrae. Although there is some variation, usually no foramen for this root occurs in either vertebra. The dorsal root, however, is far posterior to the ventral root and usually leaves the vertebral canal via a large foramen in the upper part of the neural arch of the second trunk vertebra (figs. 4, 5). This foramen lies just behind the level of the articulation of the ventral rib bearer and rib, but in front of the neural pedicel rudiment. The ganglion of this nerve is larger than that of the second spinal nerve and is intersegmental. Dorsal and ventral rami are present, and the latter is a major component of the brachial plexus.

The ventral root of the fourth spinal nerve issues through a foramen near the posterior margin of the neural arch of the second trunk vertebra. The dorsal root is located a little more posteriorly and usually has a separate foramen (figs. 4, 5). However, in some cases the bone separating the foramina may be absent, producing a large elliptical foramen, or the posterior margin of the dorsal root fora-

men may be absent, so that the nerve issues through a notch in the posterior margin of the neural arch. Occasionally the dorsal root lacks a foramen and leaves the cord intervertebrally. In some individuals the ventral root may have more than one rootlet. In a 17.4 mm specimen the ventral root passes through two foramina, the smaller of which is located anteriorly, below the nerve cord support and behind the transverse processes. The more anterior foramen is identical in position to the ventral root foramina of all remaining trunk vertebrae.

The ganglion of the fourth spinal nerve is enormous and extends posteriorly to a point just dorsal to the ventral rib bearer of the third trunk vertebra. The ganglia of the fourth and fifth spinal nerves lie on either side of the transverse processes of the third trunk vertebra (fig. 4). The ventral ramus of the fourth spinal nerve is a major part of the brachial plexus.

The fifth spinal nerve issues from single or paired foramina posterior to the transverse processes of the third trunk vertebra. The foramen for the dorsal root is slightly posterior to that of the ventral one. The two foramina are separated by

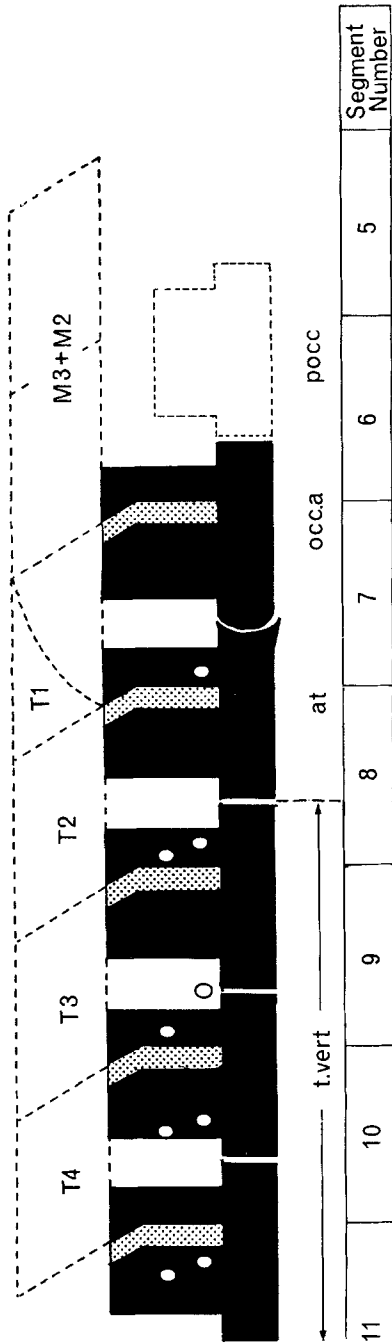


Fig. 5 Diagrammatic representation of the pattern of nerve routes illustrated in figure 4. Anterior to the right. The occipital and preoccipital arches are indicated as independent, but in reality they are a unit.

the nerve cord supports (cf. fourth spinal nerve). The cord support separates the dorsal and ventral roots as they leave the neural canal on this and all posterior vertebrae. The ganglion of this nerve is not so large as that of the fourth spinal nerve. The ventral ramus contributes to the brachial plexus (fig. 4).

The remaining presacral spinal nerves resemble the fifth, but do not contribute to the brachial plexus.

The transitional nature of the region between the head and trunk of salamanders is reflected in the structure of the vertebral column (fig. 5). There is a ventral root for every segmental muscle of the trunk, and in *Eurycea* the first ventral root supplies the musculature derived from the metotic and first trunk segments. At the anterior end of the trunk the ventral roots are located at the posterior end of the segment they supply. On succeeding vertebrae, the ventral roots are located progressively in a more anterior position. By the fifth spinal nerve the ventral root is at the anterior end of the segment it supplies, an organization retained posteriorly. While the second spinal nerve penetrates the second vertebra (the first trunk vertebra), the fifth penetrates the fourth vertebra (the third trunk vertebra) (figs. 4, 5). This forward movement relative to the segments is anticipated in the first few vertebrae by anteriorly placed rootlets of the ventral roots. These rootlets may have their own foramina (see above). Perhaps such an occurrence could explain the appearance in some species of "transient" nerve roots ("suboccipital" nerve) at the level of the posterior margin of the occipital condyles, such as reported by Hoffmann ('02) and Froriep ('17).

*Caudosacral and caudal regions*

The seventeenth (occasionally eighteenth) vertebra is the sacral in *Eurycea bislineata*. It is followed by a series of three transitional or caudosacral vertebrae (Wake, '63; Wake and Dresner, '67). The first of these differs from trunk vertebrae in having very long transverse processes and no ribs. The last is the first vertebra to have a large, complete haemal arch, but it differs from caudal vertebrae in lacking an anterior keel on the arch. Functionally these vertebrae are part of

the body, for the pelvic musculature extends to the third vertebra.

At the 10.3 mm stage no evidence of a sacral rib or rib bearer is present. However, a well developed cartilaginous rudiment of the transverse process of the first caudosacral vertebra is attached to the bony neural arch at a point slightly in advance of the neural pedicel rudiment. It thus resembles a ventral rib bearer of trunk vertebrae.

A short rudiment of the transverse process is present on the second caudosacral vertebra, but here the cartilaginous element is attached by bony struts both to the neural arch and to the bony centrum. The dorsal attachment is opposite the neural pedicel rudiment, but the ventral strut is far anterior. A pair of short cartilaginous rods (haemal arch rudiments), covered by a thin bony layer, is attached to the ventral surface of the centrum, anterior to the bases of the neural pedicel rudiments and the transverse processes. The caudal artery and vein pass between these elements, which may remain as low ridges throughout life. The arch is incomplete ventrally.

The last caudosacral vertebra bears a complete haemal arch which is attached to the centrum far in front of the neural pedicel rudiments. This cartilaginous arch has a large block of cartilage in the ventral midline (the infraventral of Mookerjee, '30). We have no evidence that this block arises as a separate center of chondrification. There are no rudiments of transverse processes on this vertebra.

At the 12.0 mm stage the first caudosacral vertebra bears a large, well developed transverse process which is a bony cylinder with a cartilaginous core. The process is attached to the neural arch by bone at the level of the neural pedicel rudiment. The transverse process has a large, anterolaterally directed extension near its base. A pair of small cartilaginous rods is attached to the lateroventral margins of the centrum just posterior to the neural pedicel rudiments. They represent serial homologues of the haemal arches. The second caudosacral vertebra has an exclusively bony transverse process which attaches to the neural arch at the level of the base of the neural pedicel rudiment. A pair of cartilaginous ventral processes are present in the same position

as on the first caudosacral vertebra. They are connected below the haemal artery and vein by a small bony bridge. The third caudosacral vertebra is similar to the second, but the haemal arch is large and complete. Bone surrounds the continuous cartilaginous arch except midventrally. Succeeding caudal vertebrae have complete arches, with a median anterior bony keel, but with poorly developed, bony transverse processes.

The transverse processes of the first two caudosacral vertebrae at the 13.0 mm stage have cartilaginous cores. Haemal arch rudiments occur only on the last caudosacral vertebra.

By the 16.9 mm stage, the ventral processes on the centrum are absent on the first two caudosacral vertebrae, but a complete haemal arch is present on the third vertebra. The cartilaginous arch is undergoing erosion and ossification. Ventrally a large cartilaginous mass remains. The transverse process of the first caudosacral vertebra has a cartilaginous core. Cartilage in the transverse processes of the second vertebra is reduced to a few cells. Processes on succeeding vertebrae are exclusively bony.

Haemal arch rudiments may appear on all caudosacral vertebrae, but they rarely persist to metamorphosis on the first two. Occasionally adults are found to have low ridges, or even small arches on the second caudosacral vertebra. In adults of *Eurycea* the cloaca has a fibrous attachment to the centra of the caudosacral vertebrae. Disappearance of the haemal arch elements of the first two vertebrae provides space for the cloaca, but the haemal arch on the third enlarges to support the posterior cloacal wall.

The transverse processes of the first two caudosacral vertebrae resemble those of more anterior vertebrae, and are laid down in cartilage. Based on topography, they probably correspond to ventral rib bearers. The third caudosacral resembles caudal vertebrae in the morphogenesis of transverse processes.

#### REGIONAL VARIATION IN THE ADULT VERTEBRAL COLUMN

##### *Quantitative aspects*

Patterns of regional variation in the

vertebral column of diverse groups of salamanders have recently been discussed elsewhere (Worthington and Wake, '72). Materials and methods utilized here are exactly the same as those reported for *Eurycea bislineata* in that paper, with one exception. While Worthington and Wake ('72) dealt with transverse processes as a unit, we here separate the treatment of the dorsal (diapophysis) and ventral (parapophysis) rib bearers. Measurements of five adult animals between 42 and 46 mm, standard length, were taken for four dimensions, and the means and 90% confidence intervals are presented in figure 7. Certain objective criteria can be used to group the vertebrae in five categories. The cervical region consists solely of the atlas, which has no ribs and bears articular cotyles. The sacral region includes only the sacral vertebra, whose ribs articulate with the ilia. The trunk region, consisting mainly of rib-bearing vertebrae, lies between. Immediately behind the sacrum is the caudosacral region, comprised of three vertebrae which function to support the cloaca and the tail. The caudal region includes all remaining vertebrae. General features of vertebrae from different parts of the column are illustrated in figure 6.

These five categories are not at all obvious when measurements alone are considered. These measurements reflect certain functional parameters, and it can be seen that, for example, the second verte-

bra is more a part of a neck than of the body proper. Other functional implications have been considered by Worthington and Wake ('72) from a comparative viewpoint, and we will limit further comments to a consideration of variation in the individual dimensions within *Eurycea bislineata*.

#### *Centrum length*

The second vertebra is short, and length increases abruptly from it to the third vertebra. A slow lengthening trend continues to the eighth vertebra. Centrum length is about constant through the twelfth vertebra, following which a shortening trend ensues which continues into the tail.

#### *Distance between tips of transverse process*

This measurement is complex, in that it includes both the width of the neural arch and the combined length of a pair of transverse processes. Thus, the measurement is relatively large for the second vertebra, where the transverse processes are, in fact, very short, because this vertebra surrounds part of the enlarged brain stem. In other parts of the column, intervertebral differences are mainly due to differences in transverse process length, although the neural arch does narrow steadily posterior to the sacrum.

Mean distance across the diapophyses

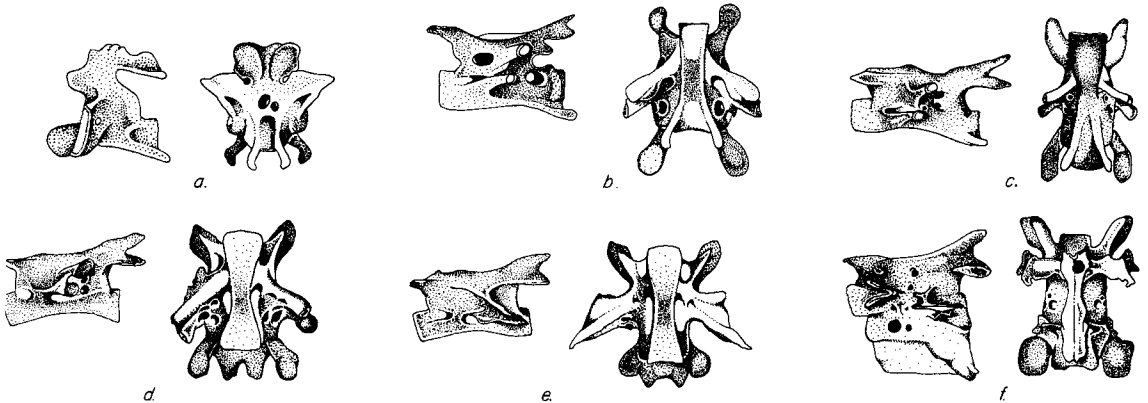


Fig. 6 Lateral (left) and ventral (right) views of selected vertebrae from the column of an adult *Eurycea bislineata*. Anterior to the left and at top, respectively. a, atlas; b, second trunk vertebra; c, seventh trunk vertebra; d, sacral vertebra; e, first caudosacral vertebra; f, second caudal vertebra. Only the bony parts are drawn, and no ribs are shown; all drawn to same scale.

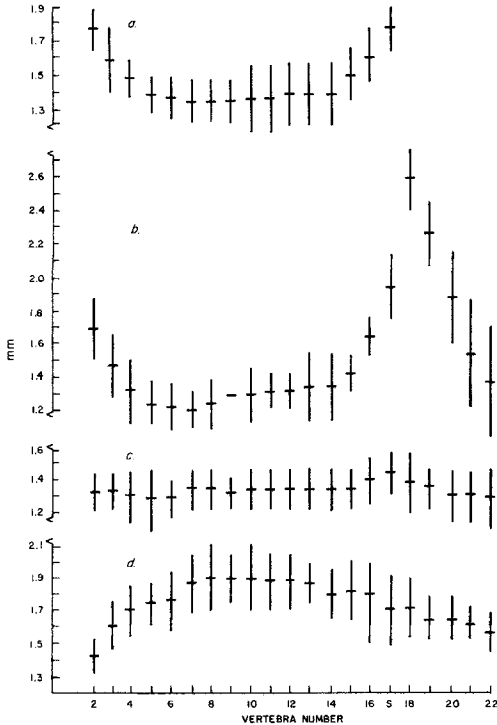


Fig. 7 Regional variation in vertebral proportions in a sample of adult *Eurycea bislineata* (40–46 mm standard length; N = 6) from Turkey Run State Park, Parke County, Indiana. Horizontal line indicates mean. Vertical line indicates 90% confidence interval of variation about the mean. a, distance from tip to tip of dorsal rib bearers; b, distance from tip to tip of ventral rib bearers through the sacral vertebra, and of the transverse processes on vertebrae posterior to the sacrum; c, distance across the prezygapophyses; d, centrum length, bony parts only.

is larger than that across the parapophyses on vertebrae 2 through 15, with the greatest difference between the two occurring on vertebrae 3 through 7. Parapophyses are longer than diapophyses on vertebra 16, and especially on vertebra 17 (the sacral).

The distance across the diapophyses is initially large, and decreases abruptly from the second through the fifth vertebrae. The mean then stabilizes until the fifteenth vertebrae, when a lengthening trend ensues. The means of the second and seventeenth vertebrae are nearly identical.

The distance across the parapophyses is large initially, and decreases from the second through the seventh vertebrae.

The mean measurement then increases gradually through the fourteenth vertebra. There is an abrupt increase in process length between vertebrae 14 and 17; while the mean of vertebra 2 is a bit larger than that of vertebra 16, by far the longest parapophyses are on vertebra 17.

Only a single transverse process is found on postsacral vertebrae, and its measurements are presented as continuations of those of the parapophyses. The processes are elongate on the first caudosacral vertebra (number 18), but then abruptly diminish in length until vertebra number 22, the second caudal vertebra. From this point the processes gradually diminish in length and disappear near the tail tip. By far the largest processes in the column are those on vertebra 18.

#### *Distance across the prezygapophyses*

This is the most uniform dimension which we measured. Means gradually decrease from the second through the sixth vertebrae, increase slightly on the seventh, then remain stabilized until the fifteenth. The largest mean is on the sacral vertebra (number 17). From this point the mean distance gradually decreases to the tail tip.

#### *Variation in discrete characters*

A detailed analysis of vertebral numbers would have to take geographic patterns into account. Nevertheless, it is of some interest to determine the general range of variation. We have a series of 27 skeletons. Because of the high incidence of tail breakage, numbers of caudal vertebrae cannot be accurately determined. In most specimens there are 15 trunk vertebrae; the sacrum is the seventeenth vertebra in all but four individuals, three of which have the eighteenth vertebra serving as the sacrum. The remaining individual has an asymmetrical sacrum (17 on one side and 18 on the other).

All 16 trunk vertebrae bear bicipital ribs in one individual. All other individuals with 16 trunk vertebrae have unicipital ribs on the last vertebra. Bicipital ribs are present on the last vertebra of half of the 22 individuals which have 15 trunk

vertebrae. The remaining adults have a unicipital rib on the last vertebra. In all instances it is the dorsal rib head which fails to develop when a unicipital rib is present.

The dorsal and ventral rib bearers are variably free or joined along the column. Usually they are entirely separated from each other, but several patterns of attachment occur. A fine bony web may extend between the basal parts of the processes on the first two or three trunk vertebrae. Throughout most of the trunk region there are no connections. When present, joining pieces are fine filaments of bone located near the distal tips of the processes. At the posterior end of the trunk, webs of bone may again extend between the processes, and an extensive web of bone always extends for nearly the entire length of the sacral processes.

Normally the last caudosacral vertebra is the first to have a fully developed haemal arch. The first caudal vertebra has the first arch to bear an anteromedial keel. This is the pattern in 24 of the 27 individuals. A complete haemal arch is present on the second caudosacral vertebra in the other individuals, but it is poorly developed and small.

Much variation is detected in the shape and form of the neural crests regionally and between individuals. Crests of presacral vertebrae are rather low and anteriorly placed, usually isolated from the elevated posterior parts of the arches. The highest crests tend to be on the more anterior vertebrae, but there are exceptions. Behind the sacrum, crests enlarge and both their height and basal length increase. The largest crests of the entire column occur on the first two or three caudal vertebrae. These crests may be as high as, or slightly higher than, the posterior margin of the neural arch, which on these vertebrae is high relative to more anterior vertebrae. Posteriorly the neural crests steadily decrease in size.

Basapophyses are well developed on the atlas and on most of the trunk vertebrae. The processes arise from the ventrolateral sides of the centrum and extend posteroventrally, beyond the end of the centrum. Variation is great, and the shape of the processes of the first four or five vertebrae ranges from long and spine-like to short

and ridge-like. In some individuals the processes are absent past the fifth vertebra, while in others they are present nearly to the sacrum.

Low, ridge-like hypapophyses occur on the anterior and ventral midline of the centra of the sixth and seventh vertebrae. These are very indistinct, and there is no sign of them on other vertebrae.

## DISCUSSION

### *Evolution of the cranio-vertebral joint*

Some amniotes have an independent element, the proatlas, located between the occipital and atlas arches. The odontoid process of living amniotes forms from parts associated with the atlas of early amniotes. Neither a proatlas nor an odontoid of the axis occur in modern amphibians, but there has been much speculation since the time of Albrecht (1878) and Hoffmann (1878) concerning probable homologues of structures in modern amphibians. The literature is complex and confused (see an attempt at a general review by Smit, '53). The multiple views can be summarized as falling into three general categories. Some believe that the atlas of salamanders is a composite structure, combining the proatlas and the atlas of amniotes. Others think that the atlas has added skeletogenous parts of a half segment to it, to form the so-called odontoid (or tuberculum interglenoideum). Finally a group of workers considers the atlas to be simply a specialized vertebra with hypertrophied parts. All modern authors agree that the atlas of modern amphibians is formed from a different series of segments than that of amniotes, and thus no strict application of criteria for homology is appropriate.

In order to deal with this problem it is necessary to formally consider segmentation patterns. We do so reluctantly, for we believe that such formal treatment ignores dynamic aspects of developmental processes. However, an understanding of the reasoning of previous workers depends on a general awareness of their premises.

We choose to accept Fox's ('65) view that the salamander skull shows evidence of having been derived from six segments in some distant ancestor. There is general



agreement (Fox, '65; Bjerring, '71) that there are three metotic segments. The popular conception is that each segment consists of a myotome, a ventral root nerve which innervates the myotomic derivatives, a dorsal root nerve which exits behind the myotome, and a skeletal member which lies at the posterior end of each myotome. Only Regel ('68) has ever found a myotomic derivative of the first metotic segment. Bjerring ('71) calls this the anterior basioticus muscle and claims that it is served by the abducens nerve. At the posterior end of this muscle is the hypothetical anterior otic pila, a skeletal member which has not been positively identified. The muscle of the second metotic segment, the posterior basioticus muscle, extends between the anterior and posterior otic pilae (the latter are commonly considered to form the preoccipital arch). Bjerring claims to have found a transitory innervation of this muscle by a ventral root termed tenuis. The third metotic segment produces the occipital muscle, innervated by a transitory nerve that has been found in some species of salamanders (Fox, '62). Many years ago Goodrich ('11) demonstrated that muscles of the second and third metotic segments fuse to form a single adult muscle, which shifts its posterior attachment during ontogeny from the occipital arch to the atlas. In adults the occipital nerve (also termed hypoglossal or spino-occipital) is absent, and the composite muscle is served by the first spinal nerve (which exits in front of the rudiment of the atlas arch). As one proceeds posteriorly along the vertebral column, the pattern of successive myotomes with skeletal members (vertebral arches) on their posterior borders is repeated. The regularity is such that one would think no further controversy could arise, but such has not been the case.

In amniotes each trunk sclerotome divides into cranial and caudal halves. The caudal half of one segment and the cranial half of the succeeding one merge to form the vertebral arch, at the end of each segment. For reasons unclear to us, some workers have held that the occipital arch of amphibians develops in some different manner, so that at the anterior end of the column a cranial half sclerotome is present which gives rise to "pro-

atlas" mesenchyme tissue. This tissue, according to various views, gives rise to parts of the occipital condyles, the atlantal cotyles, and the odontoid process (de Gaay Fortman, '18; Mookerjee, '30; De Beer, '37).

Another view is that the "proatlas" was a complete vertebra in ancestral groups, and that it has fused with the atlas. Albrecht (1878, 1880) discussed the nerve patterns of the neck region and noted that the first spinal nerve pierces the atlas. He and many subsequent workers (e.g., Gadow, 1896) believed that all ventral roots exited between vertebrae in the trunk region of salamanders. We now know this is not true. On that basis alone it was held that two vertebrae had fused together, around a nerve. Such a view required, however, yet another nerve and another muscle between the atlas and the occipital arch for a totally regularized pattern. Hoffman ('02) claimed to have found the nerve rudiment of the second nerve, but his observations have not been confirmed. Froriep ('17) reported the fusion of two anterior vertebrae in the development of the atlas of *Salamandra atra*, but other workers have been unable to duplicate these observations, which are of dubious validity.

In our view, the segmentation pattern is as regular as one can expect in a dynamic system. Although Fox has not formally expressed his opinion on this subject, the highly regularized and consistent patterns of segmentation which he finds in the postotic and neck regions of salamanders provides support for this view (Fox, '54, '62, '65). Modifications of the regularity are the result of selection for specific features and concomitant accommodation by surrounding tissues. Thus the musculature of the first postotic segment disappears, that of the second joins that of the third, and the resulting muscle extends from the head to the first logical point of attachment, the first vertebra or atlas. The anterior and posterior otic pilae fuse together with the occipital pila to form the basioccipital part of the skull, and the rudimentary nerves tenuis and occipitalis disappear. Much modification occurs in the region between the occipital and atlas arches, and there is a great proliferation of skeletogenous mesenchyme

during development. This is mainly directed into the production of the occipital condyles and atlantal cotyles. Tissue surrounding the notochord gives rise to the odontoid process. As a result of these modifications a rather complex joint is produced with two pairs of articulations. Probably associated with the movement of this joint region, the first spinal nerve exits through the wall of the atlas rather than in the center of the segment, the presumed ancestral route. Perhaps the nerve is more protected in this position. Despite literature reports to the contrary (Gadow, '33) this position is absolutely constant in all salamanders. The next segment is the first to have the pattern that repeats throughout the trunk. The segmental muscle extends between two vertebral arches. In the center of the segment a typical intervertebral joint is formed by the zygapophyses and the ends of the adjacent centra, which surround the notochord and intervertebral cartilage.

We can summarize our data on *Eurycea* as follows: during early stages of development a mesenchymatous mass joins the bases of the anteriorly sloping occipital arch and the posteriorly sloping atlantal arch. These bases are closely approximated. Only a single muscular segment extends between the occipital and atlantal arches during early development, and it is much shortened ventrally. The mesenchyme mass is continuous for the entire length of the shortened segment, and does not correspond to the cranial half only. There is no evidence of an intercalated arch at this point, or at any later stage. As this region is converted to skeletal tissue an area of fibrocartilage appears in the region of the future joint. Only during later stages of development, following initiation of head movement, does a synovial cavity appear. We have seen no evidence that the region is invaded during early stages of development by cells which give rise to the joint, as Mookerjee ('30) has reported in *Triturus*.

The odontoid condyles form from paired masses of procartilagenous cells in the region below the level of the occipital arch. There is no direct connection between these cells and the tissue that gives rise to the atlantal cotyles, but one is known to exist in *Ambystoma* (Worthington, '71) so the condition in *Eurycea* may be a re-

cent specialization. The cartilage of the occipital cotyle, derived from the same tissue that gives rise to the hypochordal commissure, is separated from the cartilage which becomes the occipital condyle. The odontoid process is formed by the appearance of perichordal ossification around the dorsolateral, lateral and ventral portions of the cartilage-filled notochord in front of the neural pedicel rudiment of the atlas. This ossification proceeds simultaneously with that of the remainder of the centrum of the atlas, with which the bone of the odontoid is in continuity from its first appearance. During later stages of development, the dorsal part of the notochordal sheath disintegrates and the cartilage within it gradually erodes, producing the U-shaped structure of adults.

Our observations are similar to those of Teege ('57) on *Ambystoma*. She also reported that the occipital condyles develop as direct outgrowths of the occipital arch cartilage. However, she thought that atlantal cotyles are produced from a modified intervertebral cartilage rudiment between the atlas and occipital arches. The odontoid was said to be formed from the anterior portion of this cartilage and from the notochordal cartilage. In *Eurycea*, the cartilage that gives rise to the occipital condyles and atlantal cotyles is continuous between the neural pedicel rudiments of the occipital and atlas arches. The cartilage of the odontoid condyle develops from paired segmental masses, possibly serial homologues of the more posterior perichordal rings. These masses do not give rise to a complete ring and may be continuous at a later stage with the cartilage of the neural pedicel rudiment of the atlas. In these features the paired masses differ from an intervertebral cartilage.

In our view, the occipital-atlantal region evolved as a result of selection for a joint region in terrestrially feeding animals. The embryonic tissue of the neural arches has the potency to produce anterior and posterior cartilagenous processes (which give rise to zygapophyses) on trunk vertebrae, and this potential under the influence of selection, provided the basis for the formation of occipital condyles and atlantal cotyles. These structures are essentially derived from the same material

that gives rise to the neural pedicel rudiments and the zygapophyses. As the joint evolved, the occipital arch shifted somewhat posteriorly, at least in development, relative to the atlas, and the tissue that forms the condyles, cotyles, and pedicels of both arches forms as a single mass.

It is likely that the early appearance of cartilage in the notochord of the atlantal-occipital region is a larval adaptation. This cartilage, extending across the incipient neck joint in a notochord that is continuous from the hypophysis to the tail tip, provides an area of relatively great strength and adds to the resilience of the notochord in the region prior to the formation of the occipital condyles and atlantal cotyles. The cartilage is eroded away following formation of the joint, and the notochord becomes discontinuous.

The odontoid may have evolved to provide additional strength and flexibility to the region as a result of the anterior growth of the tissue at the end of the atlas, relative to the occipital arch. In more posterior vertebrae, tissue from the segment immediately in front of the neural pedicel rudiment is incorporated into the vertebra, and the atlas seems to differ only in degree from this situation.

The possibility remains that the atlas is composite, since tissue associated with the notochord directly below the occipital arch does contribute to the odontoid. This tissue may be serially homologous with that which forms the centra of more posterior vertebrae. There is a separate, anterior center of ossification in *Notophthalmus* (Wake, '70). Thus tissue associated with the occipital skeletal segment may be associated with the next posterior segment to form the odontoid of the atlas, but no separate, intercalated skeletal elements seem to be involved with either the occipital or atlas skeletal members. We can find no evidence whatever of any rudimentary vertebra between the atlas and the occipital arches.

#### *Evolution of transverse processes and ribs*

##### Historical perspective

The development and evolution of the transverse processes and ribs have been

discussed by many authors and are the subjects of continuing controversy. Following the early work of Göppert (1895, 1896) and Mayerhofer ('09), several important papers appeared in which evolutionary aspects were considered.

Gamble ('22) thought both primitive and derived conditions were represented in *Necturus*. He considered the arrangement of the anterior trunk vertebrae to be primitive. Here a "basal stump" of cartilage forms on the ventrolateral margins of the centrum, ventral to the vertebral artery. A lateral cartilaginous outgrowth of the basal stump, called the parapophysis, extends from below the vertebral artery in a dorsolateral direction to join, or nearly join, a ventral cartilaginous process of the "rib bearer." He described the rib bearer as a band of cartilage attached to the ventrolateral margin of the neural arch above the vertebral artery. The ventral head of the rib attaches to a ventral process of the rib bearer, a structure which is continuous with the parapophysis in some cases and joined to it by fibers in others. Presumably derived conditions occur on the more posterior vertebrae, where the ventral rib head connects to the parapophysis, and the ventral process of the rib bearer attaches to the dorsal surface of the parapophysis near the distal tip of the latter. On both anterior and posterior trunk vertebrae the dorsal rib head is connected to a lateral outgrowth from the dorsal part of the rib bearer. On the postsacral vertebrae the basal stump gives rise to the haemal arch and "parapophyses" develop as dorsolateral projections from the base of the haemal arch.

Gray ('30), working with *Triturus*, stated that the rib grew toward the centrum and the ventral head joined a downward growth of the "lateral process" (rib bearer of Gamble). It then proceeded toward the centrum, below the vertebral artery, forming a second attachment for the ventral rib head. Gray reported that both attachments were fibrous. He did not distinguish the bearer for the ventral rib head from the rib head *per se*. The ventral attachment was considered to represent the lower head of the amniote rib.

Mookerjee ('30), also working with *Triturus*, reported one outgrowth of the neural arch (for the attachment of the dorsal rib head) and two from the centrum (for the

attachment of the ventral rib head). The lower attachment of the ventral rib head (below the vertebral artery) was considered to represent the "true head of the rib in amniota." The upper attachment of the ventral head (above the vertebral artery) was termed the "diaapophysis" and the attachment for the dorsal head the "diapophysis." These two processes were said to correspond to the "diapophysis of the amniote vertebra."

Gadow's book of 1933 was written before the work of Gray and Mookerjee, but published posthumously at a later date. His scheme of rib development and evolution in the Amphibia (his fig. 40) emphasizes a tendency for upward movement of the rib attachments to the vertebra in ontogeny and phylogeny. His basiventral seems to be approximately equivalent to the basal stump or *Zentrapophysis* of other authors, and to our ventral rib head bearer. The ribs are unicipital and articulate with the basal stump alone in his first stage. Next, the ligamentous attachment of the rib to the neural arch (his basidorsal) becomes strengthened, as a result of the outgrowth of the neural pedicel rudiment, to form the diapophysis and tuberculum, but the ventral connection is maintained. In his third state, the diapophysis divides to form a secondary dorsal diapophysis. The original ventral attachment is reduced to a bony strut or is lost. His primary diapophysis is equivalent to the diaapophysis and his secondary diapophysis to the diapophysis of Mookerjee ('30). These two processes are the "*Rippenträger*" of Göppert (1896). Gadow states that it is possible that this complex structure arose from the tendinous anchoring of the rib shaft to the neural arch (cf. Schmalhausen, '58) rather than from the neural pedicel rudiment. A final stage in salamanders is the loss of all attachments to the centrum and the dorsal movement of the rib bearers along the neural arch.

The most comprehensive approach to the problem of rib evolution in recent years has been by Schmalhausen ('58, '68), who studied the embryology of *Hynobius retardatus*. His interpretations, which are in broad agreement with those of Emelianov ('36), are strongly influenced by recapitulation theories. Developmental

states of salamanders are equated with the situations encountered in various groups of fossil amphibians. The primitive arrangement is viewed as a single attachment to the centrum. As the tetrapods became terrestrial, functional requirements associated with the weight of the thorax led to the evolution of the dorsal rib head. This formed by the differentiation of tissue in the intermyotomal septum (termed "ligament"). The dorsal rib head was attached to the ventral part of the neural arch in early amphibians, and has moved dorsally during phylogeny. The distal parts of the parapophysis (original ventral head) and the ventral part of the diapophysis (derived from the rib-vertebral ligament) fused lateral to the vertebral artery in salamanders to form the main rib-bearer, and this structure subsequently became bifurcated. These events are said to be recapitulated in the ontogeny of *Hynobius*. There, in early stages, only a ventral head is present, and it is connected to a block of cartilage that projects downward from the neural arch. On the first few vertebrae, this cartilage may extend around and under the vertebral artery, attaching to the notochord, in the region that will become the centrum. As development proceeds, the dorsal rib head forms, and the cartilaginous connection to the notochord, if ever present, is replaced by a bony strut to the centrum. On the first three vertebrae, rib development is independent of the bearer development, but Schmalhausen affirms that complete continuity of the rib and transverse process Anlagen is maintained on other vertebrae, with chondrification of the region proceeding from the vertebra distally into the ribs. He views the transverse processes of salamanders as having been derived from a combination of a distal remnant of the original parapophysis and a neomorphic dorsal bearer which is formed from septal mesenchyme at the base of the ribs. The structure is said to have a dual origin, and only during late ontogeny are dorsal and ventral portions clearly differentiated as the cartilaginous mass bifurcates. Even in adults, the ventral bearer is said to be represented by a distal piece of cartilage, formed from the ancestral parapophysis, which has become fused to the neomorphic dorsal bearer.

The bony struts that may extend below the vertebral artery to the centrum are viewed as secondary specializations.

#### Pattern in *Eurycea*

In *Eurycea* different modes of development characterize different parts of the vertebral column. Nevertheless, certain generalizations can be made. Rib development precedes that of the rib bearer along the entire column. Distal parts of the rib develop first, then tissue in the intermyotomal septum differentiates to produce proximal growth. The ventral rib head forms long before the dorsal, but both develop independently of their respective bearers. On the anterior vertebrae, development of the dorsal rib head proceeds from the rib towards the vertebra; more posteriorly rib head development is initiated near the vertebra and proceeds toward the rib. The tissue forming the sheet-like intermyotomal septum gives rise to a variety of skeletal elements and differentiation of these structures varies in rate and sequence.

The ventral rib bearer in *Eurycea* develops as a block of cartilage lying lateral to the vertebral artery and vein. On the first two trunk vertebrae, the bearer gains two cartilaginous attachments to the vertebra. The first is by means of a ventromedially directed process that differentiates below the vertebral artery and vein and extends from the bearer proper to the ventral margin of the centrum, near the anterior end of the latter. The second attachment is by means of a thick band of cartilage that lies along the neural arch, adjacent to the neural pedicel rudiment (fig. 8a). This cartilage lies lateral and dorsal to the vertebral artery and vein. Dorsally, the cartilage extends as a thin band to the center of the neural arch, where it expands to form the dorsal rib head bearer. From information presented earlier concerning *Hynobius*, *Necturus* and *Triturus*, it seems likely that the ventral attachment of the lower rib head corresponds in position with the original single rib bearer of tetrapods. The dorsal attachment of the ventral head and the dorsal rib head and its bearer are secondary structures.

In adult *Eurycea* the link between the more dorsal connection of the ventral rib head bearer and the dorsal rib head bearer is converted to bone. The two structures are well separated, except near their bases, where a thin web of bone, a secondary connection, may extend between them. A double attachment to the vertebra is retained by the ventral bearer. The vertebral artery and vein extend between these attachments (fig. 8a). A cylinder of bone with a cartilage core, which is progressively eroded at its proximal end, forms the adult bearer.

Rib development on the more posterior trunk vertebrae differs from that on the first two in lacking the more dorsal connection of the ventral rib bearer to the neural arch. The sole attachment is a piece of bone extending below the vertebral artery and vein to the anterior end of the centrum (fig. 8b). This bone is preformed in cartilage only on some of the more anterior vertebrae. In adults, the ventral rib bearer is a cylinder of bone filled with cartilage. Proximally it is open and the cartilage is eroding as ossification of the cylinder proceeds. The ventral lip of this cylinder extends below the vertebral artery and vein as a flattened sheet of bone. This is a very weak attachment, and the ventral rib bearer tends to break when vertebrae prepared by maceration are handled. On occasional vertebrae, a secondary strut that extends from the dorsal rib head bearer may develop late in life, but it is never preformed in cartilage.

During development the ventral rib head bearer of the sacral vertebra has cartilaginous attachments to both the neural arch, above the vertebral artery, and to the centrum, below the vertebral artery. Cartilage of the dorsal rib bearer and of the dorsal part of the ventral rib bearer is not continuous at any stage of development, but blastemal tissue is continuous between the two areas in the 12.0 mm stage. Stout, bony, dorsal and ventral attachments of the ventral rib bearer are present in adults (fig. 8c).

The haemapophyses of the caudosacral and caudal vertebrae are preformed in cartilage. A small nodule of cartilage first appears at the ventrolateral margins of the centrum near the neural pedicel rudiment.

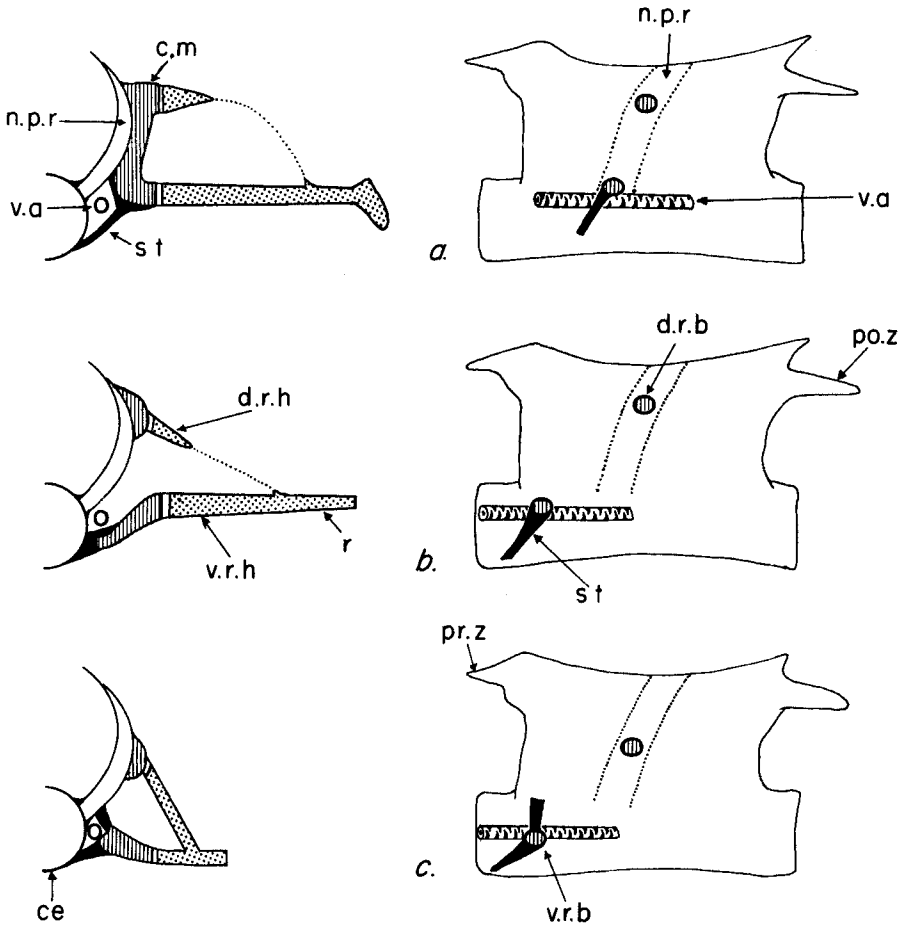


Fig. 8 Diagrammatic representations of the relationship of the rib bearers and ribs to other vertebral parts in *Eurycea bislineata*. Transverse sections on left and lateral views on right. a, condition on first and second trunk vertebrae; b, condition on mid-trunk vertebrae; c, condition on sacrum. See text for explanation.

This position is somewhat posterior to that for the ventral attachment of the ventral rib bearer on the first two trunk vertebrae and the single attachment of the ventral rib bearer on remaining trunk vertebrae. Gamble ('22) showed that in *Necturus* both the ventral attachment for the ventral rib bearer (his parapophysis) and the rudiment of the haemal arch arise from the same block of tissue (his basal stump). On the contrary, in *Eurycea* the haemopophyses arise far posteriorly, in contact with the notochordal sheath, while the transverse processes first appear as condensations of cells that do not contact the vertebra or notochord. As development proceeds, the cartilage of the transverse

process first gains a posterior and dorsal bony attachment to the neural arch, then an anterior and ventral attachment to the centrum. The dorsal connection is at the level of the neural pedicel rudiment. At no stage during development is there any cartilaginous or bony connection between the transverse and the haemal arch. The transverse processes of more posterior vertebrae (all caudal) do not preform in cartilage, but all haemal arches do.

In *Eurycea* the anterior two trunk vertebrae retain a condition that is similar to that postulated by Schmalhausen ('58) as an ancestral urodele pattern. Dorsal and ventral attachments for the ventral rib bearer lie respectively above and below the

vertebral artery and vein. The dorsal attachment of the ventral bearer is a part of the same piece of cartilage that gives rise to the dorsal rib bearer. More posteriorly along the column, the dorsal attachment of the ventral rib head to the neural arch, when present, is by a slender bony strut. This strut forms late in ontogeny and does not preform in cartilage. It is independent of that found on the two anterior vertebrae. Both dorsal and ventral attachments are preformed in cartilage on the sacral vertebra, and both ossify in the adult. Finally, the transverse processes of those postsacral vertebrae that are preformed in cartilage have no cartilaginous attachments to the vertebra, but acquire both dorsal and ventral bony struts that extend to the neural arch and centrum. No sign of the dorsal rib bearer is present posterior to the sacrum.

Some authors have made much of the connection or separation of the cartilaginous rib bearers and neural pedicel rudiments (cf. Schmalhausen, '68), but the presence or absence of bone between the two areas seems to be quite simply related to the stage at which ossification of the neural arch is initiated. In generalized species, like *Ambystoma*, differentiation of the bearer tissue precedes ossification of the vertebrae on the anterior vertebrae, and on those the cartilage of the arch and the bearers are in broad continuity. A clear gradient of development is evident, with anterior vertebrae being relatively advanced in development. The gradient is discontinuous in the sacral region, which also develops early. As a result of this gradient, the ossification process, in effect, catches the vertebrae at various stages of development, and the neural arch is ossified prior to differentiation of rib bearers on the mid-trunk vertebrae. Thus, a layer of thin bone intervenes between the cartilage of the neural pedicel rudiment and the rib bearer. This layer persists until remodeling of the vertebral canal results in its erosion from its medial border. In *Eurycea* osteogenesis of the vertebral column is much advanced relative to other aspects of development, and rib bearer material lies outside a bony layer on all vertebrae.

#### Comparisons

Observations on other salamanders are

instructive in completing the picture of rib and rib bearer development and probable evolutionary trends. In *Notophthalmus viridescens* the situation on midtrunk vertebrae is very similar to that described above for the first two trunk vertebrae of *Eurycea*. The ventral rib bearer is preformed in cartilage that extends in two directions: (a) below the vertebral artery, to the centrum, and (b) above and lateral to the artery, to the neural arch (as in fig. 9c). The cartilage of the dorsal rib bearer is continuous with that of the dorsal attachment of the ventral rib bearer. In adults there are two distinct bony attachments of the ventral rib bearer which correspond to the earlier cartilaginous structures (as in fig. 9e). These observations are consistent with those of Göppert (1896), Mookerjee ('30), Emelianov ('33, and earlier) and Mauger ('62), on salamandrid genera, and of Keller ('46) and Teege ('57) on ambystomatids, though the interpretations of these authors differ extensively in certain regards.

Larval *Ambystoma opacum* (17.5 mm) have a very large neural pedicel rudiment on the first two vertebrae which grows distally and bifurcates to give rise to both dorsal and ventral rib bearers (as in fig. 9g). Göppert (1896) figures a similar condition on the second vertebra of *Triturus alpestris* and *Salamandra salamandra*. No attachment below the vertebral artery is present, but the rib is oriented in that plane and a line of fibroblasts marks the position of the future bony attachment. On the third and succeeding vertebrae the cartilage that gives rise to the bearers is mainly independent of the neural pedicel rudiment. The rudiment of the ventral bearer forms first and attaches to the dorsal end of the ventral rib head. Condensations of undifferentiated cells mark the position of the future dorsal rib head and its bearer, and of the ventral attachment of the ventral rib bearer. Distal portions of the latter condensation may chondrify, but the proximal parts ossify directly. In the adult the ventral rib bearer has bony attachments above the vertebral artery to the neural arch and below the artery to the centrum (as in fig. 9e). The main attachment is the more dorsal one, which is much larger than the ventral one.

In *Plethodon cinereus* dorsal and ventral bearers differentiate from a block of

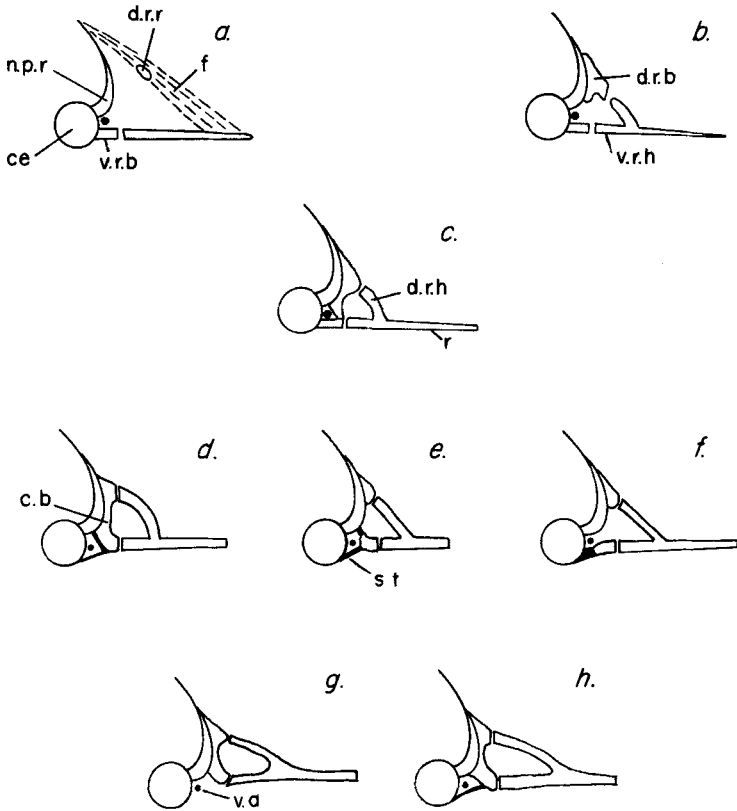


Fig. 9 Some examples of rib evolution in salamanders. Schematic representations of transverse sections through one side of the vertebrae. a, pre-salamander condition with unicipital head; hypothetical; b, ancestral salamander condition; hypothetical; c, generalized condition, found in anterior trunk vertebrae of *Hynobius*, *Eurycea* and other genera; d, mid-trunk vertebrae of *Hynobius* and some other genera; ventral rib bearer has only a bony connection to the centrum below the vertebral artery; e, widespread condition in salamanders; ventral rib bearer has only a bony connection to the centrum both above and below the vertebral artery; f, mid-trunk vertebrae of *Eurycea*; ventral bearer joins to the centrum below the vertebral artery only, and this is by a bony connection that typically does not preform in cartilage; g, mid-trunk vertebrae of *Plethodon*; ventral rib bearer joins only to neural arch; h, some vertebrae of *Plethodon* have a neomorphic bony strut which connects to the centrum, producing a secondary condition similar to that in d. This situation differs from d in that it is not represented in the embryo.

cartilage that lies along the ventrolateral margins of the neural arch. The cartilage extends laterally and divides. In adults both processes are located above the vertebral artery (as in fig. 9g). Some ventral struts may appear late in development, but these are not preformed in cartilage and appear to be neomorphic (fig. 9h).

If one takes into account the varied conditions of rib bearer and rib development encountered in modern salamanders, a general evolutionary pattern emerges. Although modern species are insufficiently

known to be able to describe the situation in detail, broad outlines seem apparent. A process extending from the ventral head of the rib to the centrum apparently represents the original position of the rib in salamanders (fig. 9a). A dorsal head arose later (cf. Schmalhausen, '68) and as it became increasingly larger, in response to functional demands, a dorsal bearer also evolved from tissue of the myoseptum opposite the neural pedicel rudiment. Theoretically the simplest and most primitive condition for a salamander with bicipital



ribs would find the dorsal bearer attached to the neural arch and the ventral bearer only to the centrum (as in fig. 9b). Among salamanders the closest approach to this model is that described by Gray ('30) for *Triturus*. Here, however, the dorsal and ventral bearers are connected by fibers extending from a ventral process of the dorsal bearer to the tip of the ventral bearer (as in fig. 9c). Gamble ('22) describes a condition in the trunk vertebrae of *Necturus* where the dorsal and ventral bearers are in cartilaginous continuity, with the cartilage extending to the neural arch and centrum respectively. On the anterior vertebrae of *Necturus*, the ventral bearer has its main attachment to the dorsal cellular mass. In the anterior two trunk vertebrae of *Eurycea* and in the trunk vertebrae of *Notophthalmus* a continuous cartilage mass develops along the neural arch. During ontogeny, this mass extends laterally to include the cartilage of the ventral bearer, and extends ventrally, below the vertebral artery, to contact the centrum (fig. 9c). This is close to the condition described for some of the anterior trunk vertebrae of *Hynobius* by Schmalhausen ('58, '68), who thinks this condition is a generalized one in salamanders. It can be viewed as resulting from the distal fusion of the ventral rib bearer and dorsal rib bearer lateral to the vertebral artery. The conditions considered below may be derived conditions.

There is an apparent tendency in some lineages for the ventral attachment of the ventral bearer to be reduced, first during development and later during phylogeny. The connection may preform in cartilage but subsequently resorb, or it may even fail to develop. In either case, an attachment to the centrum by membrane bone appears late in ontogeny (fig. 9d). Such a pattern has been reported in the trunk vertebrae of *Hynobius* by Schmalhausen ('58) and variations have been seen in several species by many authors. In *Ambystoma opacum*, the ventral attachment is present on most vertebrae, but it does not preform in cartilage. The extreme of this trend is found in *Plethodon*, where no cartilaginous ventral attachment is present on the trunk vertebrae of adults (fig. 9g), though some bony struts may

form secondarily. While the condition we have described as widespread in salamanders is encountered on the two anterior trunk vertebrae of *Eurycea* (fig. 9c), the majority of its trunk vertebrae have a different condition. The dorsal attachment of the ventral bearer is absent and the ventral attachment may or may not preform in cartilage (fig. 9f). In late stages of development a new bony dorsal attachment may form, but it is not related except in a functional sense to the structure that preforms in cartilage at an early stage on the anterior vertebrae. The sacral vertebra is similar to the anterior two trunk vertebrae.

The condition that prevails on the mid-trunk vertebrae of *Eurycea* (fig. 9f) is superficially similar to the theoretically primitive condition for all salamanders (fig. 9b), but the connection of the ventral bearer to the centrum is never cartilaginous. Further, the ventral bearer is displaced anteriorly. Thus the condition in *Eurycea* should be viewed as derived.

In summary, in modern salamanders ribs are bicipital except in certain specialized species (Wake, '66). Dorsal and ventral bearers are present. The dorsal bearer is always on the neural arch, opposite the neural pedicel rudiment. The ventral bearer has a variety of positions, and its homologies are not established to satisfaction. It may be viewed as first appearing during development in a distal position and gaining attachments either with the centrum (by way of a cartilaginous connection near the vertebral midpoint primitively, or by a bony secondary growth which attaches to the anterior region of the centrum) or with the neural arch (by way of a cartilaginous connection with the ventral part of the dorsal bearer), or various combinations of both. The most generalized condition seen in modern salamanders is likely one in which, during development, the dorsal and ventral bearers are in cartilaginous continuity and the ventral bearer is attached to the centrum by a piece of cartilage. The vertebral artery and vein pass between these two attachments. Many derived, specialized conditions are encountered, but these tend to be on the mid-trunk vertebrae, and in forms like *Eurycea bislineata* the most

generalized conditions are encountered on a few anterior trunk vertebrae and the sacral vertebra.

*Segmentation and the development  
of vertebrae*

The basic body plan of vertebrates involves segments in the form of a series of somites. The inner borders of the somites produce areas of sclerotome cells, lying ventromedial to the myotome cells. The sclerotome is responsible for the formation of the vertebral column, and it is often termed the skeletogenous layer. A continuous cylinder of cells in a layer of varying thickness surrounds the notochord, and it is this perichordal tube which differentiates to form the individual centra. In amniotes the segmental arrangement of the myotomes (primary segmentation) is reflected in the sclerotomes. The extensive amniote sclerotome often develops a sclerocoel, which corresponds in position to the myocoel and divides the sclerotome into two parts, the cranial and caudal halves (Schauinsland, '05; Sensenig, '49; Williams, '59; Werner, '71). These two parts are recognized readily since the caudal half has a denser concentration of cells. Following sclerocoel formation, sclerotome cells from the cranial half of one segment join those from the caudal half of the preceding segment, and a continuous perichordal tube is formed. Diameter of the tube is smallest at the segmental borders and greatest near the midpoint of each segment. The vertebral centrum of the adult thus forms from the caudal and cranial halves of adjacent sclerotomes, while the thickened midsegmental portion (the perichordal ring) forms the intervertebral cartilage. The segmental arrangement of the vertebral column does not correspond to the primary segmentation of the somites. There has been, in effect, a resegmentation.

Resegmentation is a demonstrable feature of amniotes, but the occurrence of such a phenomenon in fishes and modern amphibians is highly equivocal (see Williams, '59; Schaeffer, '67; Wake, '70). In salamanders, evidence of resegmentation is slight (Wake, '70) and in *Eurycea* we are unable to find any indication of such a phenomenon. The sclerotome is

poorly developed in frogs and salamanders, and it is difficult to locate. There is no evidence of segmental sclerotomes in *Eurycea*, but scattered cells, not segmentally arranged, lie along the notochord prior to formation of the perichordal tube. No indication of sclerocoel has been seen. The perichordal tube and perichordal rings are the first indication of discrete sclerotomal tissue. The rings are segmental in position, but tend to be near the posterior ends of the somites (relative to the medial borders of the somites). The area between rings gives rise to the bony centrum and becomes continuous with the neural pedicel rudiment. This rudiment is located in a crevice between adjacent myotomes, i.e., intersegmentally.

In salamanders, the centra form by differential morphogenesis of the continuous perichordal tube, and little ontogenetic adjustment is required. Each centrum is thus a trans-segmental structure, a functional necessity in living tetrapods. Since we have been unable to find any evidence of an original segmentation of the sclerotome, it is quite inappropriate to speak of a resegmentation in *Eurycea*, and to do so is to argue by analogy with the condition in amniotes. However, the trans-segmental position of the centrum suggests that it is formed by cells ultimately derived from two adjacent segments.

We see no evidence, from our study of *Eurycea*, that an amniote pattern of resegmentation need ever have occurred in the ancestry of salamanders. Amniotes and the three long separated lineages of modern amphibians all have specialized vertebrae, and there is no reason for assuming that a developmental process of any of these lineages was the primitive one. The increased amount of sclerotome in amniotes may be related to the development of the increasingly massive vertebral centra that evolved in the antracosaur, the group generally assumed to have given rise to amniotes (Romer, '66; Carroll, '69). The developmental feature of significance common to all tetrapods is the formation of a perichordal tube and its subsequent differentiation into trans-segmental vertebrae.

*Evolution of the vertebral centrum*

The question of the relationship of the

salamander centrum to that of other amphibians and early tetrapods has recently been discussed by Wake ('70). Little need be added here. Basically the arguments involved interpretation of data and questions of logic. Our position is as follows: the only conclusive evidence of centrum homology in salamanders relative to other tetrapods will come from relatively complete sequences of fossils. In the absence of such sequences the most logical conclusion is that vertebrae of all tetrapods are homologous (see also Thomson and Bossy, '70). However, different groups have different arrangements of the centrum. In early rhachitomes the centrum was dual. In some groups the anterior or intercentrum has become dominant (e.g., stereospondyls), while in others the pleurocentrum dominates (some dissorophoids, anthracosaurs, amniotes). Williams ('59) believed that developmental data suggested that modern amphibians have a centrum derived from a pleurocentrum. Wake ('70) examined the logic of this suggestion and was unable to accept the conclusion. Basically, the argument is as follows. Reptiles can be shown to have been derived from anthracosaurs, and a reasonably good fossil record shows that the pleurocentrum became progressively large in the lineage leading to amniotes. Living amniotes show a clear sclerotomal resegmentation, which produces a centrum (pleurocentrum) in the adult that is derived from halves of two adjacent sclerotomes. Williams argued that this pattern of development was probably characteristic of all forms with a dual centrum, possibly right back to rhipidistian fishes. He felt that evidence was good for such a developmental pattern in the centrum of modern amphibians. Reasoning that the pattern of development was conservative, he argued that the centrum of modern amphibians was therefore the homologue of the resultant structure of the same developmental process in amniotes, namely the pleurocentrum.

In contrast, Wake ('70) has argued that the resegmentation seen in amniotes is an adaptive feature, and probably not conservative in evolution. Functional demands require location of a centrum at somitic borders. The simplest developmental solution to the problem of formation of a skeletal unit straddling two somites is the contribution of cells from adjacent sclero-

tomes to the element. If the volume of sclerotome is large, the separating cell masses may be discrete, as in amniotes and caecilians. In most salamanders (e.g., *Eurycea*) the sclerotome is scanty and no sclerocoel appears. It so happens that all living groups in which large volumes of sclerotome occur also have either a monospondylous centrum, or one in which the pleurocentrum is by far the dominant element. We suspect that a hypothetical group with a dominant or exclusive intercentrum, determined on the basis of fossil sequences, would have a developmental pattern similar to that in amniotes, provided that sclerotomes of large volume were present. In other words, questions relating to general patterns of development and to homologies of adult structures seem to be quite independent. Functional considerations were likely of major importance in the evolution of various arrangements of adult centra in lower vertebrates (Parrington, '67; Thomson and Bossy, '70).

In the case of plethodontid and most other salamanders, development is characterized by the appearance of virtually no sclerotomal units. Thus there is nothing to resegment. Further, there is no evidence in the fossil record to suggest that the single centrum is either a pleurocentrum or an intercentrum. We suggest that the most logical approach is to consider centra as centra (Panchen, '67; Thomson and Bossy, '70; Wake, '70). Further attempts to assign exact homologies are futile without conclusive evidence from the fossil record, or new criteria for their establishment.

#### ACKNOWLEDGMENTS

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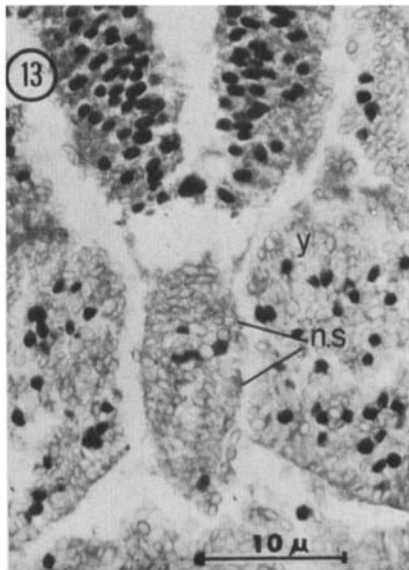
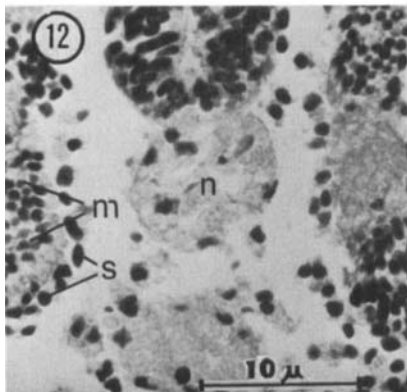
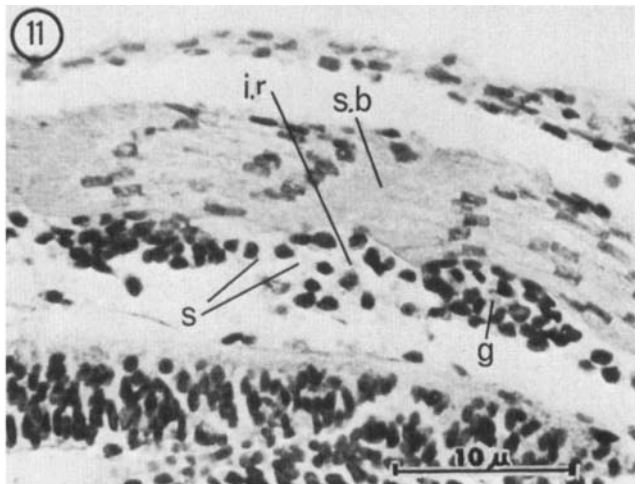
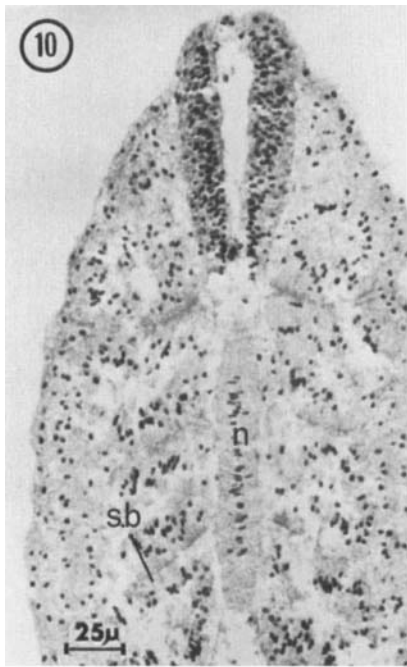
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PLATE 1

EXPLANATION OF FIGURES

- 10 Frontal section through 3 mm embryo of *Eurycea bislineata*. The somites are differentiated, but there is no clear sclerotome.
- 11 Longitudinal section through the anterior part of the trunk of a 4 mm embryo of *Eurycea bislineata*. Pro-muscle cells are elongated, but sclerotome cells are few in number and rather unorganized.
- 12 Transverse section through the anterior trunk region of a 5 mm embryo of *Eurycea bislineata*. There is little sign of sclerotomal tissue.
- 13 Obliquely transverse section through the anterior trunk region of a 3 mm embryo of *Eurycea bislineata*. The notochord and surrounding tissues are richly supplied with yolk granules, and there is little tissue differentiation as yet.

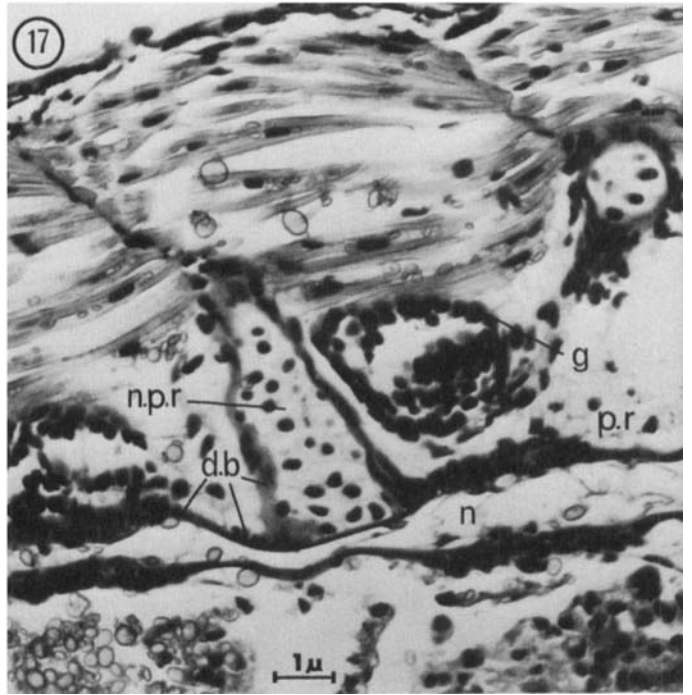
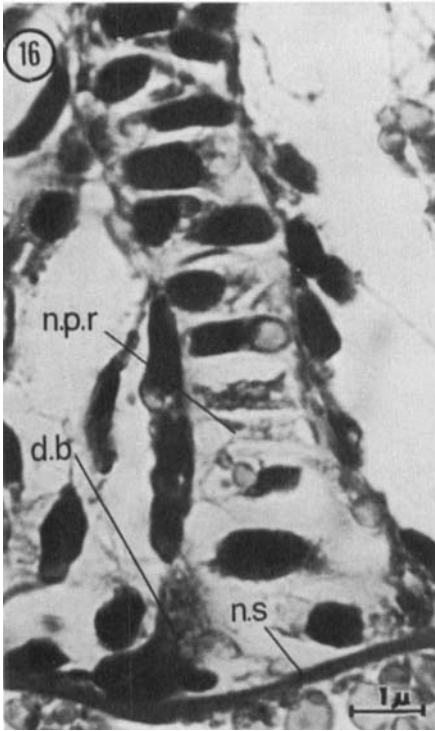
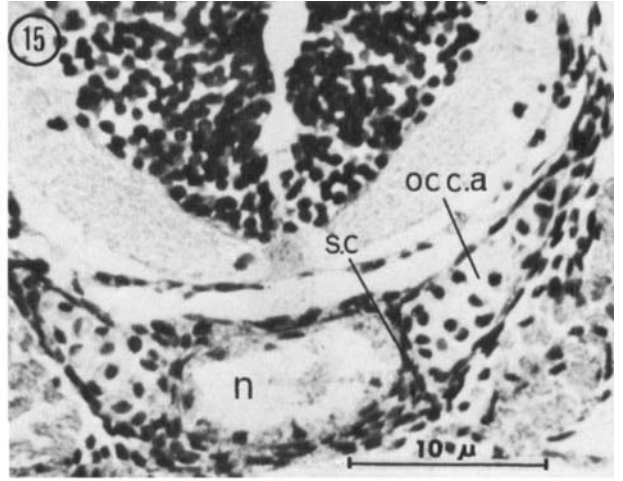
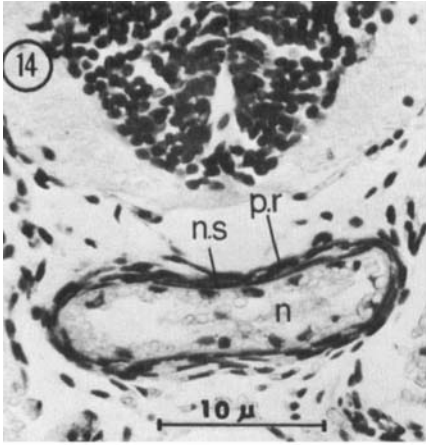


## PLATE 2

### EXPLANATION OF FIGURES

- 14 Transverse section through the developing vertebral column in a 7.4 mm embryo of *Eurycea bislineata*. The perichordal ring is present but it is very small compared with the condition typical of amniotes.
- 15 Transverse section through the occipital arch in a 7.4 mm embryo of *Eurycea bislineata*. The notochord now contains large, "vacuolated" cells. The cartilage of the occipital arch is well differentiated.
- 16 Longitudinal section through the developing cartilaginous neural arch (neural pedicel rudiment) of an anterior trunk vertebra in a 7.4 mm embryo of *Eurycea bislineata*. Osteogenesis is commencing at the base of the pedicel.
- 17 Longitudinal section through the developing vertebral column of a 7.4 mm embryo of *Eurycea bislineata*, just behind the head. Anterior to the right. The neural pedicel rudiment is well developed, and bone formation is proceeding at its base as well as in the immediately adjacent perichordal tissue.





### PLATE 3

#### EXPLANATION OF FIGURES

- 18 Transverse section through an anterior vertebra of a 10.3 mm larva of *Eurycea bislineata*. The perichordal ring is well developed, and tends to be asymmetrical. Bone of the centrum is well developed.
- 19 Frontal section through the developing first rib of a 10.2 mm larva of *Eurycea bislineata*. The ossification process is just beginning near the midpoint of the largely cartilaginous rib.
- 20 Frontal section through the cranio-vertebral joint in a 10.2 mm larva of *Eurycea bislineata*. Anterior to the right. The notochord is filled with cartilage from about the midpoint of the atlas across the joint and into the posterior part of the head. This cartilage material gives rise to parts of the odontoid process of the adult.
- 21 Frontal section through a rib rudiment in a 10.2 mm larva of *Eurycea bislineata*. Rib formation is initiated laterally, some distance from the vertebra.
- 22 Frontal section through a developing zygapophyseal joint in a 10.2 mm larva of *Eurycea bislineata*. The pre- and post-zygapophyses form from chondrification centers.
- 23 Transverse section through the cranio-vertebral joint of a 10.3 mm larva of *Eurycea bislineata*.
- 24 Transverse section through the dorsal vertebral roof of an anterior trunk vertebra in a 10.3 mm larva of *Eurycea bislineata*.

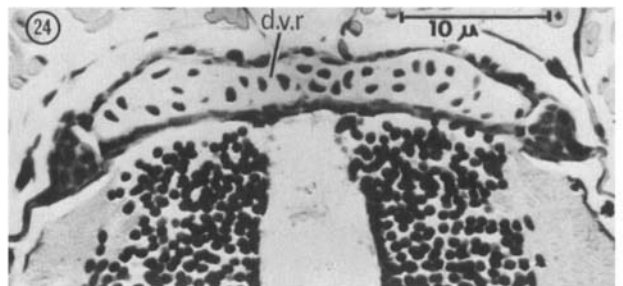
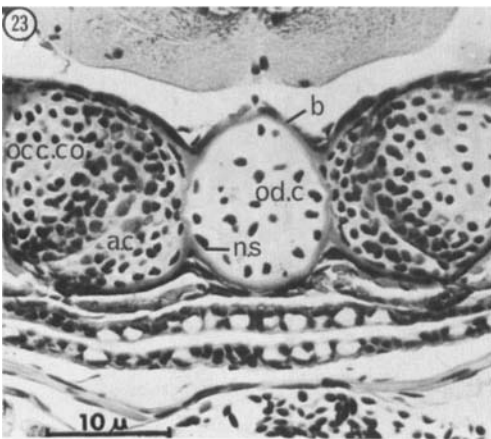
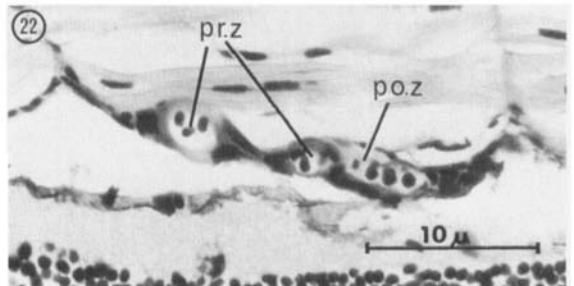
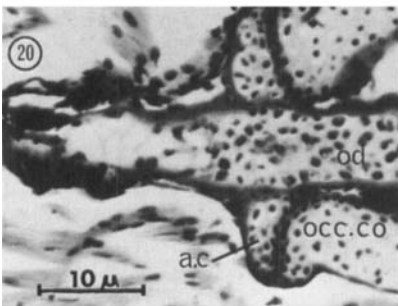
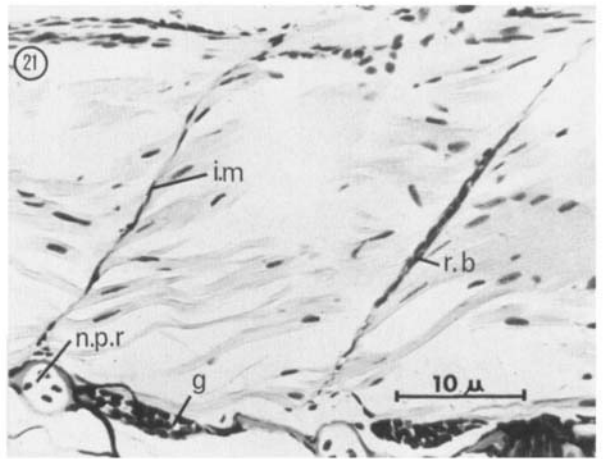
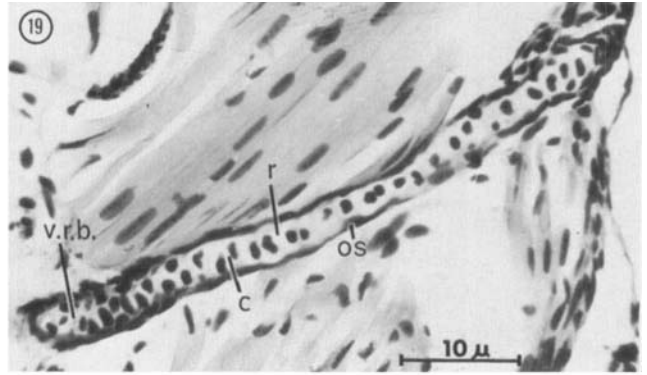
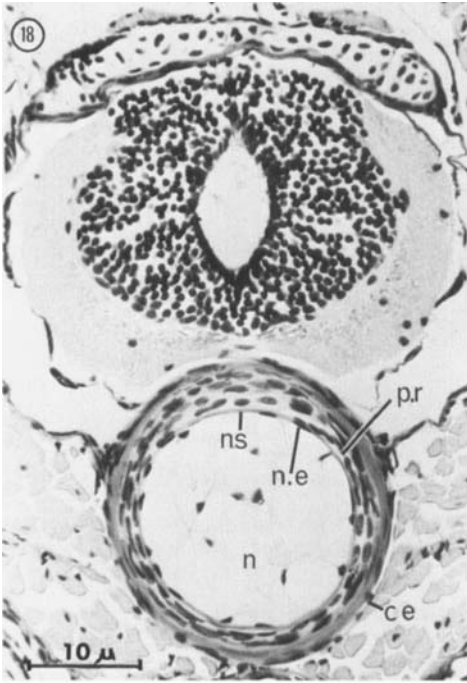
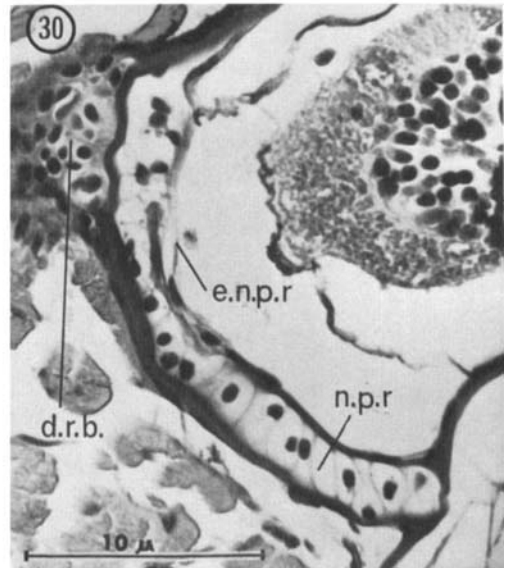
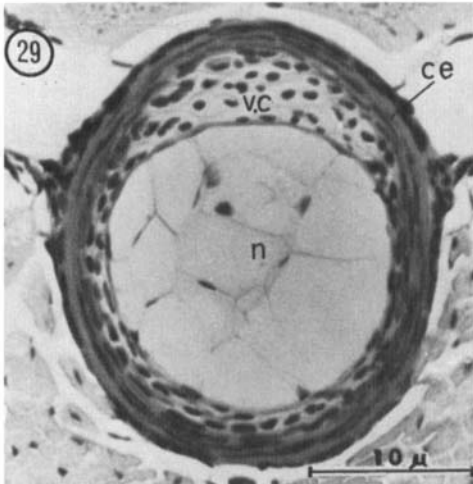
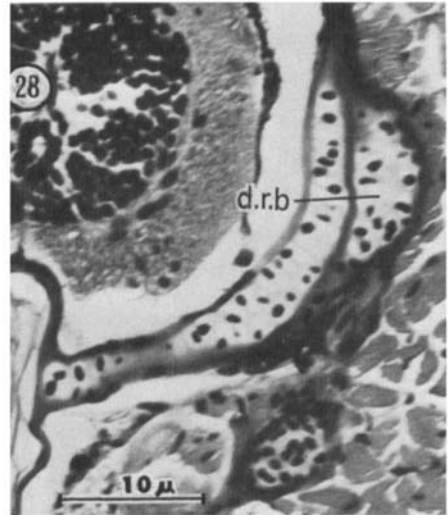
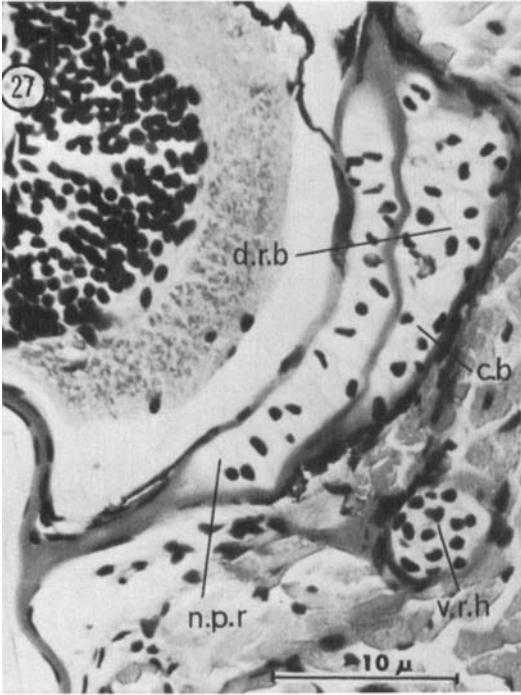
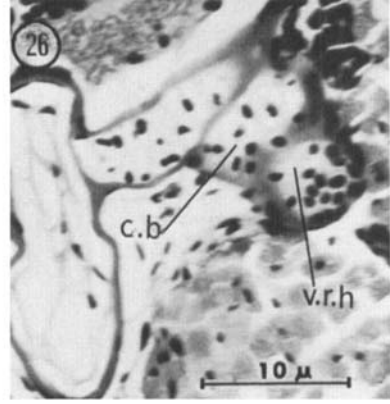
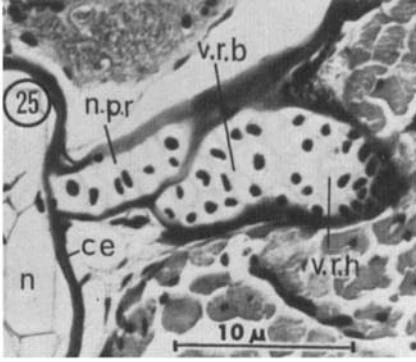


PLATE 4

EXPLANATION OF FIGURES

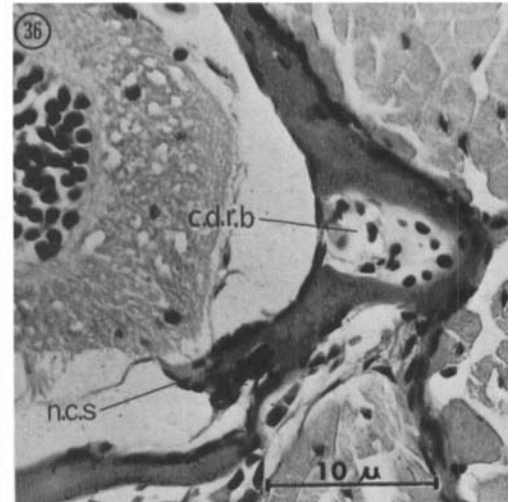
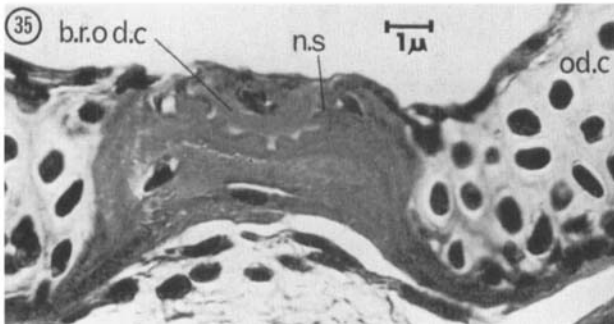
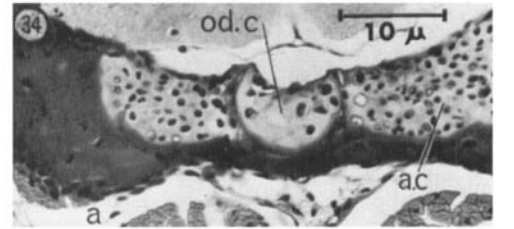
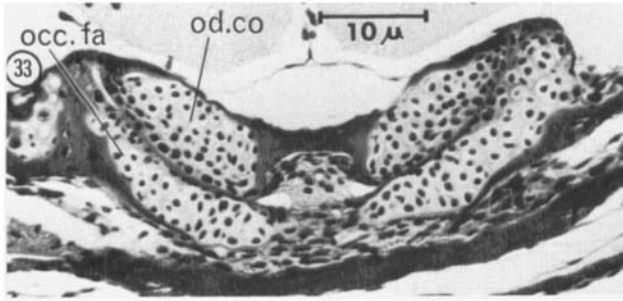
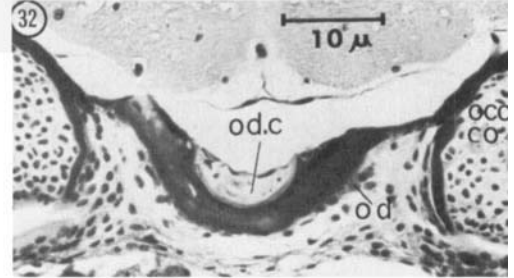
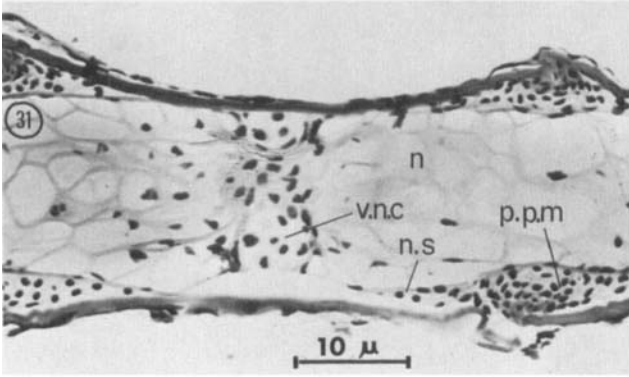
- 25 Transverse section through the ventrolateral part of the second trunk vertebra of a 12.1 mm larva of *Eurycea bislineata*. The ventral rib head is in continuity with its bearer.
- 26 Same as 25, but slightly posterior, illustrating the connection between the dorsal and ventral rib bearers.
- 27 Slightly posterior from 26.
- 28 Slightly posterior from 27.
- 29 Transverse section through the centrum and intervertebral cartilage in a 12.1 mm larva of *Eurycea bislineata*. Note increased thickness of the intervertebral cartilage (derived from the perichordal ring, (cf. figs. 14, 18) and asymmetry of the notochord in the cartilage.
- 30 Transverse section through the lateral side of the neural arch in a 13 mm larva of *Eurycea bislineata*. Note that the neural pedicel rudiment has begun to erode medially (cf. fig. 27).



## PLATE 5

### EXPLANATION OF FIGURES

- 31 Frontal section through the centrum of an anterior trunk vertebra of a 15.5 mm larva of *Eurycea bislineata*. Cartilage is present as a ring in the center of the centrum, inside the notochordal sheath. It is compressing the notochordal cells. Most of the vertebra is cartilage and notochord, with a thin shell of bone at this stage.
- 32 Transverse section through the odontoid process of the atlas vertebra at the level of the occipital condyles. The notochord is present as an eroding remnant in the heavily ossified process.
- 33 Same as 32, but somewhat more anteriorly, illustrating the joint between the odontoid condyles, at the anterior end of the odontoid process, and the occipital facets, on the ventromedial margins of the foramen magnum, directly above the posterior end of the parasphenoid bone.
- 34 Same as 32, but somewhat more posteriorly, showing the relationship of the base of the odontoid process and the cartilage of the atlantal cotyles. Here the odontoid process consists basically of the cartilage filled notochord and a surrounding thin bony ring.
- 35 Same as 33, but slightly posteriorly, showing remnant of notochord near tip of odontoid process.
- 36 Transverse section through the lateral side of the neural arch in a 17.4 mm larva of *Eurycea bislineata*. Note the skeletal structure which extends medially from the wall of the arch to support the nerve cord.



## PLATE 6

### EXPLANATION OF FIGURES

- 37 Frontal section through the neural canal of a 41.6 mm adult *Eurycea bislineata*, illustrating nerve cord support (cf. fig. 36) and erosion of cartilage in the dorsal rib bearer.
- 38 Frontal section through the intervertebral joint of a 41.6 mm adult *Eurycea bislineata*. Cartilage plays a dominant structural and functional role in the adult centrum. The intervertebral cartilage is large and differentiated, with an arc of fibrocartilage cells marking the plane of movement. Anterior to the left. The notochord persists as a well organized structure throughout life, but is compressed by the intervertebral cartilage in the anterior end of the centra and filled by cartilage in the center of each centrum. Compare with figure 1, d.



