Neurulation in *Xenopus laevis*. An analysis and model based upon light and electron microscopy

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It is a matter of general knowledge that neurulation, as it occurs in most chordate embryos, proceeds by longitudinal in-folding of the neural plate. Løvtrup (1965) ably described such morphogenetic movements as they occur in several neurulating amphibians. The mechanical causes of these movements are not clearly understood, however. In his review of the prominent theories of neurulation, Curtis (1967) points to their various inadequacies and concludes that 'possibly the solution of this problem is to search for contractile movements in the cells involved in neurulation' (p. 310).

The present paper seeks to identify the causal mechanisms of neurulation in the African clawed toad *Xenopus laevis*. The study was originally undertaken specifically to test Cloney's (1966) prediction that the presumed contractility of neural plate cells is associated with the morphological presence of fine cytoplasmic filaments which actually constitute the molecular agents of contraction and cellular shape-changes. A large part of that prediction was borne out when highly ordered arrays of 60 Å filaments were found in the constricted necks of neural plate cells of *Hyla* and *Xenopus* (Baker & Schroeder, 1967). The circumferential alignment of the filaments permitted, but did not prove, a 'purse string' mechanism of apical cell constriction based upon some form of filament-dependent contraction. If this model were correct, the aggregate effect of such constrictions could conceivably oblige the entire neural plate to in-fold by virtue of its ever-reduced apical surface area.

Clearly, apical contraction fails to explain all aspects of neurulation, regardless of its ultimate significance. Why, for example, does the neural plate fold only as seen in the transverse plane and not lengthwise as well? Moreover, how can one account for the various subtle sculptural details of the changing neural plate beyond mere in-folding?

As this study progressed, two separate problems emerged. It was recognized that there was wide morphological variability between various species of

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neurulating embryos; one problem, therefore, was to describe in detail what movements of tissues and cells characterize neurulation in a single given species. The second problem was to use these morphological data as clues to the causes of movement and thereby develop a comprehensive mechanical explanation of neurulation.

![Fig. 1. Sequence of developmental stages of *Xenopus laevis*. Drawings and stage designations (13–36) are taken from Nieuwkoop & Faber (1956). Views shown include: anterior (A), dorsal (D), lateral (L), and posterior-dorsal (P–d) views. All transverse sections used in this study were obtained from trunk levels (arrows). Scale lines = 1 mm.](image)

**MATERIALS AND METHODS**

Embryos of *Xenopus laevis* (African clawed toad) were obtained by artificially induced spawning (Gurdon, 1967). Developmental stages were determined by external criteria, as pictured and described by Nieuwkoop & Faber (1956). Embryonic stages employed in this study are illustrated in Fig. 1. Recognizing the high degree of regional variation in structure along the neural plate (see Nieuwkoop & Florschutz, 1950), observations reported here refer exclusively to mid-trunk regions, as indicated by arrows in Fig. 1.
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The fixation protocol was as previously described (Baker & Schroeder, 1967). During glutaraldehyde fixation, large wedges of yolky endoderm were removed with fragments of razor blades as an aid to infiltration and subsequent orientation. Thick sections (\(\frac{1}{4}-1 \mu\)) were stained according to Richardson, Jarret & Finke (1960) and photomicrographed. Thin sections were double stained with lead and uranyl solutions. Electron micrographs were made with a RCA EMU-3G electron microscope.

Fig. 2. Diagrams based upon low magnification light micrographs of transverse sections at levels indicated by arrows in Fig. 1. Photomicrographs of the same sections are shown in Figs. 3-9. Stippling: ectoderm (finest), notochord (fine), somitic mesoderm (medium), endoderm (coarse). df = dorsal fin; ic = intraectodermal cleft; m = myocoelic space. Scale = 100 \(\mu\).

OBSERVATIONS

General features

Nearly 100 embedded embryos were examined, 34 in particular detail. They spanned stages 13 (late gastrula) to 36 (hatching) and were sectioned in various precisely determined planes. Only transverse sections through the trunk neural plate (spinal cord region) are described here. Fig. 2 illustrates the general topography of dorsal tissues at key developmental stages.

Neural in-folding begins at stage 13 and is completed at the time of neural closure, or stage 20. Thereafter, the definitive neural tube detaches from epidermis; complex migrations of neural crest commence, and an epidermal dorsal fin makes its appearance (Fig. 2, df).

Figs. 3-9 are light micrographs of neural plates and adjacent tissues of Xenopus embryos. Familiar landmarks such as notochord, somites, ectoderm and endoderm are clearly demarcated. They are generally separated by patent spaces (Figs. 3-9, s). The reproducible presence of these spaces argues against their being artifacts of preservation. They probably constitute blastocoelic remnants and presumptive mesenchymal compartments. Two other conspicuous
spaces are (1) myocoelic slits, which are transitory within myotomes (Figs. 2, 4, m), and (2) separations between the two layers of ectoderm, here called 'intraectodermal clefts' (Figs. 2, 3, ic). These spaces also occur reproducibly at early stages in the ectoderm covering the neural folds.

Ectoderm in Xenopus is manifestly bilayered (composed of two distinct cell layers) from blastula stages to hatching; this generalization applies to all ectoderm except some regions of the neural tube which become unilayered after stage 20. Where identifiable, the two layers may be called 'superficial ectoderm' (the apical layer) and 'deep ectoderm' (basal layer), after the usage of Nieuwkoop & Florschutz (1950).

Table 1. Topographical zones of the dorsal ectoderm

<table>
<thead>
<tr>
<th>Topographical zone</th>
<th>Abbreviation</th>
<th>Fate in tadpole</th>
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<tbody>
<tr>
<td>Superficial ectoderm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>MS</td>
<td>Parts of floor plate and lateral walls</td>
</tr>
<tr>
<td>Intermediate</td>
<td>IS</td>
<td>Roof plate</td>
</tr>
<tr>
<td>Lateral</td>
<td>LS</td>
<td>Superficial layer of epidermis</td>
</tr>
<tr>
<td>Deep ectoderm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>MD</td>
<td>Part of floor plate</td>
</tr>
<tr>
<td>Intermediate</td>
<td>ID</td>
<td>Part of lateral walls</td>
</tr>
<tr>
<td>Intermedio-lateral</td>
<td>ILD</td>
<td>Neural crest</td>
</tr>
<tr>
<td>Lateral</td>
<td>LD</td>
<td>Deep layer of epidermis</td>
</tr>
</tbody>
</table>

The ectoderm by itself is highly heterogeneous with respect to constituent cell morphology (see Figs. 3-9), so, for the purposes of precise analysis, the ectoderm has been 'dissected' into seven topographical zones, five of which are paired. Zonal boundaries are drawn in Figs. 3-9, not on purely arbitrary grounds, but because the neural plate indeed appears to be composed of several distinct and internally homogeneous populations of cells, as determined by close and careful inspection of cross-sections. The names of the zones are based upon topographical considerations alone (see Table 1). The method of delineating exact borders for some zones (see Figs. 3-9) depends upon unmistakable morphological homogeneity among the constituent cells. However, in other cases, some zones are defined by exclusion from other zones, as determined by cell morphology and/or known fate later in development.

In other words, individual ectodermal zones in Xenopus represent results of analysis as well as the bases for further analysis into the nature of embryonic organization during neurulation. Both of these aspects are described below for each zone, and adjacent mesodermal structures are likewise considered.
Fig. 3. Light micrograph (above) and diagram (below) of a cross-section of stage 13 embryo showing cell outlines in the superficial and deep layers of the neural plate (medium and fine stippling, respectively) and underlying mesodermal structures (coarse stippling). Notice the patent intraectodermal cleft (ic) and remnants of blastocoelic spaces (s). bc = bottle cell; l = lipid droplets; n = nucleus; y = yolk granule; vm = vitelline membrane. For zonal abbreviations see Table 1. Scale = 10 μ.
Fig. 4. Light micrograph and diagram of stage 16 embryo. Note the dramatic changes in cell shape since stage 13 (Fig. 3). $m = $ myocoel; $nf = $ neural fold; $ng = $ neural groove; $s = $ space. Scale = 10 μ.
Fig. 5. Light micrograph and diagram of late neural groove stage embryo (stage \(18^\frac{1}{2}\)). Bottle cells are maximally deformed. \(m\) = myocoel; \(ng\) = neural groove; \(s\) = space. Scale = 10 \(\mu\).
Fig. 6. Light micrograph and diagram of the recently closed neural tube of a stage 20 embryo. Note the radical change in shape of the myotome cells since Fig. 5. nc = neurocoel; ps = pseudopodia of epidermal cell at point of suturing between neural folds; s = mesenchymal space. Arrow = point at which bilateral epidermal (LS) zones meet. Scale = 10 μ.
Fig. 7. Light micrograph and diagram of early neural tube (stage 22). Note that the floor of the neural plate (MS + MD) is unilayered unlike earlier stages. Neural crest is indicated by cross-hatching. c = ciliated epidermal cells; nc = neurocoel; s = mesenchymal space. Scale = 10 μ.
**Median superficial (MS) zone**

The median superficial (MS) zone sustains most of the longitudinal in-folding which characterizes neurulation in *Xenopus*. This is evident by stage 16 when the MS zone clearly forms the floor of the neural groove (Fig. 4, ng). The

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**Fig. 8.** Light micrograph and diagram of cross-section of stage 26 embryo. The neurocoel (nc) is reduced from the previous stage. The developing marginal zone (mz) consists of discrete patches of longitudinal axons, as verified by electron microscopy. c = ciliated epidermal cell; df = dorsal fin of epidermis; s = mesenchymal space; v = intracellular vacuole of notochord cell. Scale = 10 μ. 
Fig. 9. Light micrograph and diagram of cross-section of recently hatched embryo (stage 36). Area of neural tube is reduced, but marginal zones (mz) have enlarged since previous stages. Cross-hatching indicates neural crest cells. $c =$ ciliated epidermal cells; $s =$ mesenchymal space; $v =$ vacuoles of notochord cells. Scale = 10 $\mu$. 
identifying criterion for MS cells, by light microscopy, is their shape. Between stages 15 and 18½, MS cells are deformed as bottle-shaped cells whose narrow necks border upon the lumen of the neural groove. The term ‘bottle cell’ was coined by Ruffini (1907) to describe blastoporal cells and has been since applied to neural plate cells of similar shape. ‘Flask cells’, ‘wedge-shaped cells’ and ‘cuneate cells’ are synonyms which occasionally appear in the literature.

Bottle cells of the MS zone are maximally deformed at stage 18½ (Fig. 5). Their immediate neighbors within the superficial ectoderm—cells forming the lateral walls of the neural groove—are quite squamous at that time. This fact permits accurate demarcation of the MS zone. Also, the lateral boundaries of MS zones coincide precisely with geometrical inflection points in the curvature of the open neural groove (Figs. 4, 5). Like other zones to be described here, the MS zone extends longitudinally in the embryo, although its longitudinal variations will not be considered here.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Shape</th>
<th>Luminal*/total cells</th>
<th>Height: width at apical pole: width at basal pole (μ)</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Cube</td>
<td>17+/17+</td>
<td>20:20:20</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>Truncated pyramid</td>
<td>15/17</td>
<td>23: 6: 8</td>
<td>4</td>
</tr>
<tr>
<td>18½</td>
<td>Tall bottle</td>
<td>8/16</td>
<td>40: 4:15</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>Short bottle</td>
<td>10/17</td>
<td>28: 5: 8</td>
<td>6</td>
</tr>
</tbody>
</table>

* Luminal cells are those which actually contribute to the luminal surface of the neural groove in a given cross-section.

Table 2. Shape changes in cells of the MS zone

In Fig. 4 (stage 16) the MS zone is 15 bottle cells in width. By stage 18½ (Fig. 5 is representative) there are only six to ten such cells whose apices contribute directly to the luminal surface of the MS zone in a single cross-section (see Table 2). Isolated basal portions of additional cells commonly appear at this stage, suggesting that many cells are either tilted or distorted relative to the plane of sectioning; this appears to be a normal occurrence during stages of acute neural in-folding. Basal poles of such cells are included in the ‘total cell’ column of Table 2. Prior to stage 15 and again after stage 18½, the lateral borders of the MS zone are no longer indicated as points of discontinuity of cellular shape, so the identification of the MS cells at these times must be inferred from considerations beyond those of mere cell shape. Following are some of the inferential arguments for identifying MS cells before and after the time of their characteristic deformation.

Cells of the superficial ectoderm, including MS cells, are typically cuboidal in shape at stage 13 (Fig. 3). Nevertheless, a few cells in the midline of the superficial ectoderm are indeed bottle-shaped by this stage. They indicate that shape changes in the MS zone have just begun by stage 13.
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During neurulation, cells of the MS zone are dimensionally altered, as summarized in Table 2. The number of MS cells per cross-section which contact the apical or luminal surface decreases with the stage of development; therefore, by inference backward in developmental time, the MS zone at stage 13 is probably more than 17 cells wide; all the cells in the field of view of Fig. 3 (a total of about 20 cells) are incipient bottle cells of the yet uninvaginated MS zone. This inference has been supported by preliminary electron microscopic studies which identify presumptive bottle cells at stage 13 by their unique distribution of organelles (Schroeder, 1968a).

Between stages 18½ and 20, bottle cells of the MS zone have lost some of their taper; they are now wider apically and narrower basally. Basal poles of some adjacent MS cells are now seen to be spread apart by partial interdigitation or wedge-like intrusions from the median deep zone (Fig. 6, MD); earliest indications of this process can be traced back to stage 17½.

By stage 22 the floor plate (ventralmost neural tube ectoderm) is unquestionably unilayered (Fig. 7), whereas previously it had been bilayered (Fig. 6). It is possible to explain this change by assuming that the intrusions mentioned above actually culminate in the complete mutual interpénétration of median deep (MD) and median superficial (MS) zones. Individual identities of MS and MD zones are then lost (Fig. 7, MS + MD).

Table 3. Changes in thickness of the floor plate

<table>
<thead>
<tr>
<th>Stage</th>
<th>Thickness of floor plate (µ)</th>
<th>Number of layers</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>80</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15–17½</td>
<td>50</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>18½–20</td>
<td>40</td>
<td>2</td>
<td>5, 6</td>
</tr>
<tr>
<td>22–26</td>
<td>20</td>
<td>1</td>
<td>7, 8</td>
</tr>
<tr>
<td>36</td>
<td>10</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

After stage 22, the floor plate becomes continually thinner, as tabulated in Table 3. Cellular shapes in the floor plate range from pyramidal to columnar during this time, except at hatching (stage 36) when they are cuboidal and few in number.

Intercellular spaces in light micrographs give a clue as to the extent and nature of intercellular adhesion. Cells of the definitive MS zones (Figs. 3–6) are closely apposed throughout neurulation. Intercellular adhesion between bottle cells is, therefore, thought to be high, particularly at luminal or apical cell margins. The ultrastructural basis for such adhesion will be discussed presently. Along the lateral margins of MS cells, one occasionally sees narrow intercellular spaces.

Between MS zones and other nearby zones, intercellular adhesion varies with the specific case. Between median superficial (MS) and median deep (MD) zones, there are very few intercellular spaces at any time; therefore, a high level
of intercellular adhesion probably holds the two layers of the neural plate together at the midline. A different situation obtains farther laterad, however; the aforementioned intraectodermal clefts (Fig. 3, ic) indicate very low levels of adhesion between MS and intermediate deep (ID) zones in most early stages. Shortly thereafter, intraectodermal clefts disappear, and there seems to be increased contact between MS and ID zones (Figs. 4–9).

Within MS cells, whether they are deformed as bottle cells or not, one can easily identify nuclei, yolk platelets, lipid droplets, mitochondria and pigment granules using the light microscope (Figs. 3, 12, n, y, l and p). These organelles and inclusions occupy significant volumes of the cells but are not specific to stage or cell type.

**Ultrastructure of bottle cells**

Besides the ubiquitous subcellular structures mentioned above, bottle cells possess ultrastructural features which are distinct enough to set them apart from other cell types. Whereas some of the distinguishing structures are obvious in electron micrographs, others are rather subtle. For example, rough endoplasmic reticulum in MS cells is very sparse and undeveloped (Figs. 11, 14, er). Cisternae of this membranous organelle invariably occur singly rather than in stacks of several cisternae, as in other cell types mentioned below. Ribosomes are confined to small patches on the membranes.

Another negative distinction of bottle cells is their nearly total lack of Golgi complexes. When observed in cells of stages 13–20, Golgi complexes are usually small, indistinct and randomly located. Later, Golgi complexes may proliferate, for they are more frequently found in neuroepithelial cells of the hatching embryo, approximating the luminal surface. However, since one is not absolutely certain of the zonal origins of individual cells at this time, the observed Golgi complexes may occur primarily in non-MS cells of the neural tube.

**Apical filaments.** The most remarkable organelle exhibited exclusively by cells of the MS zone is a transient system of aligned apical filaments. The material composing the filamentous array first appears immediately beneath the apical cell membranes of cuboidal neural plate cells at stage 13 as a thin, occasionally discontinuous layer of dense cytoplasm from which organelles and particulate cellular elements of all types are excluded (Figs. 10, 11, dl). The dense layer is

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**Fig. 10.** Electron micrograph of cross-section through apical ends of three neural plate cells at stage 13 before in-folding has begun. A thin layer of dense material (dl) appears discontinuously beneath the apical membranes, being thickest at cell margins (arrows). There are rather few apical vesicles (v) at this time. l = lipid droplet; p = pigment granule; ps = perivitelline space; y = yolk platelet. Scale = 1 μ. **Fig. 11.** Similar section through neural cells, as in Fig. 10 at higher magnification. The dense layer (dl) appears to be composed of fine fibrils or filaments 40–60 Å in diameter. er = rough endoplasmic reticulum; g = glycogen and/or ribonucleoprotein particles; pv = prevesicular membranes. Scale = 0.1 μ.
about 0·1 µ in thickness in most areas. At the margin of a cell, the dense layer is often 0·25 µ in thickness. In favorable cross-sections (probably tangential to a cell margin), its composition appears only vaguely fibrillar or filamentous at an early stage (Fig. 11, dl). Fibrils of the dense layer, although difficult to measure, are about 40–60 Å in diameter and are oriented parallel to the apical surface.

As bottle cells form in the MS zone, the dense layer thickens progressively, accumulates or condenses at the cell margin, and exhibits greater fibrillar organization. It is soon prominent enough to be seen even in light micrographs (Fig. 12, dl). Without a single exception in this study, the dense layer was confined to cells lining the floor of the neural groove; that is, the dense layer or its constituent filaments may be considered as diagnostic of cells comprising the median superficial (MS) zone.

Immediately adjacent to the bottle cells, as in Fig. 5, are the completely undeformed cells of the intermediate superficial zones of ectoderm. Even as immediate neighbors, they possess neither a dense layer nor its ultrastructural manifestation of apical filaments (Fig. 19). Such cells will be described in greater detail later in this paper.

The substructure of the dense layer was the specific subject of an earlier paper (Baker & Schroeder, 1967). It was there shown to be composed of thin cytofilaments 40–60 Å in diameter aligned in skeins circumferentially around the apical necks of *Xenopus* bottle cells. Due to their precise orientation these cytofilaments are ordinarily seen end-on in cross-sections (Fig. 13, dl); as expected, they predominate near peripheral cell margins rather than in the center of a cell. Some filaments appear to enter and perhaps terminate upon the lateral cell membrane in subapical junctional regions of increased electron density (Fig. 13, ij). These regions resemble intermediate junctions characteristic of adult epithelial junctional complexes into which terminal webs are thought to attach (Farquhar & Palade, 1963).

*Cell junctions.* The fine structure of apical junctional regions provides evidence for strong intercellular adhesion between MS cells. As mentioned
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above, closely apposed (~300 Å) membranes recall intermediate junctions by virtue of the associated amorphous dense material (Fig. 13, ij). Desmosomes also occur, usually above the level of the filamentous dense layer, as shown by Baker & Schroeder (1967). Tight junctions have not been observed. Away from apico-lateral contact regions, adjacent cell membranes are closely apposed, but specific adhesion devices are not in evidence.

Disappearance of apical filaments. By stage 20 the neural groove has been roofed-over and MS cells have become wider apically (Table 2). Coincidently, the apical dense layer is no longer observable and all traces of apical filaments are gone. Pigment granules are now found in the most apical regions of ependymal cells indicating that the granule-excluding capacity of the dense layer has also disappeared.

From these observations, one may conclude that the presence of the dense layer and its component filaments coincides both in time with neural in-folding and in space with the specific zone of cells sustaining the clear majority of observed apical contraction during neurulation. The proposed functional significance of this correlation will be discussed later.

Apical vesicles. Coincident with the appearance of the filamentous apical dense layer in bottle cells is the appearance of numerous membrane-bound vesicles. They are present in greatest numbers when cells of the MS zone are maximally deformed. Such apical vesicles are roughly spherical, measure about 0.25 μ in diameter and their contents are electron lucent, in contrast to the contents of the perivitelline space nearby (Fig. 13, v). Apical vesicles ordinarily occur in a discrete zone beneath the dense layer of bottle cells (see fig. 7, Baker & Schroeder, 1967). Fully spherical vesicles are characteristic of MS cells from stages 15 to 18½.

At stage 13 apical vesicles occur rather infrequently (Fig. 10, v). However, at this stage sac-like structures of flattened or biconcave membranous elements are dispersed above and within the felt-like material of the forming dense layer (Fig. 11, pv). Such structures appear to represent precursors of apical vesicles and therefore are labeled ‘prevesicular membranes’. They are of unknown origin; expected continuities between prevesicular membranes and other cell...

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Fig. 14. Basal end of a bottle cell (neural ectoderm) at stage 17½ (see the boxed area in Fig. 12). Note the numerous microtubules (m) running parallel to the apico-basal cell axis. er = rough endoplasmic reticulum; es = extracellular space; l = lipid; p = pigment granule; y = yolk. Scale = 0.5 μ

Fig. 15. Apical surface of an epidermal cell at stage 13 characteristically displaying apical pigment granules (p), tonofilament bundles (tf), stacks of rough endoplasmic reticulum (er), and prominent Golgi complexes (g). Compare this cell with neural cells in Fig. 10. Scale = 1 μ.

Fig. 16. Similar cell as in Fig. 15 showing one of the infrequent epidermal vesicles (ev) of this early stage. Scale = 1 μ.

Fig. 17. Higher magnification of tonofilaments (tf) of Fig. 15. Scale = 0.1 μ.
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membranes have not been observed. As in Fig. 11, the prevesicular membranous sacs occasionally appear to be in the process of filling with some electron-lucent material. That condition may represent an intermediate stage in the formation of fully spherical apical vesicles.

Following neurulation, apical vesicles are retained by former bottle cells for a short time. At stage 20 apical vesicles have lost their spherical shape, however, and are distributed randomly within the cytoplasm. Shortly thereafter, apical vesicles may no longer be identified, either in ependymal cells or elsewhere.

Microtubules. Cytoplasmic microtubules are found in most MS cells during the stages of maximal deformation. As in many other anisometric cells (Porter, 1966), microtubules in neural plate cells are oriented predominantly parallel to the axis of greatest dimension. In later neural groove stages, there are an estimated 150 parallel microtubules per cell (Schroeder, 1968a). They course from just below the apical dense layer to the very base of the cell (Fig. 14, m). Close associations between microtubules and other cell structures were not observed.

When the neural plate is still flat (i.e. stage 13), cytoplasmic microtubules are less frequently encountered. The few microtubules one can find are invariably in the most medial cells—the first cells to become deformed, as in Fig. 3.

Lateral superficial (LS) zones

Light microscopy

The definitive epidermis of a hatching tadpole is a bilayered epithelium (Fig. 9). Outer and inner layers of epidermis are derived from superficial (LS) and deep (LD) ectoderm, respectively, of earlier stages (Figs. 4–8). Presumptive epidermis in a gastrula is situated laterad of, but confluent with, ectoderm which will form the neural tube; by definition, it represents those areas of ectoderm whose fate is to remain on the embryo’s exterior through neurulation.

Neural folds are covered by cells of the LS zones (Fig. 4, nf). Progressive elevation of neural folds, relative to the floor of the neural groove, is simultaneously associated with a mediad migration of LS zones (compare Figs. 4, 5). By stage 20 this migration had resulted in contact between bilateral LS zones at the dorsal midline of the embryo (Fig. 6, arrow). Fusion of neural folds is

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Fig. 18. Superficial cells of the epidermis at stage 26 (late embryo). Epidermal vesicles (ev) are now very abundant. The outer surface of the cell is underlain with a finely fibrillar dense zone (fz) which excludes most organelles, with the exception of epidermal vesicles. d = desmosome; er = rough endoplasmic reticulum; g = Golgi complexes; n = nucleus. Scale = 1 μ.

Fig. 19. An intermediate superficial (IS) cell at the crest of a neural fold at stage 18. Note how cellular contents resemble those of epidermal cells except for the absence of epidermal vesicles. Such a cell forms the roof plate of the neural tube. er = rough endoplasmic reticulum; g = Golgi complex; n = nucleus; p = pigment granules; tf = tonofilaments. Scale = 1 μ.
perhaps facilitated by pseudopodial activity which has been observed in LS cells at this point of fusion (Fig. 6, ps).

By stage 22 a few LS cells have developed cilia (Figs. 7–9, c). Also by this stage a bilayered epidermal dorsal ridge begins to appear. From this ridge a dorsal fin gradually emerges (Fig. 8, df); it extends as much as 0.3 mm above the neural tube by stage 36 (Fig. 2, df). The presence and growth of the dorsal fin seem to be under the exclusive control of superficial (LS) and deep (LD) epidermis of which it is composed.

Ultrastructure of superficial epidermis

Membranous components of the synthetic apparatus are far more abundant in epidermal cells than in the neural cells already described. Rough endoplasmic reticulum and Golgi complexes are plentiful in cells of LS zones (Fig. 18, er and g). The former ordinarily occurs in stacks of two to five tortuous cisternae which are rather densely studded with ribosomes, in contrast to the endoplasmic reticulum in MS cells.

Epidermal vesicles. The Golgi complexes and endoplasmic reticulum both appear to be involved in the production of ‘epidermal vesicles’ which comprise a class of bodies restricted in distribution to presumptive and definitive epidermal surface cells. They occur nowhere else in the Xenopus embryo.

Epidermal vesicles are found in superficial ectoderm (LS) cells at all stages examined. They are spherical membrane-bound bodies approximately the size of mitochondria. They were first described by Eakin & Lehmann (1957) as ‘secretory vesicles’. Each epidermal vesicle encloses a flocculum of intermediate electron density (Figs. 16, 18, ev).

Epidermal vesicles are found only in cells of the LS zones, according to the zonal designations of Figs. 3–9. This fact was carefully verified by electron microscopy. They are found in early stages (Fig. 16) before epidermal and neural ectoderm may be distinguished by cell shape alone.

In definitive epidermis the free surface is filled with epidermal vesicles (Fig. 18), except in the small number of ciliated cells where they are excluded from the actual surface. Even after hatching, however, epidermal vesicles do not seem to be everted to the exterior through the apical plasma membrane. Their biological function, therefore, remains obscure, for it is not unequivocally clear that they are secretory. Regardless of their function, epidermal vesicles are valuable morphological aids in distinguishing epidermal cells from other cells. Such a distinction has been useful for determining boundaries for certain topographical zones.

Tonofilaments and cell junctions. From stages 13 to 18½, superficial epidermal cells display tight fascicles of ~80 Å tonofilaments which course like ropes through the subapical cytoplasm (Fig. 17, tf). A bundle of tonofilaments may be traced for several microns into the interior of a cell, always at a distance of about a micron from the apical surface. Fascicles of tonofilaments can be
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followed directly into desmosomes which are also situated about a micron from the surface. Tonofilaments are never observed in the subnuclear cytoplasm.

From stage 20 onwards, tonofilaments become less and less prominent in LS cells, and by stage 26 they are apparently no longer present, although desmosomes persist as the most obvious junctional devices from early stages right through to hatching (Fig. 18, d). In place of tonofilaments in non-ciliated cells at such late stages, one finds a diffuse apical zone of finely fibrillar material intermingled with cytoplasmic particles and pigment granules (Fig. 18, f2). Larger organelles are excluded from this zone, except for epidermal vesicles (Fig. 18, ev). This zone is about one micron in thickness; its constituent fibrils are extremely thin, short, randomly oriented and lightly staining, and its function is unknown.

Intermediate superficial (IS) zone

A logical and morphological necessity

In the course of this study, it became apparent that bottle-shaped cells were not the only superficial ectodermal cells to be included in the definitive neural tube. A small number of squamous superficial cells, bilaterally distributed just lateral to the MS zone of bottle cells, is also incorporated into the neural tube, as will be shown. In the light microscope these squamous cells are indistinguishable from epidermal cells; but, by definition, they cannot belong to the epidermis and the neural tube. Therefore, it was recognized that these few squamous cells necessarily constitute bilateral zones of their own. These new zones are designated intermediate (IS) zones (Figs. 4, 5) and are interposed between the MS zone (of bottle-shaped neural cells) and LS zones (of squamous epidermal cells).

The basis for establishing IS zones may be seen by interpolating the developmental events which must occur between stages 18½ (Fig. 5) and 20 (Fig. 6). It is thereby apparent that apposition and initial fusion of the neural folds involves neither bottle-shaped cells of the MS zone nor LS cells, whose fate is to remain strictly exterior. Hence, it is logically necessary to postulate the existence of non-epidermal, neural ectoderm zones located near the crests of the neural folds. The constituent cells are squamous, not bottle-shaped. These criteria define the IS zones.

Morphology of IS cells

One may be absolutely assured of observing IS cells by limiting one’s attention to the one or two cells immediately laterad of the lateralmost bottle cell underlying a well-formed neural groove, as in Fig. 5. Such IS cells never possess the elaborate apical specialization or extreme anisometry of bottle-shaped neural cells (Fig. 19). They are entirely devoid of apical protrusions, filamentous apical dense layer, apical zone of exclusion and parallel microtubules, and in general they have no apical vesicles. Occasionally a few apical vesicles are seen near the contact region adjacent to a bottle cell.
Before neural closure, intermediate superficial (IS) cells resemble epidermal cells in shape (Fig. 5), though, of course, not in fate. Like cells of the epidermis, however, cells of IS zones possess bundles of tonofilaments, frequent Golgi complexes, stacked cisternae of rough endoplasmic reticulum, and apical pigment granules (Fig. 19) at a time when bottle cells have none of these features. For all their likenesses, IS and LS cells are clearly dissimilar by one major ultrastructural criterion: epidermal vesicles are never observed in intermediate superficial cells. Therefore, IS cells are truly intermediate between superficial epidermis and neuroectoderm in terms of both topography and morphology.

The neural groove is roofed-over by median fusion of the bilateral IS zones (Fig. 6, broken line). The roof plate thus formed is the dorsalmost wedge of neural tube tissue. Later, disengagement of epidermis from the neural tube separates IS from LS zones (stage 22, Fig. 7). Neural crest cells from ILD zones (see below) then fill in the intervening spaces (Figs. 6, 7). Cells of the thin roof plate (10–15 μ) in embryos beyond stage 24 extend long cell processes ventrolaterally along the outside of the neural tube. Such processes may be neuronal in function, in which case the roof plate cells may indeed be sensory Rohon-Beard cells as postulated by Hughes (1957). Such roof plate cells are considered to be derived from IS zones.

**Median deep (MD) zone**

Neurulation movements pivot about an inconspicuous longitudinal column of deep ectodermal cells comprising the median deep (MD) zone. These cells look and behave differently from adjacent cells of the deep ectoderm. The MD zone is distinguished by the intimacy of its contacts with the MS zone (dorsad) and the notochord (ventrad) (Figs. 3–6). Although intercellular spaces between these tissues are minimal, no definite attachment devices have been observed. At an early stage, MD cells demonstrate little in the way of polarity or definable shape (Fig. 3). Formal organization develops slowly up to stage 20, whereupon cells of the MD zone appear distinctly triangular in cross-section (Fig. 6). There are a few microtubules in these cells. Subsequent to stage 20, the MD zone ceases to exist as an integral zone as a result of its mutual interpenetration with the median superficial (MS) zone, as previously described. Cells of the MD zone apparently contribute to the unilayered floor plate of the definitive neural tube. The notochord remains in very intimate contact throughout this period (Figs. 7–9).

**Intermediate deep (ID) zones**

Already by stage 13, cells of the intermediate deep (ID) zones are characteristically columnar in shape (Fig. 3). These tall cells are situated directly beneath the barely elevated neural folds in this early stage. In cross-sections of various stage 13 embryos, ID cells measure 10–20 μ wide and 25–40 μ tall. By stage 16 and thereafter the ID zones appear as regular palisades of columnar cells. They possess some microtubules oriented parallel to their length. Each
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fully palisaded ID zone is about 100 μ wide and consists of 10–15 cells in a given transverse plane.

During neurulation each ID zone tilts dorso-mediad, like a pair of wings pivoting about the median deep (MD) zone. By stage 18½, ID zones subtend angles of about 30° with the frontal plane (Fig. 5). Also by this stage the process of mutual interpénétration of layers is under way, which tends to mingle ID and MS cells. It is not until stage 22 (Fig. 7), however, that the distinction between ID and MS cells becomes impossible due to fusion of the zones. Lateral walls of the neural tube thence gradually lose their clear-cut, bilayered stratification in favor of a pseudostratified epithelial organization (Figs. 7–9). Some of the cells span the entire thickness of the wall of the neural tube. Unlike the process of thinning in the floor plate (Table 3), however, the actual thickness of the lateral walls does not appreciably decrease. They are 40–50 μ thick right up to the time of hatching.

As early as stage 26, ‘marginal zones’ or ‘marginal layers’ (Arey, 1965) have begun to form. These are comprised of bundles of axonal processes along the distal edges of the lateral walls (Figs. 8, 9, mz). Neural tube cells which contribute axonal processes to these bundles possess rather numerous microtubules aligned in the direction of growth. Axons themselves contain many neurotubules.

Intermedio-lateral deep (ILD) zones

The neural crest can be positively identified in thick sections of embryos from stage 26 onward (Figs. 8, 9). At these late embryonic stages, the neural crest consists of a small number of cells in the following locations: (a) between epidermis and roof plate of the neural tube, (b) between myotomes and the epidermis, (c) between myotomes and the neural tube and (d) between myotomes and the notochord.

Proceeding backwards in developmental time, Fig. 7 shows neural crest cells in locations (a) and (b). Their absence from locations (c) and (d) by stage 22 is perhaps explained by the neural crest not having begun to migrate down the lateral walls of the neural tube.

At first glance the exact location of the neural crest might seem impossible to determine before stage 22. However, by examining late neural groove stages (Fig. 5; omitting stage 20 for the moment), one sees several cells beyond the edges of the deep layer of the neural plate that share features with identifiable neural crest cells later on. That is, the cells are rather spherical in shape or pleiomorphic, unlike the nearby columnar cells of ID zones. Moreover, the many extracellular spaces around each cell suggest that they are only tenuously attached to each other. In electron micrographs these cells display many single cisternae of densely studded endoplasmic reticulum as well as microtubules located in their extended cell process. These cells at stage 18½, therefore, have been identified as intermedio-lateral (ILD) cells or presumptive neural crest (Fig. 5).
Since there are few intercellular spaces in the stage 20 embryo of Fig. 6, it is more difficult than usual to specify the location of neural crest cells there. Nevertheless, by knowing where they are before and after this stage, the ILD zones can be reasonably identified as shown (Fig. 6, ILD).

At stages earlier than 18½, the positions of ILD zones are similarly inferred. If they are indeed as drawn in Fig. 5, then ILD zones must reside approximately as shown in Fig. 4, for it is unlikely that their topographical relations to other parts of the neural plate change very drastically in the brief intervening time. At stage 13 (Fig. 3) the ILD zones are of course excluded from the field of view along with other lateral ectodermal tissues. The locations of presumptive neural crest at such very early stages is not known, except to say that the neural crest resides in the deep layer of the ectoderm.

After the neural tube disengages from the epidermis, the neural crest is exhibited as two distinct populations of cells. A median aggregate appears coincidently with disengagement and remains closely associated with the roof plate at the neural tube. This presumably derives from fused ILD zones (Figs. 7–9). The other population is comprised of individual migratory cells which squeeze between tissue layers in a diffuse exodus away from the neural tube toward more ventral regions of the embryo.

The median dorsal population of neural crest cells composes a column consisting of about six cells in cross-sections (Figs. 7–9). They give rise to tendril-like cellular processes (Figs. 8, 9) which project toward myotomes and lateral walls of the neural tube. Hughes (1957) has referred to these cells as 'extra-medullary cells'.

**Lateral deep (LD) zones**

Beyond or laterad of prospective neural crest zones at early embryonic stages are components of deep ectoderm which later contribute to the epidermis. These components exist as the paired lateral deep (LD) zones, small portions of which are seen in Figs. 5, 6.

Median edges of LD zones apparently move mediad during neurulation until stage 22, when they can progress no farther, having met at the midline (Fig. 7). Thereafter they move with the superficial epidermis in forming the dorsal fin.

The superficial and deep layers of epidermis remain in very close contact throughout development. From stage 22 to 26, which coincides with the formation of the dorsal epidermal fin, cells of these two layers touch at numerous narrow points separated by gaps of intercellular spaces (Figs. 7, 8). By hatching, the two epidermal layers display broad areas of contact and little in the way of intervening spaces (Fig. 9). Specific attachment devices were not observed in electron micrographs.

**Myotomes**

Because they are dorsal tissues intimately appressed against the neural plate, the myotomes must be considered here for their possible role in neurulation.
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According to Nieuwkoop & Faber (1956), longitudinal somite segmentation is initiated by stage 17. Prior to this stage presumptive somite mesoderm pinches itself off from lateral mesoderm and forms rod-like columns parallel to and immediately beside the notochord (see Fig. 2). These are columns of myotome mesoderm.

A myocoelic slit separates a thicker inner sheet of splanchnic mesoderm from the thinner outer somatic mesoderm. The myocoel is most apparent between stages 16 and 18½ (Fig. 5). During this same period somites display their greatest dorso-ventral thickness, which is about 180 μ. As illustrated in Fig. 2, the somites extend as much as 50 μ above the frontal plane of the notochord at stage 18½. This fact is sufficient to implicate the somites in elevating the neural folds, probably by providing part of the force which tilts the bilateral palisades of intermediate deep (ID) zones in a dorso-mediad direction. The presence of blastocoelic spaces between the myotomes and the neural plate argues against the opposite mechanism, that the neural plate elevates the myotomes.

The thickened condition of the somites is due to the presence of tall columnar cells in the myotomes. They measure nearly 100 μ in length by about 10 μ in width (Figs. 4, 5). They possess numerous parallel microtubules (Schroeder, 1968 a).

Prior to their elongation, myotome cells appear as irregular polygons (Fig. 3). Likewise, at the end of neurulation they are again irregular in shape (Figs. 6–9). In cross-section they measure only about 15 μ in diameter after stage 20, although they are known from serial frontal sections to be undergoing longitudinal cell elongation as myoblasts (presumptive muscle cells). Myofibrils are first observable at stage 22.

Notochord

The notochord provides a structural axis for the growing embryo. Throughout development, it is closely abutted by the neural plate, myotomes, and roof of the gut (Fig. 2). The relationship between the neural plate and notochord is especially close (Figs. 3–9). It is an accepted fact that these two structures do adhere to each other when living embryos are dissected; however, the mechanism of adhesion was not elucidated by these light or electron microscopic studies.

A cross-sectional profile of the notochord at stage 13 is a trapezoid about 150 μ high by 100 μ in width at the base and 50 μ at the top (Fig. 2). By stage 15 the profile is more oblong (100 μ by 75 μ). Thereafter, the notochord appears round in cross-section, enlarging slightly as development proceeds.

There is a conspicuous lack of intercellular space within the notochord from stage 13 onward. Contact relations between constituent cells are very tight, but no specialized attachment devices such as desmosomes are present.

At stage 24, intracellular vacuoles first appear in notochord cells. These vacuoles progressively enlarge and contain a material which stains orthochromatically (Fig. 9, v).
DISCUSSION

Although there are several minor aspects of this study which deserve some discussion, my particular attention will be on the mechanism of neurulation as reflected in morphology. Neurulation is such a fundamental aspect of chordate development that, judging from the present study, it simultaneously involves the co-operation of diverse embryonic tissues. Therefore, it is highly improbable that such complex changes are accomplished by any single, univalent mechanism.

Whereas the progress of neurulation is macroscopically visible, one is curious to know what macromolecular mechanisms are responsible for it. Hence, a complete analysis of neurulation must demonstrate the dynamic interrelationships of many levels of morphological organization, from subcellular up to the tissue level.

In order to dissect the complex movements of neurulation into more easily understandable parts, the trunk neural plate of *Xenopus* has been divided into five topographical zones (three of them paired) on the basis of constituent cell shape, fate during development and fine structure. Each zone undergoes its own unique morphogenesis. The logical and biological *raison d'être* of each zone has been individually defended. It has also been demonstrated that morphogenesis of the neural plate results in part from, or at least is associated with, specific changes in the notochord, myotome mesoderm and epidermis.

![Diagram](image)

**Fig. 20.** Diagrammatic portrayal of five possible sources of mechanical force affecting the progress of neurulation. Autonomous forces acting within the neural plate include: apical contraction of the MS zone (empty double arrows) and cellular elongation of ID zones (empty circled arrows). Extrinsic forces include: elongation of myotome cells (solid double arrows), mediad convergence of epidermis (solid circled arrows), and the adhesive relationship between notochord and neural plate (JLT-L). See Discussion for descriptions of how these forces operate, as conceived in a model of neurulation.

Various kinds of zonal morphogenesis involve corresponding changes in tissue organization: thickening, thinning, in-folding, elongation, fusion and detachment. Not only do these parameters embody the probable immediate causes of neural folding and closure, but they are in their turn the results of specific cellular events, e.g. elongation, shortening, constriction, enlargement,
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adhesion, dissociation and cell migration. The bases for such cell activities are still largely unknown; but as these cell activities occur during neurulation, one may see correlations between cellular dynamics and subcellular function such as microtubular support, filament-dependent contraction, cell vacuolation, specific cell recognition, attachment devices and amoeboid movement. These processes most likely constitute the ultimate mechanisms of neurulation, as presently envisioned. This study, therefore, permits the enunciation of the following model.

Proposed model of neurulation (see Fig. 20)

1. Cells of the MS zone (see Table 1) in *Xenopus* effect their own deformation (cuboidal to bottle-shaped) by the combined efforts of lengthwise microtubules assisting cellular elongation and apical filaments which constrict the narrow cell necks by an intrinsic contractile capacity. Such cellular deformation initiates the formation of the neural groove, whose axial character is determined by the longitudinal disposition of the MS zone resulting from the antecedent events of primary induction.

2. Myotomes and ID zones contribute dorso-mediad components of force which help to elevate the neural folds. Cellular elongation is intrinsic to these tissues, being achieved by microtubular growth or support. Microtubules, therefore, provide an important force at the subcellular level which ultimately elevates the neural folds.

3. Epidermal ectoderm (LS and LD zones) migrates mediad by a mechanism akin to mass amoeboid movement which forcibly aids closure of the neural tube by pushing the neural folds together.

4. Longitudinal shortening of the neural plate during in-folding is actively opposed by the elongating tendency of the notochord, to which the neural plate is physically attached along its midline.

Defense of the model

*Autonomous in-folding of the neural plate.* Portions of neural plates, when cleanly excised from salamander embryos, have been maintained *in vitro* and observed to undergo in-folding movements without the aid of neighboring tissues (Giersberg, 1924; Boerema, 1929; Holtfreter, 1939; Burt, 1943; Horstadius & Sellman, 1946; Jacobson, 1962). Such experiments convincingly demonstrate the autonomous capacity of neural plates to infold upon themselves, at least when they are taken from salamanders. Unfortunately, comparable demonstrations do not exist for anuran neural plates, let alone for *Xenopus*. In the absence of experimental evidence, which is technically difficult to obtain from anuran embryos, I am obliged to rely on morphological evidence for a demonstration that in-folding is also autonomous in *Xenopus*.

Bottle cells of the *Xenopus* neural plate transiently display a high level of subcellular organization which has been interpreted as evidence for cellular
self-deformation. Their apical filaments, in particular, suggest an intrinsic capacity for apical contractility without which regular patterns of bottle cells, and therefore the production of a gently concave neural groove, would be impossible.

Apical contractility in *Xenopus* cells originally gained support by analogies to filament-dependent contractions in other systems, as previously indicated by Baker & Schroeder (1967). As a result of subsequent investigations, additional analogies may now be drawn. Contractility in cells based upon cytoplasmic filaments operating as ‘purse strings’ has been proposed for pancreatic bud cells (Wessells & Evans, 1968), marine eggs during first cleavage (Schroeder, 1968b, 1969; Szollosi, 1968; Arnold, 1969; Tilney & Marsland, 1969; Schroeder, 1969), developing lens cup cells (Wrenn & Wessells, 1969), and ampullar epidermal cells of ascidians (De Santo & Dudley, 1969).

In the neural plate, precise correlations, both temporal and spatial, between apical filaments and deformed bottle cells, as well as correlations between such bottle cells and neural in-folding, urge a causal interpretation. Because of increased evidence for contractility in non-muscular cells, as mentioned above, the argument that contractile filaments cause neural in-folding is more persuasive than its converse.

Changes in shape at the cellular level were long ago conceived as the cause of invagination in a variety of embryonic tissues (Ruffini, 1907; Glaser, 1916; Boerema, 1929). Lewis (1947) devised working models whereby co-ordinated apical contractions of cells resulted in concave in-folding. The present contribution to Lewis’s model merely draws attention to the probable subcellular basis for apical contractions as he envisioned them.

Although Waddington & Perry (1966) observed an apical dense layer in bottle cells of salamander neural plates, they observed no fibrillar structures in it and felt that it played no active role in neurulation. Rather, its amorphous appearance led them to conclude that the dense layer was ‘the result of a passive accumulation of superfluous cortical material’. In subsequent studies, I have since observed very similar apical dense layers in another salamander (*Ambystoma*); as in *Xenopus*, it is composed of fine filaments, and I believe it to be similarly contractile (Schroeder, 1968a).

To explain cell deformations associated with neural folding, Waddington & Perry (1966) appealed to the existence of numerous microtubules in bottle cells. It is difficult to conceive of microtubules causing apical constriction, but they probably are significant in other ways. Microtubules are now widely believed to provide mechanical forces effective in cellular shape-changes, especially cell elongation or extension (Byers & Porter, 1964; Porter, 1966; Gibbins, Tilney & Porter, 1969), and they may also confer structural rigidity to anisometric cells. Their presence in bottle cells may indicate either or both of these functions. An elongational role for microtubules would aid bottle cell deformation, at least in its elongational aspects (see Table 2 for the amount of elongation in
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these cells). Microtubular rigidity would also serve to maintain cell rigidity when external pressures would otherwise result in passive deformation.

I conclude that cytoplasmic filaments and microtubules work hand-in-hand to produce bottle-shaped cells, whose presence dictates the inward bending of the neural plate.

**Forces elevating the neural folds.** Formation of a neural groove simultaneously initiates neural folds as visible ridges, but active elevation of neural folds implies additional mechanisms. Whereas MS cells achieve neural in-folding, and their basal poles push up against the undersides of the squamous cells of the IS zones, the actual degree to which neural folding can be attributed directly to bottle cells is very modest. The greater part of elevation of the neural folds correlates better with changes in the myotomes than any changes in the neural plate proper.

The myotomes in *Xenopus* undergo marked thickening by cellular elongation. The points of maximum thickening occur directly beneath the elevated neural folds. This cannot be entirely fortuitous, for even in microscopic sections one can see that the effects of myotome thickening are not lost on the neural plate: the intervening ID zones are themselves thickened at the same time, as if to further transmit the dorso-mediad components of force from the myotomes to the neural folds.

The highly anisometric cells of both ID zones and myotomes contain numerous oriented microtubules during the time of neural folding. If the above arguments for the causal involvement of microtubules in cell elongation hold true, then one can claim that microtubules are part of the molecular machinery responsible for generating the forces of neural closure as I have proposed in the above model.

**Neural closure.** Mediad 'convergence' of epidermis in early amphibian embryos is now a demonstrated reality (Jacobson, 1962; Burnside & Jacobson, 1968). Giersberg (1924) previously illustrated by experiment that these movements are forceful. They may, therefore, be significant during neurulation by pushing upon the neural folds. The evidence for this, however, pertains primarily to urodeles, not to anurans like *Xenopus*.

In *Xenopus* there is but a single observation to be marshalled in favor of active epidermal 'convergence'. That an epidermal dorsal fin forms immediately after neural closure implicates the epidermis in mediad movements at least during that late period. Though the evidence is admittedly meager, it is possible that the same forces causing the epidermis to buckle into a dorsal fin also operate earlier as horizontal mediad forces pushing against the neural folds.

Unfortunately, no ultrastructural clues for the underlying mechanism of this mass movement of epidermis were found. Lentz & Trinkaus (1967) were likewise unable to rationalize similar epibolic movements in fish with any specialized cell ultrastructure.

In achieving actual closure of the neural folds, cell recognition between IS zones must certainly take place prior to fusion of the folds. As IS cells are
subsequently drawn ventrad with the neural tube, it is clearly necessary that LS
cells of the epidermis also recognize one another. Part of the recognition and/or
attachment behavior of these latter cells may be reflected in the pseudopodial
activity which was described. Such activity may further indicate the amoeboid
character of epidermal cells during this time of migration.

Elongation of the neural plate. It is a demonstrable fact that the neural plate
in *Xenopus* elongates during neurulation (see Fig. 1, or the various median
longitudinal sections portrayed by Nieuwkoop & Florschutz, 1950). Between
stages 13 and 20 the extent of elongation approaches 65% which is equivalent
to the amount of elongation observed in salamander embryos (Jacobson, 1962).

Microsurgical operations on *Xenopus* embryos are difficult to perform, and
the role of the notochord in axial elongation has not yet been elucidated by
experiment in this species. Such operations have been performed on salamanders,
however, and by analogy, the conclusions are believed to hold equally well for
*Xenopus*. Excised urodele notochords are capable of self-elongation *in vitro* by
a mechanism of cell enlargement involving the production of intracellular
vacuoles (Mookerjee, Deuchar & Waddington, 1953). Neural plates, on the
other hand, fail to elongate when underlying mesoderm is removed (Holtfreter,
1939; Jacobson, 1962): in fact, such neural plates shorten, the significance of
which will be shown below. In urodeles, therefore, adhesive interactions
between a self-elongating notochord and an overlying neural plate apparently
result in the elongation of both.

All features of neurulation in *Xenopus* are consistent with a mechanism of
elongation of the neural plate which originates in the notochord. The neural
plate is always in close apposition to the notochord, and functional tight
adhesion between them may be easily verified by trying to separate these tissues
surgically. Both layers of the neural plate are also in close contact (MS and MD
zones). Furthermore, intracellular vacuoles are seen in the notochord as
evidence of its self-elongating capacity.

According to Lewis (1947), in the absence of outside mechanical constraints,
apical cell constriction should result in cup-like invagination of the entire neural
plate. Since the notochord apparently resists longitudinal shortening—permit­
ting only a trough-like in-folding instead of a full invagination—one must
postulate that drastic rearrangement of bottle cells occurs in order that their
narrow apical poles be accommodated to the contours of the longitudinally
folded neural plate.

Because apical constriction in the frontal plane is nearly equal transversely
and longitudinally (Baker & Schroeder, 1967), many cells must be rearranged
longitudinally in order to maintain a given length of the neural plate, let alone
to allow it to elongate. This perhaps explains why the number of luminal bottle
cells seen in single cross-sections decreases as neurulation occurs (Table 2). The
unaccounted cells are merely lost to view in a given plane but presumably occur
in other planes of section. The rearrangement (or torsion) of bottle cells
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apparently involves only the apical poles, for their basal poles remain in the same plane; this explains why the 'total cell' count in Table 2 remains unchanged.

An excised neural plate shortens as it in-folds, as mentioned above. This important fact is best explained by, and gives credence to, the dual mechanisms of apical constriction and cellular rearrangement which occur in the normal neural plate. Only the former mechanism apparently operates after excision.

Without a notochord to which the neural plate can adhere, cellular rearrangement would be impossible, according to the model. Since the relative positions of neural plate cells would then be fixed, apical constrictions of bottle cells would produce equal contractions both transversely and longitudinally. Narrowing and shortening would then be the expected result, which in fact is what was observed. Since the contractions are restricted to the apex, the invagination would be cup-like rather than trough-like, as also occurs after the neural plate is excised.

In the cinematographic studies of Burnside & Jacobson (1968), no rearrangements of neural plate cells were reported. Upon examination, the stages and sectors of the neural plate which they studied were undergoing very little apical constriction, without which rearrangements are largely unnecessary and would not be expected. Later stages of embryos have not been studied in the same way, for technical reasons (M. B. Burnside, personal communication).

While rearrangement of MS cells is not a clearly demonstrated event, as a hypothesis it helps to explain some important aspects of neurulation in Xenopus. However, it also places some special burdens on intercellular adhesion devices. It is not known how strong adhesions between MS cells also permit relative movements and rearrangement.

SUMMARY

1. In this analysis of neurulation, embryos of Xenopus laevis (S. African clawed toad) are examined by light and electron microscopy at selected developmental stages. Particular attention is given to the region of the presumptive spinal cord.

2. For purposes of analysis, the two-layered ectoderm is subdivided into seven topographical zones, each of which is characterized by distinguishing histological or ultrastructural criteria. The fate of each zone during neurulation is followed. Of the seven zones, four belong to the neural plate proper at early stages, since they later contribute to the neural tube. One bilaterally paired zone gives rise to neural crest material. The remaining two zones comprise the epidermis.

3. An extensive model is presented whereby the movements of neurulation are explained by specific changes in cellular configuration within topographical zones. Each zone is unique in its proposed mechanical contribution to neurulation.
Initial in-folding of the neural plate results when cuboidal cells become deformed as bottle-shaped cells constituting an unpaired median zone, according to the model. ‘Contractile’ cytoplasmic filaments in the cell apices and microtubules aligned lengthwise within the cells are the suspected agents of the deformations.

Progressive deepening of the neural groove and elevation of the neural folds occur simultaneously. The model attributes these events to (a) persistent adhesions between the neural plate and notochord, (b) thickening at the edges of the neural plate associated with the presence of cytoplasmic microtubules, and (c) thickening of the myotomes, also linked to microtubules.

Neural closure is held to result from active convergence of the epidermis. In later stages the eventual elongation of the definitive neural tube is largely a product of notochordal elongation, which proceeds by intracellular vacuolation.

RÉSUMÉ

La Neurulation chez Xenopus laevis. Une analyse et un modèle basés sur la microscopie photonique et électronique

Dans la présente analyse de la neurulation, des embryons de Xenopus laevis (Amphibien, Anoure) sont examinés aux microscopes photonique et électronique à des stades choisis. Une attention particulière est portée à la région de la moelle épinière présomptive.

Pour les besoins de l’analyse, l’ectoderme à deux couches cellulaires est divisé en sept zones topographiques, chacune d’entre elles étant caractérisée par différents critères histologiques et ultrastructuraux. La destinée de chaque zone est suivie au cours de la neurulation. De ces sept zones, quatre appartiennent à la plaque médullaire des premiers stades puisqu’elles contribuent ultérieurement à la formation du tube neural. Une paire bilatérale de zones donne les crêtes neurales. Les deux dernières zones sont épidermiques.

Un modèle détaillé est présenté dans lequel les mouvements de neurulation s’expliquent par des changements de configuration cellulaire dans chaque zone topographique. Chacune de ces zones se singularise par la contribution mécanique à la neurulation qui leur a été proposée.

L’enfoncement initial de la plaque médullaire se manifeste quand des cellules cubiques se déforment en cellules à col de bouteille en constituant une zone impaire médiane d’après le modèle. Des filaments cytoplasmiques ‘contractiles’ à l’apex des cellules et des microtubules alignés dans le sens de la longueur de la cellule seraient les agents présumés de ces déformations.

Le creusement progressif du sillon neural et l’élévation des replis neuraux sont synchrones. Le modèle attribue ces événements à (a) des adhésions permanentes entre la plaque neurale et la choride dorsale, (b) des épaissements au niveau des bords de la plaque neurale associés à la présence de microtubules, (c) des épaissements des myotomes également liés aux microtubules.
6. La fermeture du névraxe est considérée comme le résultat d'une convergence active de l'épiderme. Dans les stades ultérieurs, l'élongation éventuelle du tube neural définitif est, dans une large mesure, due à l'élongation de la chorde, laquelle résulte de la vacuolisation intracellulaire.

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