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Morphogenesis of the Head of a Newt: Mesodermal Segments, Neuromeres, and Distribution of Neural Crest

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Segmentation of the mesoderm in the head of a newt embryo is revealed by scanning electron microscopy. By the end of gastrulation, the newt embryo is already segmented from one end to the other, with additional segments added later by the tail bud. This metameric segmentation appears long before the first "somite" can be seen in the late neurula by light microscopy. The six segments found in the newt head look much like the six most-cranial segments described decades ago in shark embryos. Mesodermal segments in the newt head are similar to somitomeres in amniote embryos, but in amniote embryos, the numbers and relationships of head segments are quite different from those of the newt. In both amniote and newt, the first segment abuts the prosencephalon, but for each more caudal head segment, where the newt embryo has one segment, the amniote has two. Although the pattern and distribution of cranial neural crest is quite similar in newt and amniote embryos, there are different relationships between migrating crest masses and mesodermal segments due to the doubling of most of the cranial segments in amniotes. It now appears that all vertebrate embryos, regardless of their mode of gastrulation, form similar mesodermal segments from one end of the embryo to the other, and this metameric pattern is established during gastrulation.

INTRODUCTION

Close study of the structure and development of the head "soon brings the conviction that the head region of the Craniate is truly segmented that it is composed of a number of segments essentially similar to those of the trunk, and that segmentation originally extended to the anterior end of the body as it still does in *Amphioxus*" (Goodrich, 1930).

Questions about the segmentation of the heads of vertebrate animals have a long history. According to Goodrich's account (1930) of this history, it began in 1792 when Frank compared the skull to a single vertebra. Goethe and Oken, a few years later, proposed the "vertebral" theory of the skull, suggesting that the skull was composed of from three to six vertebrae similar in plan to the vertebrae of the trunk. This vertebral view was supported by embryologists such as Rathke and Reichert.

In his Croonian Lecture of 1858, T. H. Huxley demolished the vertebral theory and it was replaced with the "segmentation" theory. Segments differ from vertebrae in that each vertebra forms from parts of two adjacent segments or somites. The segment is the original metameric unit, and to avoid confusion, Jarvik (1980) prefers to call the segments "metameres." In this paper we use the term "segment" in the metameric sense.

In amphioxus and in larval lampreys, the parachordal mesoderm is completely segmented from dorsal to ventral edges, and the segments extend forward to the tip of the head (Jarvik, 1980). In all vertebrates with jaws (gnathostomes), the parachordal mesoderm is obviously segmented dorsally in much of the body as somites, but ventrally the hypomere or lateral plate, which surrounds the coelom, is incompletely or not obviously segmented (Jarvik, 1980). The dorsal segmentation of the parachordal mesoderm extends to the tip of the head in sharks (Balfour, 1878, 1881; de Beer, 1922; Goodrich, 1930). Balfour (1878) found eight mesodermal head segments in the shark *Scyllium* and de Beer found nine head segments in the shark *Squalus*. Sharks have three preotic segments, a fourth beneath the otic vesicle, and four or five more postotic segments in the head. Without interruption, segments continue in the trunk as somites (Fig. 1).

There is a large inferential literature about the putative segmentation of the amniote head based upon arguments of relationships of cranial nerves, muscle masses, bones of the skull, brain compartments and neuromeres, evolutionary considerations, etc. We will not discuss those works because direct observations of metameric segmentation of the mesoderm in the heads of embryos of a variety of amniotes have been made in the past few years by Meier and his collaborators, and the actual segmentation is not as suggested by any previous author.

Meier (1979) first discovered, using stereo scanning electron microscopy, that discernible metameric mesodermal units exist in the head of the chick embryo,

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continuing the segmentation craniad to the tip of the head from the first somite visible with light microscopy. He termed these metameric units "somitomeres." Each somitomere is a squat, cylindrical domain of cells arranged in concentric circles. The lateral extent of the somitomeres is rather exactly coextensive with the lateral edges of the overlying neural plate.

Meier (1981) traced the origins of these segmental units to gastrulation stages. The first somitomeres appear at the anterior-most extent of the primitive streak, just lateral to Hensen's node. A prechordal plate, looking much like a medial somitomere, forms in front of Hensen's node at about the same time as the first pair of somitomeres appear (Triplett and Meier, 1982). The second, third, etc. pairs of somitomeres are sequentially formed as the primitive streak regresses. Somitomeres always first appear just lateral to the regressing node; segmentation thus proceeds from anterior to posterior.

The eighth segmental unit to form looks, at first, exactly like the more cranial somitomeres, but it continues differentiation to the point of forming deep clefts anterior and posterior to itself and thence being recognizable as the "first" somite. All the rest of the somites as well begin as typical somitomeres. The seven pairs of units located cranially (and the prechordal plate) remain dispersed and not separated by deep clefts, so they do not become recognizable as "somites" by the usual criteria of light microscopy. Nevertheless, as long ago as 1907, Patterson noted "shallow transitory depressions situated at regular intervals" in mesoderm anterior to the "first" somite, and he suggested that they might be "vestigial clefts, separating cephalic mesoblastic somites."

Meier and Tam (1982) and Meier and Packard (1984) have subsequently shown that for mouse and snapping turtle embryos, the first somite forms from the eighth segment (somitomere) added to the axis.

Since primitive streak gastrulation is peculiar to the amniote embryo, we have examined an amphibian embryo that gastrulates through a blastopore. This paper describes the metameric segmentation of the mesoderm of the head of a newt embryo, the interrelationships between the mesodermal segments and the segmentation of the brain, and the distribution of the cranial neural crest.

MATERIALS AND METHODS

Taricha torosa, a California newt, was chosen for this study because we are familiar with its embryos, and there are numerous studies of its early development. Fate maps of external features are available (Schechtman, 1932), and the anatomy of early *T. torosa* embryos is figured by Witschi (1956) from sections and from dissected specimens that had been hardened in alcohol. Stages of development are from the staged series for *T. torosa* devised by Twitty and Bodenstein (Rugh, 1962) and modeled after the stages for *Amblystoma punctatum (Ambystoma maculatum)* by Harrison (1969).

Embryos and gravid females were collected in the San Francisco bay area and shipped air freight to Texas in cooled Thermos jugs. Embryos were removed from their jelly masses and vitelline membranes as described before (Jacobson, 1967). Embryos were fixed for scanning electron microscopy (SEM) in one-half strength Karnovsky's fixative (Karnovsky, 1965) made up in 0.1 *M* cacodylate buffer. Fixation time was 1 hr for intact embryos and 1/2 hr for opened embryos. Specimens were then rinsed in 0.1 *M* cacodylate buffer and further cuts and dissections made in this buffer. Many embryos were stripped of their epidermis with electrolytically sharpened tungsten needles and watchmaker's forceps to reveal the underlying mesoderm. After dissection, embryos were postfixed for 1 hr in

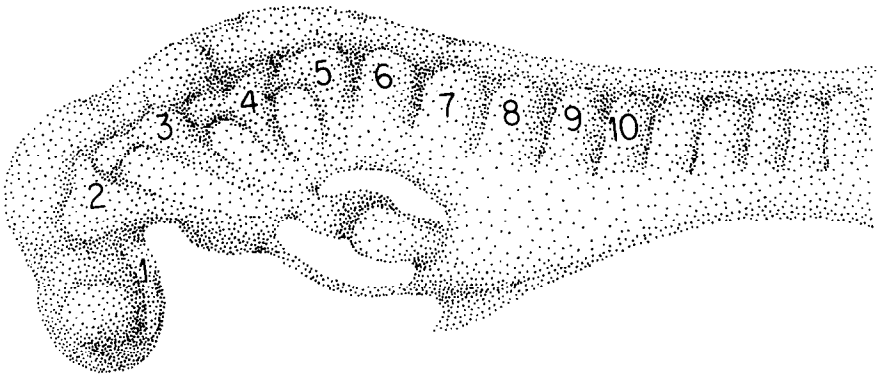


FIG. 1. A diagram of mesodermal segments in the head and trunk of a shark embryo (after Goodrich, 1930). The epidermis is removed and the mesodermal segments are numbered.

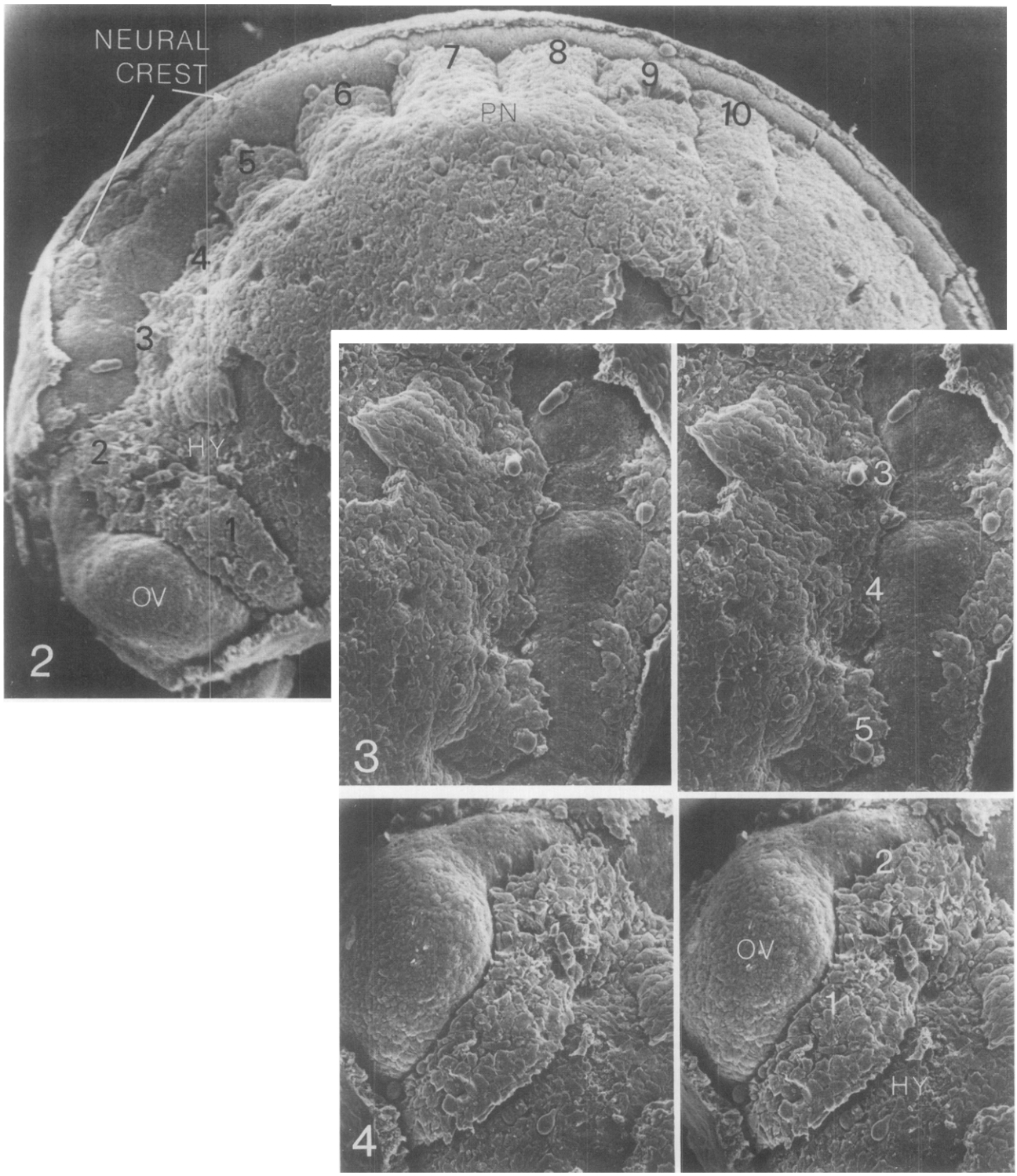
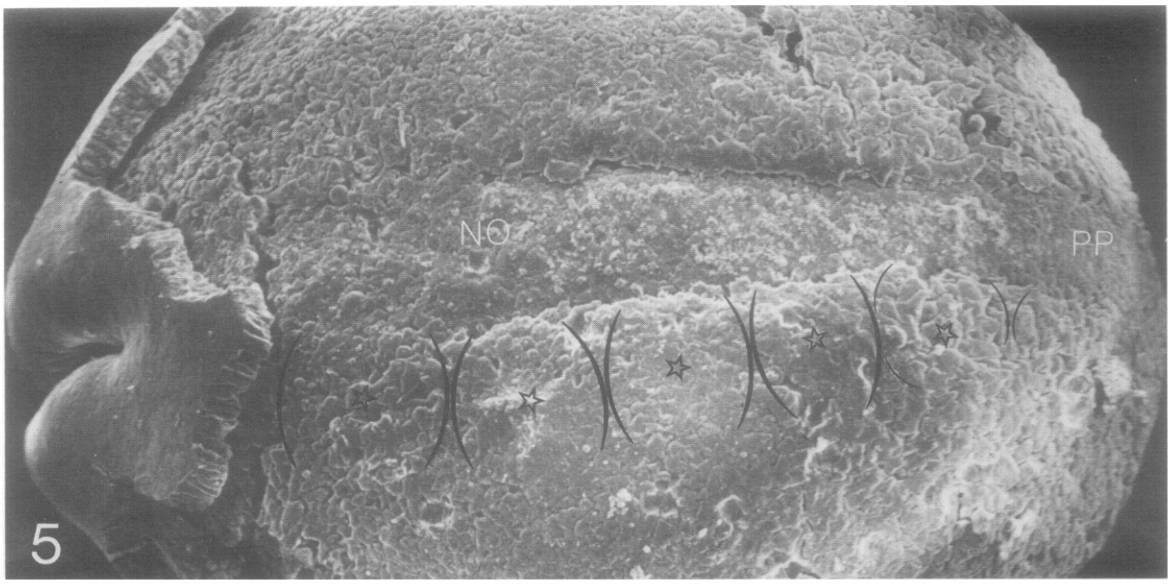
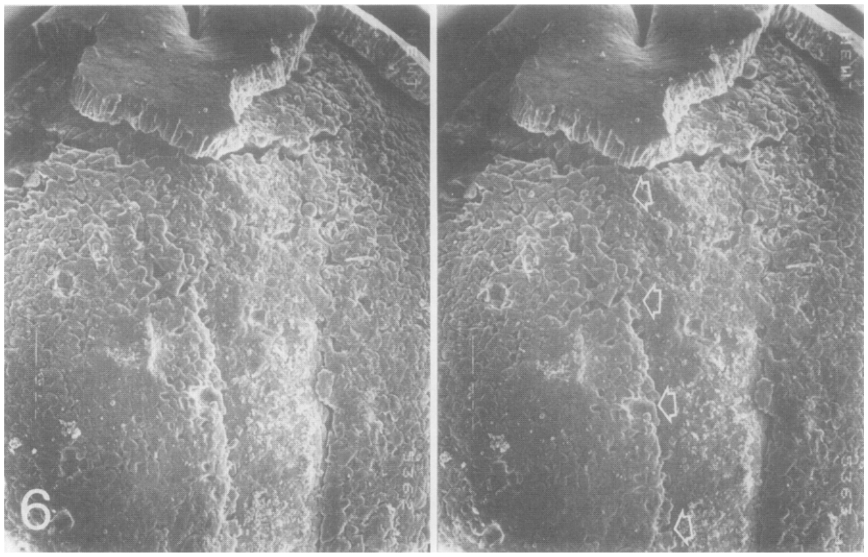


FIG. 2. A lateral view of the left side of a newt embryo at stage 23 (epidermis removed). Mesodermal segments of the head and trunk are labeled 1-10. The neural crest is gathered in a dorsal ridge along the neural tube. The neural tube, between the crest and the mesodermal segments, is free of mesenchymal cells. OV, optic vesicle; PN, pronephros; HY, hyomandibular pouch endoderm. ×58.

FIG. 3. A stereo view of the third, fourth, and fifth (3, 4, 5) mesodermal segments of the head of the embryo shown in Fig. 2 (cranial end at top). The boundaries and centers of mesodermal segments coincide with the boundaries of neuromeres of the hindbrain. ×80.

FIG. 4. A stereo view of the first and second mesodermal segments (1, 2) of the embryo shown in Fig. 2 (cranial end at top). The first segment curves under the optic vesicle (OV) and borders the hyomandibular pouch endoderm (HY). ×80.





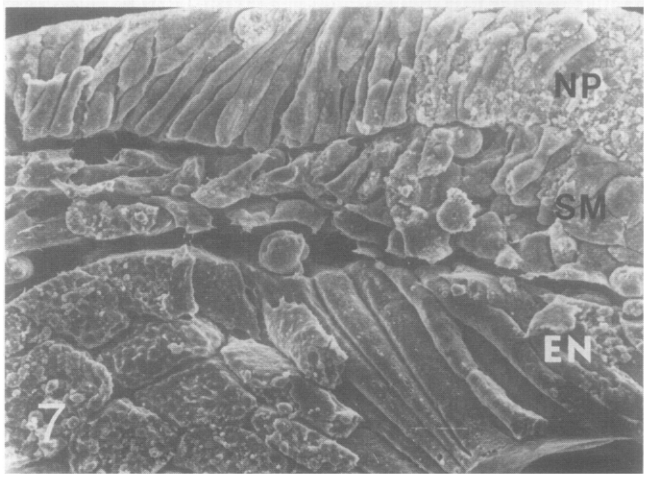


FIG. 5. A dorsal view of a newt embryo at early neurula stage 14 (ectoderm removed, cranial end to right). The broad midline groove is occupied by notochord (NO) and prechordal plate (PP). The paraxial mesoderm is segmented into circular domains of oriented cells. These segments lie in tandem to either side of the midline depression, but are marked only on the right side (star at center, curved lines at periphery of unit) ×65.

1% osmium tetroxide, then washed in buffer, dehydrated through ethanols (30% to 100%), and critical-point-dried using carbon dioxide as the exchange fluid. Dried specimens were mounted on double-stick tape, transferred to SEM stubs, and sputter-coated with 8 to 10 nm of gold-palladium alloy. Specimens were examined at 15 kV with an ISI Super III A SEM. All stereo micrographs were mounted for viewing with a standard stereo viewer, and all were taken at a tilt angle of 10°.

Embryos were examined with SEM at each stage from stage 9 (early gastrula) to stage 38 (larva near hatching). Altogether, 919 SEM photos, of which 282 were stereo pairs, were analyzed in this study. A total of 346 specimens were examined.

RESULTS

Segmentation of the Cranial Mesoderm

Embryos at tail-bud stage 23 were chosen for initial examination because this is the latest stage at which the cranial neural crest has not yet migrated over, and obscured, paraxial head mesoderm.

At stage 23, the *T. torosa* embryo has six obvious somites, just as Harrison (1969) described for the stage 23 embryo of the salamander *Ambl. punctatum (Amb. maculatum)*. Closer examination of the *T. torosa* embryo with SEM reveals a set of segments in the head that are very similar to the head segments described in a shark (Fig. 2, compare to Fig. 1). The first segment underlies the optic bulge of the prosencephalon and the second segment lies alongside the mesencephalon. The third segment lies beside the metencephalon and the first neuromere of the myelencephalon. The fourth segment is beneath the nascent ear placode.

The fifth segment is the most anterior of the "somites" that have formed by this stage, and the sixth through tenth segments have formed somites as well. Posterior to segment 10 (somite 6), the paraxial mesoderm is discernibly segmented back to the tail bud when viewed in stereo SEM (not shown), and there are at least six segments posterior to the last "somite" at this stage. Altogether, from the tip of the head to the tail bud, there are 16 segments in the embryo at stage 23.

The first four segments do not form rigorously defined somites and each is a different shape depending upon its position along the axis. As seen in stereo (Fig. 3), the fourth segment is larger than the fifth (a somite) and its dorsal surface is concave, rather than convex. Cells at the outer surface of the segment form a curved border along intersegmental interfaces, whereas those cells in the interior of the segment seem to form circles and rings about the center of the unit. Like the fourth segment, the third segment is also quite large and circular, but only the center of the unit is concave on its dorsal surface. The second segment (Fig. 4) is elongated along the craniocaudal axis and its dorsal surface is somewhat convex. The first segment is much distorted in the direction of the long axis and therefore is nearly oval. Nevertheless, component cells appear to be aligned in concentric rings within the unit.

Since the stage 23 newt embryo is segmented from cranial to caudal end, we must look at earlier stages to see when segmentation begins, and in what order. Therefore, we begin with midgastrula stages and work back up to stage 23.

At stage 12, the blastopore is complete and there are hints of segmentation in the paraxial mesoderm when viewed in stereo from the archenteron surface. However, it is not definite enough to identify the extent of segmentation cranially and caudally. The appearance of the paraxial segments is more definite at the small-yolk-plug stage, stage 13, when at least 6 segments can be discerned. By stage 14, an early neurula (Fig. 5), the paraxial mesoderm is clearly delineated from the broad notochord and prechordal plate. Most of the neural plate can be removed and the axial mesoderm appears as a midline depression in the mesoblast layer. When the paraxial mesoderm is examined in stereo (Fig. 6), it is seen to be clearly organized into circular domains of oriented cells much like somitomeres in amniote embryos. Units of cricularly oriented cells lie in tandem on either side of the midline. In cross sections, the paraxial mesoderm appears as a thin bilayer that extends between the roof of the gut and the neural plate (Fig. 7). While our specimens do not show the complete cranial-caudal sequence of segmental units, our estimate is that about 11 segments are present at this stage.

The metamerism of the paraxial mesoderm becomes more pronounced, and a stage 17 embryo (Fig. 8) has one somite set off by deep segmental clefts. Harrison (1969) noted that the stage 17 *Ambl. punctatum* has one somite pair. Stereo examination reveals six segments cranial to the definitive somite, so the first-formed somite originates from segment 7. The first two head segments, and the cranial end of the third,

FIG. 6. A stereo view of the mesodermal segments of the embryo seen in Fig. 5 (cranial end down). Mesodermal segments are flat, circular domains of concentrically arranged cells (arrowheads indicate boundaries between tandem segments). ×50.

FIG. 7. A cross section through the paraxial region (left side) of an embryo at stage 14. The neural plate (NP) and the endoderm of the gut (EN) enclose a thin bilayer of segmented mesoderm (SM). ×206.

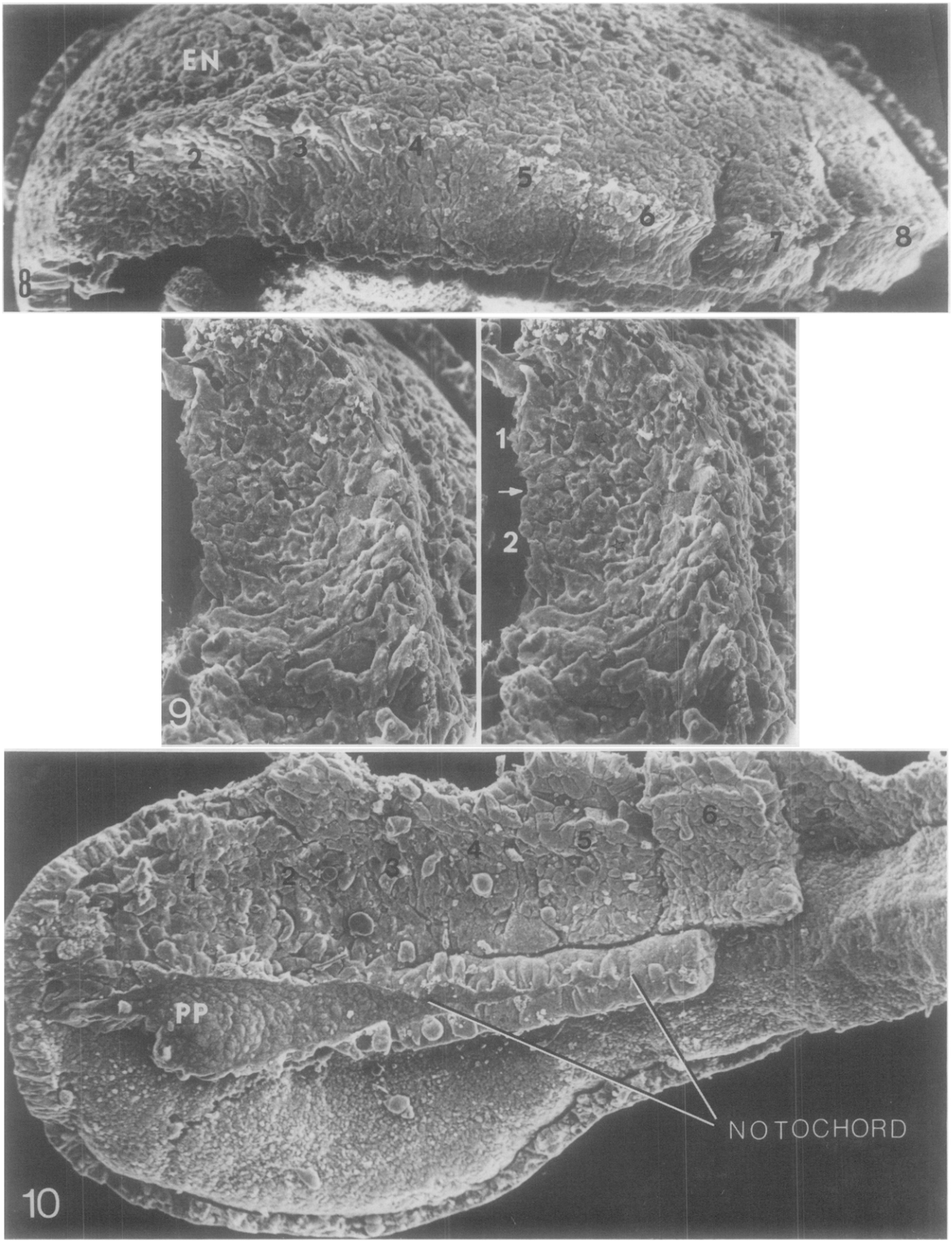


FIG. 8. A dorsal view of the cranial paraxial mesoderm of the right side of an embryo at stage 17 (ectoderm removed, cranial end left). The first eight segments are numbered (1-8). Segment 7 is already defined as a somite by deep clefts perpendicular to the midline. Bare endoderm (EN) lies lateral to the first three cranial segments, but mesoderm covers the endoderm lateral to the remaining segments. ×84.

lie above an extensive anterior region devoid of more lateral mesoderm. The edge of the mesoderm in this region forms a right angle; a horizontal edge below segments 1, 2, and part of 3, and a vertical edge extending below segment 3. As the head extends over the cranial end of the embryo and flexes ventrally, this broad, mesoderm-free region becomes reduced to a slit over the hyomandibular pouch at later stages (see Fig 12).

Viewed dorsally, the cranial segments most closely reflect the morphogenesis of the overlying neural plate. For instance, the surface of the first segment (Fig. 9), facing the optic bulge, is concave and the unit is rather oval and stretched along the long axis. Still, the unit is distinguished by the ringed arrangement of its component cells. Each cell seems slightly arched, contributing to the general "bull's-eye" appearance of the segment from this point of view.

A ventral view of the head segments in a stage 17 embryo (Fig. 10) reveals clefts that are more pronounced from this view, occurring between segments 6 and 5, and between 5 and 4, as well as on each side of segment 7. Segments 6 and 5 are about to become "somites" along with segment 7. The order of appearance of formed somites (clefts to each side when viewed laterally) is 7, 6, 5, 8, 9, etc. At the cranial-most end, the prechordal plate occupies the midline adjacent to segments 1 and 2, and its ventral surface is open to the archenteron, the opening extending into the notochord some distance, at least to segment 4 (Fig. 10). The most cranial extent of the prechordal plate mesoderm is contiguous with foregut endoderm. Stereo views of the ventral surfaces of cranial segments also reveal a rigorous cellular organization of the unit (Fig. 11). Cranial segments are no longer flat and are raised into wedge-shaped units, but cells are still tightly swirled about the center of the segment. Additional features of the cranial pattern from this view include triangular gatherings of cells that are bounded by intersegmental interfaces and the notochord. These cells are likely to be progenitors of intersegmental vascular elements.

Distribution of Cranial Neural Crest

We will now describe stages older than stage 23, paying attention to the emerging shelves of neural crest and their relationships with the metameric pattern.

A stage 24 embryo (Fig. 12) has seven somites (segments 5 to 11). Caudal to the seventh somite (11th segment) are approximately 7 additional segments, a total of 18 segments at this stage. The rostral shelf of neural crest has emerged from the mesencephalon and has extended downward over part of the second segment. The mesoderm-free area that is occupied by the hyomandibular pouch is now a long slit coursing beneath segments 2 and 3 (Fig. 13). Segments 3 and 4 are attenuated along the base of the hindbrain, and the neural crest in that region has not yet extended down the neural tube far enough to contact the mesodermal segments. A deep groove between the mesencephalon and the metencephalon can be seen, and a gap in emergence of cranial neural crest occurs at this point, at the boundary between segments 2 and 3.

By stage 25, the streams of cranial crest have descended farther ventrally (Fig. 14). The rostral shelf now covers most of segment 2 and the caudal end of segment 1. Dorsal to the hyomandibular pouch, the metencephalon, over the cranial end of segment 3, remains free of neural crest. Farther caudal, a long tongue of crest (the preotic crest) descends over the caudal half of segment 3. Posterior to this, crest emerges at the rhombencephalon, but remains at the midline to create an open crest-free area where the ear placode resides. Being still attached to the epidermis, the ear placode is removed when the epidermis is stripped off. The caudal end of segment 4 is covered by a massive postotic crest that forms a continuous shelf of neural crest that extends to more caudal somites. The formed somites, at stage 25, are epithelial and the cells are radially arranged around a central myocoel.

During the early phases of crest emigration, the cranial-most end of the embryo remains relatively free of crest (Fig. 15). A mass of mesoderm, the prechordal plate, lies cranial and ventral to the forebrain and between the pair of first segments, but crest has not been added to the area from the prosencephalon. However, by stage 26, most of the cranial crest has moved down to more lateral positions, leaving the roof of the neural tube mostly free of crest. Crest cells that originated from the rostral shelf now spread over the

FIG. 9. A stereo view of the first two cranial mesodermal segments (1, 2) in the embryo seen in Fig. 8 (cranial end up). The dorsal surface of each segment is concave, and has a definite center (star) about which the cells are arranged concentrically. An arrowhead marks the boundary between segments 1 and 2. ×163.

FIG. 10. A ventral view of the segmented mesoderm in an embryo at stage 17 (endoderm removed, cranial end to left). The first six segments (1–6) are marked on the left side of the axis. Segments have been lost in the dissection on the right side. The undersurface of the prechordal plate (PP) is contiguous with endoderm (removed) that lines the archenteron, and is part of the roof of the archenteron. At this stage, the anterior notochord also has surface that is open to the archenteron. ×85.

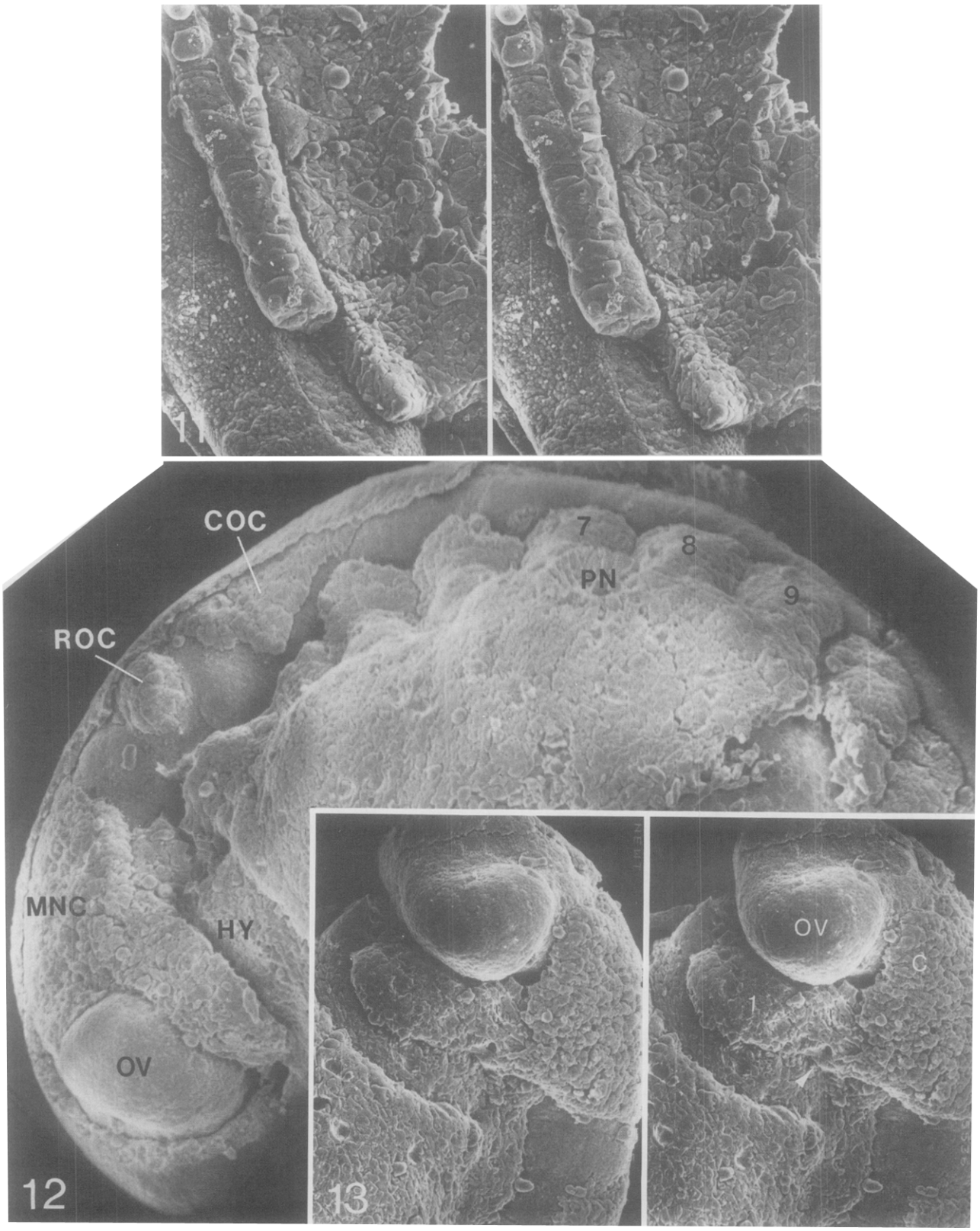


FIG. 11. A stereo view of the ventral surface of segments 4–6 of the embryo shown in Fig. 10 (cranial end up). Each unit is flattened and component cells are swirled about their centers. Triangular patches of cells (arrowhead) between segments may contribute to vascular elements ×98.

FIG. 12. A lateral view of the left side of an embryo at stage 24 (epidermis removed). The rostral shelf of mesencephalic neural crest (MNC) has migrated ventrad and craniad over part of segment 2. The rostral otic crest (ROC) has emerged over the caudal portion of

optic stalks, surrounding the optic vesicles and providing additional cells under the expanding telencephalon (Fig. 16).

DISCUSSION

Segmentation of the Newt Head

The first somite is visible with light microscopy in the stage 17 newt neurula, but with scanning electron microscopy the parachordal mesoderm of even younger neurulae can be seen to be segmented from end to end. These presomitic segmental units contain cells that are organized similarly to somitomeres described in the axes of amniote embryos. In later stages of newt development, more of these segmental units are added from the tail bud at the posterior end.

The mesodermal segmental units first appear at even earlier stages. We have traced them back to gastrula stages. During gastrulation, the cells of amphibian embryos are still getting smaller as cell divisions occur, so at earlier and earlier stages, the cells are larger and larger and fewer and fewer. While we have seen some segmental units at large-yolk-plug stage 12, their earliest appearance at earlier stages is difficult to detect because each segmental unit must be composed of a very few, very large cells whose patterned arrangement is not obvious. All we can say is that the earliest mesodermal segments appear in the newt embryo during gastrulation, just as they do in amniote embryos where they emerge next to Hensen's node of the primitive streak.

Certainly by the end of gastrulation (stages 13 to 14) the parachordal mesoderm is segmented from end to end of the newt embryo, with new units to be added from the tail bud. Since the pattern of segmentation is already present at these early stages, the events that cause segmental pattern formation must be looked for during or before gastrulation.

Comparison of Newt and Shark Segmentation

The arrangement and appearance of mesodermal segments in the head of a newt is similar to that of a shark. The two forms are alike in that the first segment lies next to the prosencephalon, the second next to the mesencephalon, and the third, fourth, fifth, etc., next to the rhombencephalon. The otocyst of a shark is located above the anterior portion of segment 4, at the junction with segment 3 (Fig. 1 and see Goodrich's (1930) Fig. 237). The otocyst of a newt is similarly located (Fig. 17). In both forms, the fifth segment of the head (lying behind the ear vesicle) is the most anterior segment to have an appearance closely approximating that of the trunk somites.

The two forms are also alike in that segments 3 and 4 have the appearance of being elongated along the long axis in later embryonic stages (e.g., Figs. 1, 2). The progress of such elongation is evident in a newt, especially during neural tube formation. At stage 18, when neural tube formation has begun but the brain plate is still open, segment 4 is just 0.25 mm long (measured from Fig. 8). By stage 23, after neural tube formation has been completed, segment 4 is 0.33 mm long (measured from Fig. 2), nearly a 30% increase in length. Elongation of the neural plate and tube occurs from the inception of the plate through larval stages, but is 10 times more rapid during neural tube formation (Jacobson, 1978). Elongation of the mesodermal segments of the head appears to coincide with that of the neural plate and tube. Parachordal mesoderm also condenses mediolaterally as the neural plate narrows and folds into a tube (Lipton and Jacobson, 1976).

The Boundary Between Head and Trunk

The position of the boundary between head and trunk can be deduced. Goodrich (1930) discusses the numbers of segments in the head of the salamander, *Amblystoma (Ambystoma).* Besides the three preotic segments, he describes three metaotic segments. The first metaotic segment (the fourth head segment) is below the ear vesicle, and the other two (the fifth and sixth head segments) are postotic. The *Ambystoma* head thus contains six segments.

Witschi (1956) illustrates a lateral view of a dissected *T. torosa* embryo (his Fig. 59) at late-tail-bud stage 29 (his stage 19). He shows three preotic and three postotic head segments (the anterior portion of segment 4 that should be beneath the ear vesicle is not shown). The seventh and eighth segments lie over the pronephros and are described as the first and second spinal somites. Our Fig. 2 also shows segments 7 and 8 overlying the pronephric bulge, so the boundary between head and trunk must be between segments 6 and 7 in *T. torosa.*

segment 3, leaving a stretch of neural tube bare of crest above the cranial end of segment 3. A triangular shelf of caudal otic crest (COC) has descended mainly toward the caudal portion of segment 4 creating a bare spot over the cranial end of segment 4, the site of otic placode formation. OV, optic vesicle; HY, hyomandibular pouch endoderm; PN, pronephros. ×80.

FIG. 13. A stereo view of the left side of an embryo similar to the one shown in Fig. 12 (cranial end up, epidermis removed). Most of segment 1 (1) is elongated beneath the optic vesicle (OV). The massive rostral mesencephalic crest (C) has covered most of segment 2. The boundary between segments 2 and 3 (marked by an arrowhead) lies at the constriction separating mesencephalon and metencephalon. ×66.

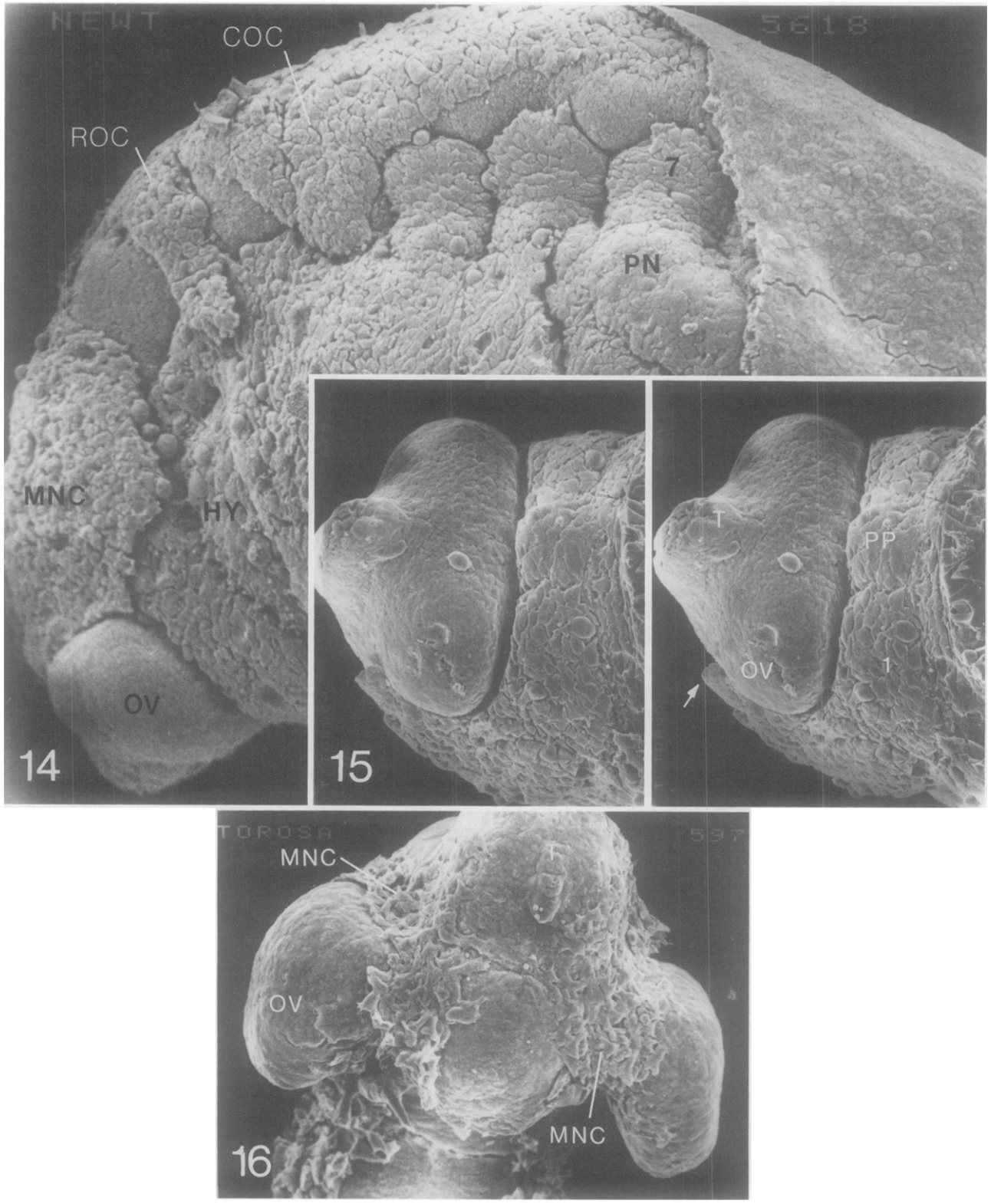


FIG. 14. A lateral view of the left side of an embryo at stage 25 (epidermis removed). The mesencephalic neural crest (MNC) now covers segment 2 and part of 1, and has begun to invest the optic vesicle (OV). Segment 3, above the hyomandibular pouch endoderm (HY), is free of crest on its cranial half, but is covered by rostral otic crest (ROC) on its caudal half. The cranial end of segment 4 is also free of crest because it underlies the otic placode (removed with the epidermis). The massive caudal otic crest (COC) covers the caudal part of segment 4, and merges with crest appearing adjacent to segment 5 and more posterior somites. The pronephros (PN) is evident lateral to segment 7 (7). ×90.

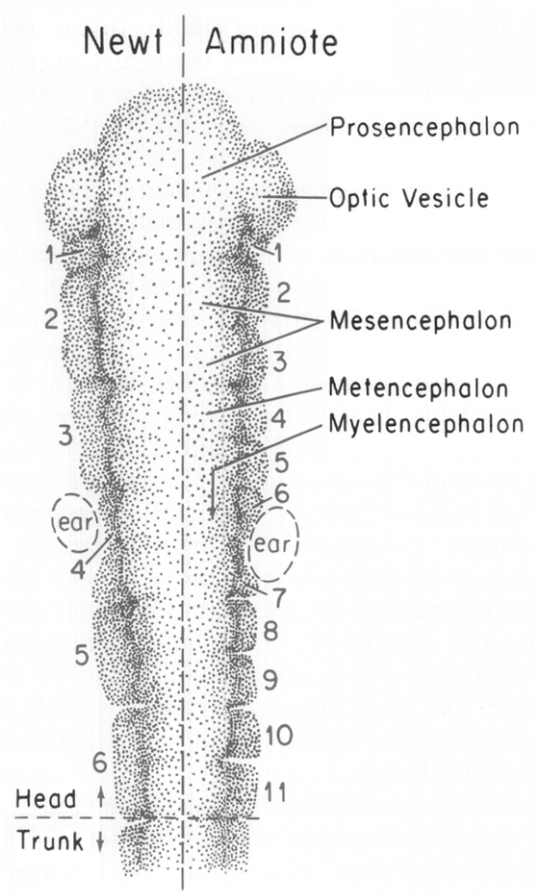


FIG. 17. Diagram comparing the head of a newt embryo to that of an amniote (dorsal view, epidermis removed). The relationships of mesodermal segments (numbered) to other head structures, such as the brain compartments and the ears, are illustrated. In both amniote and newt embryos, the first mesodermal segments are associated with the prosencephalon, but for the rest, where the newt embryo has one segment, the amniote embryo has two.

Newts thus have fewer head segments than sharks. According to Goodrich (1930), *Sq. acanthias* has nine head segments, *Scyllium* has at least seven, and at most eight head segments, *Spinax* has possibly as many as nine head segments. On the other hand, the teleost *Salmo* is said to have six head segments (Harrison, 1895), the same number as the newt.

Despite there being more segments in heads of sharks, the arrangement of the first six head segments in both shark and newt appears to be very similar. In these forms, the relationships between the first six head segments, the parts of the brain, and the position of the ear vesicle are nearly identical. In sharks, additional segments behind the sixth are part of the head.

Comparison of Newt and Amniote Head Segmentation

In contrast, a most interesting new rule emerges when head segments of newts (and sharks) are compared to those of amniotes. In all the forms, the first head segment underlies the prosencephalon, but for the second and subsequent head segments, the amniotes have two segments where the anamniotes have one (Fig. 17). The second segment of a newt underlies the entire mesencephalon, but in amniote embryos, segment 2 underlies only the cranial mesencephalic neuromere, and segment 3 underlies the caudal mesencephalic neuromere. In a newt, the third segment spans the metencephalic neuromere and the first myelencephalic neuromere, whereas in amniotes, the metencephalic neuromere and the first myelencephalic neuromere each border an individual segment (numbers 4 and 5, respectively). In a newt, segment 4 is next to the second and third myelencephalic neuromeres, and underlies the ear vesicle. In amniotes, segments 6 and 7 lie beside individual myelencephalic neuromeres (the second and third myelencephalic neuromeres), and the otic placode forms from the surface ectoderm above these segments. In a newt, segment 5 is the first to have a typical somite appearance, where in amniotes, segment 8 is the first.

Goodrich (1930) says amniotes have 5, or possibly 6, metaotic segments in the head, which should give them a total of 11 or 12 head segments. If they had started with the same number of head segments as the newt and had doubled each segment except the first, then they would be expected to have 11 segments in the head. In any event, the arrangement of head segments of the amniote in relation to brain parts and ear position cannot be explained by adding segments from the trunk or by crushing fewer around the ear (discussed in Goodrich, 1930), but rather makes sense only if additional segments are intercalated next to segments 2 through 6 of the anamniotes.

The First Segments and the Prechordal Plate

In the chick embryo, the first head somitomere is the largest of all (Meier, 1979). The first head segment

FIG. 15. A stereo view of the cranial-most end of the head of an embryo at stage 25 (epidermis removed, ventral view, cranial end left). The prechordal plate mesoderm (PP) occupies the midline beneath the incipient telecephalon (T). The first pair of mesodermal segments lie to either side of the prechordal plate (1 marked, right side), and beneath the optic vesicles (OV). The advancing edge of the rostral mesencephalic crest (arrow) can be seen dorsal and caudal to the optic vesicle, but no crest has yet reached the most rostral portions of the head. ×72.

FIG. 16. Ventral view of the head of an embryo at stage 26 (epidermis removed, cranial end up). Mesencephalic neural crest cells (MNC) have migrated rostrally between the expanded telencephalon (T) and the optic vesicles (OV), as well as ventral to the optic stalks. ×126.

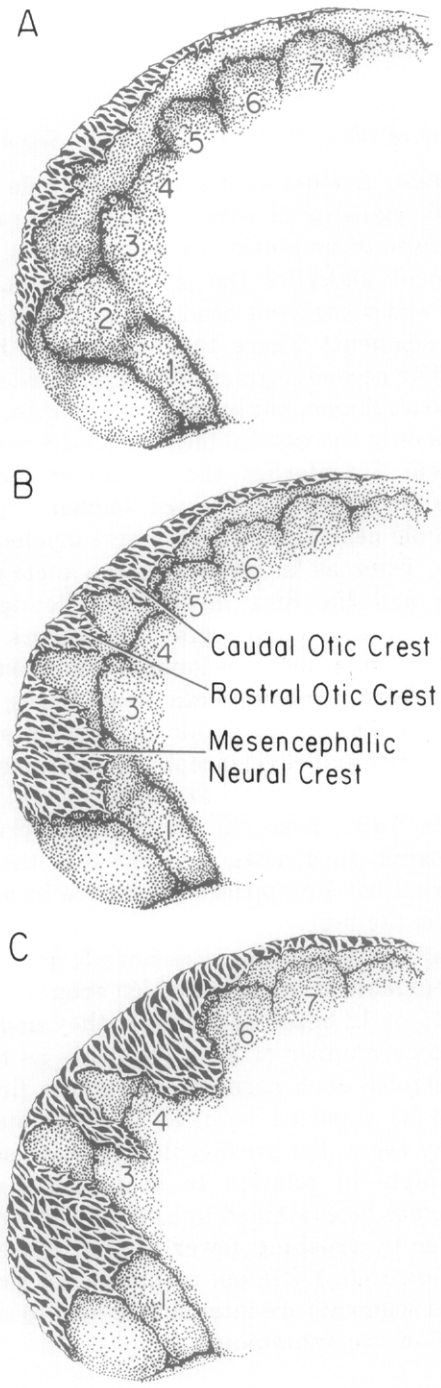


FIG. 18. Diagrammatic representation of cranial neural crest migration in the head of a newt embryo at tail-bud stages (lateral view, epidermis removed, mesodermal segments numbered). (A) Head of an embryo at stage 23. The neural crest is still gathered along the dorsal midline of the brain tube. (B) Head of an embryo at stage 24. Masses of crest cells have begun to descend over the mesencephalon and the myelencephalon. The mesencephalic crest has spread over the mesencephalon and most of segment 2, and has migrated rostrally over the prosencephalon, where it invests the optic vesicle. The metencephalon is free of crest, and so is a portion of the myelencephalon between the rostral and caudal otic crest masses. The bare region encircled by otic crest is occupied at this stage by the otic placode (not shown). (C) Head of an embryo at stage 25. The mesencephalic crest now covers segment 2 and part of segment 1, and has invested farther around the optic vesicle. The wall of the metencephalon remains free of crest. The rostral otic crest covers most of the caudal part of segment 3, whereas the caudal otic crest covers the caudal part of segment 4 and is continuous with crest descending toward more caudal segments. The ear (not shown) occupies the bare area between the otic crest masses.

of the newt is also massive (Fig. 2). In contrast, the first head segments illustrated for shark embryos are relatively small and more anterior masses of mesoderm are also shown (de Beer, 1922, his Fig. 3, "anterior head cavity of Platt"; Goodrich, 1930, his Fig. 236, "anterior proliferation of mesoblast"). These masses of mesoderm shown anterior to segment one lie beneath the eye, and together with segment one, occupy the same position as "segment 1" of a newt (Fig. 2).

Jarvik (1980) states that, in sharks, an additional segment must be added in front of the foremost one previously distinguished. This is based on the work of Bjerring (1973, 1977) who distinguishes a terminal somite, connecting across the midline and lying anterior to the premandibular segment. The terminal segment is the old vesicle of Platt (1891), and is connected with "parasomitic mesoderm" (Chiarugi's vesicle) which connects across the midline to the other side. He says the two premandibular segments and their interconnections are derived from the prechordal plate. Our observations on newt and amniote embryos suggest that all of the mesoderm of the prosencephalic region belongs either to a single pair of first segments, or to the prechordal plate.

In amniotes, a single pair of somitomeres embrace the prosencephalon, as do the first segments of a newt. The "prechordal plate" of an amniote is a midline unit between the first two segments and it has much the appearance of a somitomere (Triplett and Meier, 1982). In a newt embryo, the prechordal plate is a broad, midline sheet of mesoderm anterior to the notochord, and for a long time is part of the archenteron roof. It is distinct from the first pair of segments, and connects them across the midline. The prechordal plate remains a prominant mass of mesoderm beneath the telencephalon in later stages (Fig. 15). In newts and in amniotes, this anterior midline unit, the prechordal plate, behaves much as if it were an unpaired terminal unit of the segmental series.

The Distribution of Cranial Neural Crest

The distribution of the cranial crest has been described by Stone (1935) in *Amblystoma (Ambystoma).* We can now relate cranial crest distribution in newt

to the positions of the mesodermal segments of the head and compare these findings with those of amniotes.

When descent of the cranial crest begins in a newt embryo, the neural tube is completely closed and there is a large expanse of neural tube, bare of mesoderm, to be traversed by neural crest cells before they encounter the segmented mesoderm (compare Fig. 2, stage 23, and Fig. 12, stage 24). It seems unlikely that the segmented head mesoderm of a newt has any direct role in establishing the initial pattern of crest descent. However, once the crest cells encounter the segmental mesoderm, they contact it with cell processes.

There is strong morphological evidence to suggest that the crest is guided by the topography of the segments and their interfaces. The major portions of the rostral shelf of crest move cranially over the second and first segments (Fig 18A). The preotic crest covers the caudal portion of segment 3, whereas the cranial portion of segment 3 appears for some time to exclude crest. This creates a gap between the rostral and the preotic crest populations. A similar gap occurs between these crest populations in reptiles and birds at their segment 5, a position slightly more caudal to that of newts. The postotic crest of a newt covers the caudal edge of segment 4, but crest is excluded from most of this segment (Fig. 18B). Thus in newts, the otic placode is underlain by mesoderm of the fourth segment and the preotic and postotic crest move around its perimeter. In birds and reptiles, the otic placode forms in a comparable location over segments 6 and 7 and is similarly ringed by the preotic and postotic crest cells. Therefore, the initial pattern of cranial neural crest migration in newts and other vertebrate groups is nearly identical. Apparently, the doubling of cranial segments in the amniotes has little impact on subsequent cranial crest distribution.

Finally, in a newt, as in other vertebrates, neural crest cells do not emerge from the roof of the neural tube farther cranial than the prosencephalic-mesencephalic border (Fig. 18C). The optic vesicles evaginate from the prosencephalon and eventually the optic stalks become invested by mesencephalic crest cells that have migrated cranially from the rostral shelf of crest. Therefore in newts, as in other vertebrates, most of the ocular crest is derived from the region of the mesencephalon.

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