J. Embryol. exp. Morph. **74**, 275–295 (1983)
Printed in Great Britain © *The Company of Biologists Limited (1983)*

Neurulation in the Mexican salamander (*Ambystoma mexicanum*): a drug study and cell shape analysis of the epidermis and the neural plate

By RUDOLF B. BRUN[1] AND JOHN A. GARSON
From the Texas Christian University, Biology Department

SUMMARY

We analysed the neurulation movements in the Mexican salamander *Ambystoma mexicanum*. Embryos were exposed to colchicine or nocodazole prior to neural fold formation. Exposure to these drugs prevented the anterior neural folds from closing. Neurulation however proceeded normally in the posterior regions of the embryo. We were unable to find apically constricted cells in the neural plate of colchicine-blocked neurulae. Only rounded-up neural plate cells were present (semithin sections). This situation was typical in embryos exposed to colchicine prior to neural fold formation. Concentrations of colchicine up to $2{\cdot}5 \times 10^{-3}$ were not capable of blocking neurulation once the neural folds were formed. The wedge-shaped cells were present in similar numbers to those found in controls. We quantified the cell shape changes in the neural plate and in the epidermis in both controls and drug-arrested embryos. The comparison of these to classes of data shows that epidermal spreading is prevented by colchicine but only slightly affected by nocodazole. Embryos blocked in late neurulation by exposure to these drugs can resume neurulation following neural plate excision in nocodazole but not in colchicine. We conclude from this observation that the epidermis contributes to raising and closing of the neural folds. The presence of neural folds in absence of wedge-shaped cells in the neural plate is also taken as evidence that neurulation is not exclusively driven by forces generated in or acting on the neural plate. Our view on the concerted interplay of various embryonic components is illustrated in a summarizing diagram (Fig. 11).

INTRODUCTION

Neurulation in vertebrates has been experimentally investigated since Goette (1874), His (1894), and Roux (1885). The central problem has been to understand the mechanisms responsible for transforming the neural plate into the neural tube. Experiments isolating the neural plate from the neurulating chick embryo demonstrated that the neural plate could form a neural tube without assistance from surrounding tissues (Roux, 1885). Glaser (1914) provided evidence that the neural plate of amphibia was also capable of folding itself autonomously. He emphasized the importance of cell shape changes in the neural plate cells for neural tube formation. Extensive work to elucidate the

[1] *Author's address:* Texas Christian University, Biology Department, Fort Worth, Texas, 76129, U.S.A.

nature and mechanisms of cell shape changes has been performed in numerous systems including the amphibian neural plate (for review see Burnside, 1973; Karfunkel, 1974). Significant advance in uncovering the mechanisms responsible for cellular constriction in the neural plate has been made (Baker & Schroeder, 1967; Burnside & Jacobson, 1968; Burnside, 1971, 1973; Karfunkel, 1971). Jacobson & Gordon (1976) have investigated whether contraction of the neural plate cells is sufficient to explain early neural fold formation. Using a computer model they found that in addition to the cellular constriction, lengthening of the neural plate was also necessary to produce the key-hole-shaped early neural plate. Recently Odell, Oster, Alberch & Burnside (1981) produced a computer model of neurulation in which sequential apical constriction of the neural plate cells is sufficient to transform the neural plate into the neural tube. However this model does not take into account that neurulation continues following excision of the neural plate (Hoerstadius & Sellman, 1946; C. O. Jacobson, 1962; Jacobson & Jacobson, 1973). The probable reason is that continuation of neurulation following neural plate excision has been frequently interpreted as a wound-healing phenomenon not related to shaping the neural tube (for a review see Karfunkel, 1974).

In order to answer the question whether neurulation movements are caused by the neural plate cells exclusively, the neural plate must be eliminated from participation in neurulation without excising it from the embryo. In this paper we report our observations on neurulation in embryos exposed to either nocodazole or colchicine. These drugs prevent the formation of wedge-shaped cells in the neural plate, while allowing the formation of the neural folds.

MATERIALS AND METHODS

Axolotl (*Ambystoma mexicanum*) embryos were raised and operated as indicated by Brun (1981). They were staged according to the normal table of Schreckenberg & Jacobson (1975). For semithin sections and EM analysis, embryos were demembranated and fixed for 2–3 h at room temperature. The fixative was of the following composition: 2·5 % glutaraldehyde in 0·1 M-phosphate buffer with 1 % sucrose at pH 7·4. Specimens were washed three times with the above mentioned buffer with glutaraldehyde and postfixed 1 h at 4 °C in buffered 1 % osmium tetroxide. Specimens were dehydrated with ethanol and embedded in Spurr's low-viscosity medium (Spurr, 1969). Semithin sections (0·5 μm approximately) were stained for 0·5–5 min with a solution of 0·73 g toluidin blue, and 0·27 g pararosaniline in 100 ml 30 % ethanol. Ultrasections were stained following standard procedures and observed on a Philips EM 300.

Cell shape analysis (epidermis)

This analysis was performed on (semithin) cross sections. Sections were prepared from two areas of the embryos (Fig. 1). The anterior area was

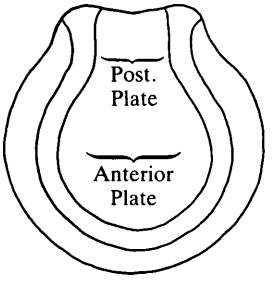


Fig. 1. Post. plate: region of the measurements performed in the posterior neural plate (folds separated by the shortest distance). Anterior plate: region of the measurements performed in the anterior neural plate (folds separated by the longest distance).

delineated by the broadest, the posterior area by the shortest distance between the neural folds. The sections were examined at 1000× using an American Optical light microscope, equipped for measuring with an ocular micrometer. We measured individual cell length at the surface of the superficial epidermal cell layer. The cell height was measured perpendicular to the cell length. The cell shape was expressed as length/height ratio. Sixty-four cells were measured in cross sections from each embryo as listed in Table 1: 32 cells (16 on the left and 16 on the right side of the embryo) in the anterior and 32 cells (16 on left and 16 on right side) in the posterior region. The superficial epidermis cell, in contact with the neural plate, provided the starting point for the measurements. The reliability of the measurements was tested in the following way: stages 14, 16 and 20 were measured first. Then stages 15 and 18 were also measured. As Table 1 shows, the interpolated stages provide interpolated values. Three colchicine- and three nocodazole-arrested neurulae were used for the epidermis cell measurements. The numbers listed in Table 1 are the average numbers for the six drug-arrested neurulae. Embryos used in the drug experiments were decapsulated prior to drug exposure. The vitelline membrane was left intact.

Measurements of cellular constriction in the neural plate

These measurements are based on the observation that the cell nuclei of an apically constricted cell population are displaced towards the cell bases as compared to the position of nuclei in non-apically constricted cells. The position of the nuclei in the zones of apical constriction (constriction zones) and in areas outside these zones are illustrated in Fig. 2. This figure is a camera-lucida drawing of a cross section through the anterior prospective brain (750×). The figure shows that the nuclei in the apically constricted cells are deeper in the neural plate than the nuclei of non-apically constricted cells. Only the first layer of nuclei beneath the surface of the neural plate was taken into consideration. The first layer of nuclei was determined in the following way: lines were drawn perpendicular to the surface line of the neural plate. The first nucleus crossed by

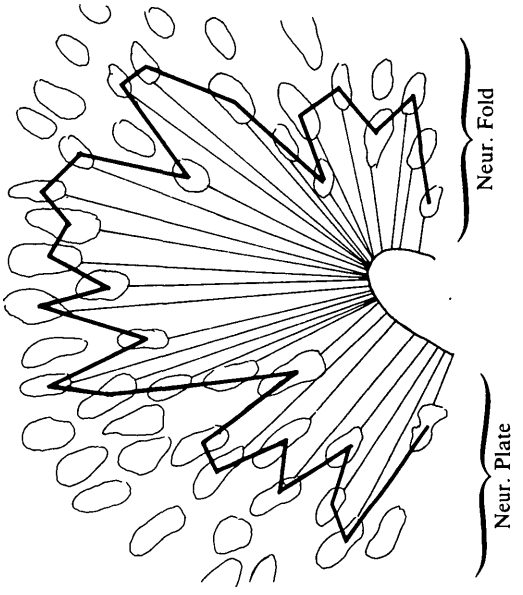


Fig. 2. Camera-lucida drawing of a cross section through the anterior neural plate (×750). The nuclei of constricted cells are deeper in the neural plate than the cell nuclei of non-apically constricted cells. The figure illustrates how the distance of the first layer of nuclei from the surface of the neural plate was determined (see text for details).

such a line was determined to be first nucleus beneath the surface of the plate. In Fig. 2, these nuclei are connected with each other by a line. In each cross section, the moving average was calculated from five nuclei in cross sections through the anterior neural plate. We started with the nuclei in position 1–5, position 1 being the neural plate cell establishing contact with the epidermis. The average of the added distances of these five nuclei provides the first data point for the dotted lines in Fig. 6. The second data point was obtained by calculating the average of the depth of the nuclei in positions 2–6, the next point was calculated by taking the average depth of the nuclei in position 3–7 etc. The rationale for calculating the moving average was to handle the problem of mitotic cells in the constriction zones. The chromosomes of such cells are located closer to the surface of the neural plate than the nuclei of the average non-mitotic cells in the constriction zones. These unrepresentative depths of mitotic cell nuclei are balanced out by calculating the moving average of the depths from all cell nuclei. To produce Fig. 7, the areas defined by the lines of the moving average and a line parallel to the x axes at the 50 μm mark of the y axes were integrated with a NUMONICS graphic calculator (Numonics Corporation, Lansdale, Pa.19446).

Counts of cells with long tonofilaments

Ultrasections from the same anterior areas as used to prepare the semithin sections were analysed at a magnification of 90 000×. The number of cells counted for each embryo is listed in Table 3. Tonofilament counts were made on the

EM screen at the magnification indicated above. Starting with the superficial epidermis cell closest to the neural fold, successively more lateral cells were screened for the presence of long (> 2·75 μm) tonofilaments. This was performed by moving the grid in such a way to always observe the outer surface of the superficial cells on the side of the screen. Only tonofilaments at least as long as the diameter of the screen were counted. At the magnification used, the screen equalled 2·75 μm. The calculations involved in obtaining the product-moment correlation coefficient (Table 4) were performed as outlined by Sokal and Rohlf (1969). Colchicine and nocodazole were purchased from SIGMA Chemical Company.

RESULTS

Analysis of the epidermis at consecutive stages of neurulation

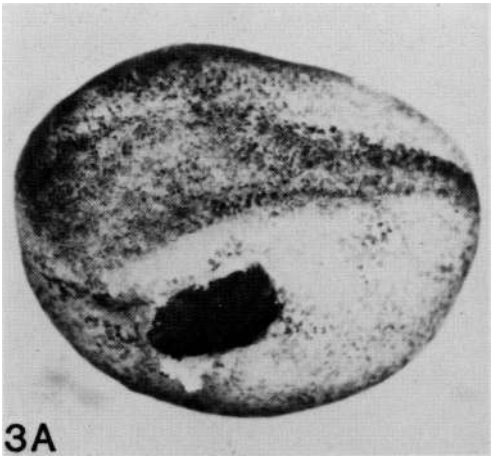
Figure 3 illustrates the transformation of a rectangular epidermis graft. Epidermis from a darkly pigmented embryo was implanted into a faintly pigmented host neurula at stage 14. Photographs were taken at intervals of 90 min. Figure 3 shows that the rectangular graft is transformed roughly into a square. The ventrodorsal side of the graft approximately doubles its original width.

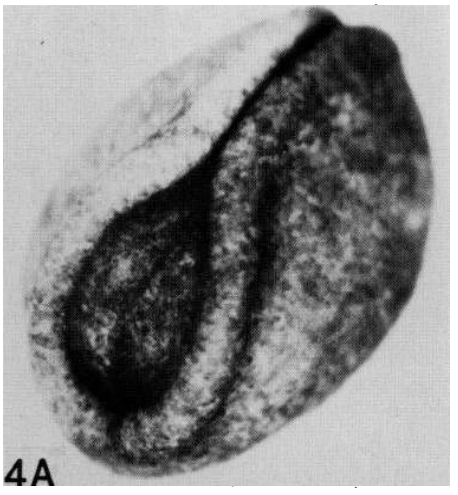
In order to obtain quantitative data pertaining to this observed dorsoventral lengthening of the epidermis, we measured the length and the height of 64 cells adjacent to the neural folds on (semithin) transverse sections. These counts were made in the cells of the superficial layer of the epidermis. The results obtained measuring the cells at consecutive stages of neurulation are listed in Table 1. This table demonstrates that the length of the superficial epidermis cells steadily

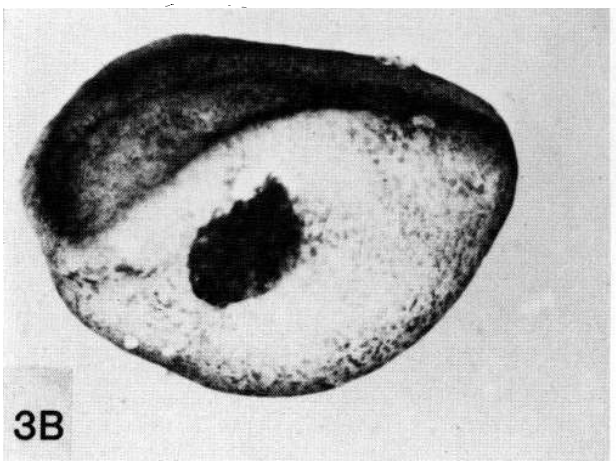
Table 1. *Cell shape changes in the epidermal surface layer in normal and drug-treated neurulae*

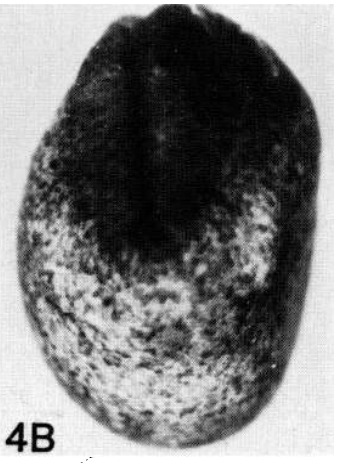
Stage	Cell length (mean) ant.	post.	Cell height (mean) ant.	post.	Standard deviation length ant.	post.	height ant.	post.	Length/height ratio ant.	post.
14	18·81	—	33·13	—	9·61	—	14·31	—	0·57	—
15	27·71	27·40	28·62	36·28	9·49	11·37	11·54	15·7	0·96	0·75
16	28·18	29·87	29·87	33·06	11·2	11·78	8·48	14·83	0·94	0·90
18	32·12	28·15	23·06	23·78	10·24	8·43	7·29	8·35	1·39	1·18
20	33·62	28·96	20·71	23·75	8·9	9·81	6·01	5·85	1·62	1·21
24	32·37	29·09	27·2	26·18	12·19	11·00	7·75	9·60	1·19	1·11
colchine arrested	28·62	25·96	22·71	24·96	9·44	7·4	7·23	6·17	1·26	1·04
nocodazole arrested	30·65	28·75	20·16	21·94	9·01	11·10	5·69	5·92	1·52	1·31

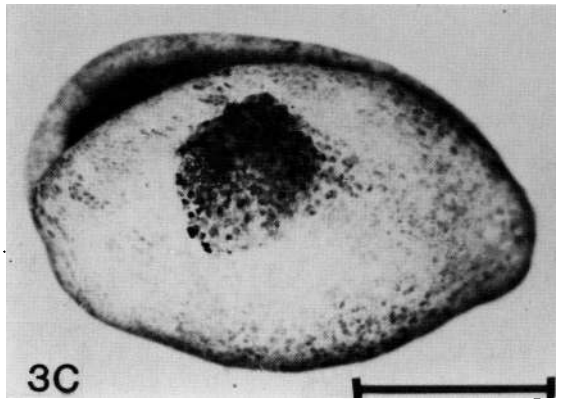
The cells were counted on transverse sections through the anterior and posterior areas of the neural plate. The starting point for the counting was the epidermal cell making contact with the neural plate cell population (n = 32).

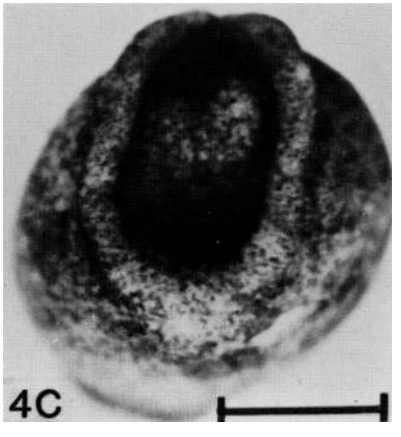












Figs 3 & 4

Table 2. *Exposure of axolotl neurulae to colchicine and nocodazole*

	Stage of embryo at exposure	Number of embryos	Stage reached after 12 h
Colchicine*	13–14	17	18 (13(17–4‡))§
		(4‡)	21 (4‡)§
	15	19	20 (19)§
Nocodazole†	13–14	4	18 (4)§
	16	3	18 (3)§
Control (DMSO 1 %)	16	4	22 (4)§

Total of three experiments.
* Concentration $2{\cdot}5 \times 10^{-5}$ M, $2{\cdot}5 \times 10^{-4}$ M, $2{\cdot}5 \times 10^{-3}$ M: same effect on neurulae.
† Concentrations 1 mg/100 ml; 10 μg/100 ml: (both in DMSO) same effect on neurulae.
‡ Colchicine was washed out of these four embryos after they had been arrested for three hours.
§ Number in parenthesis = number of embryos achieving this stage.

increases as neurulation proceeds. Concomitantly, the mean cell height decreases. The overall cell shape at stage 14 is columnar as expressed by the length/height ratio < 1. This ratio changes in the anterior and posterior areas of the embryo as neurulation proceeds. In the anterior region the ratio is close to 1 at stage 16–17. This means that the cells have changed from a columnar to an almost cuboidal configuration. The ratio further increases to 1·62 at the stage where the neural folds come in contact with each other (stage 20). This ratio represents a tissue consisting of squamous cells. At stage 24, the cells regain a more rounded shape characterized by the 1/h ratio of 1·19.

The shape of the embryos continuously exposed to nocodazole or colchicine beginning at stage 13 is illustrated in Fig. 4. The control (not drug-exposed) embryo has reached stage 20 (Fig. 4B), whereas the drug exposed neurulae are permanently blocked at stage 18 (Fig. 4A: nocodazole; Fig. 4C: colchicine). The effects of nocodazole or colchicine on neurulation are listed in Table 2. This table shows that embryos exposed to colchicine at stage 15 continue neurulation normally. This was found for all the colchicine concentrations used ($2{\cdot}5 \times 10^{-5}$ M–$2{\cdot}5 \times 10^{-3}$ M). In contrast, embryos exposed to colchicine prior to the neural fold stage (stage 13) stopped neurulation at stage 18. The prospective neural tissues of embryos blocked this way disintegrate after approximately 24 h. As Table 2

Fig. 3. Epidermis from a dark donor embryo was grafted on a faintly coloured host at stage 14+. The interval between Fig. 3A and Fig. 3B, as well as between Fig. 3B and 3C is 1 h and 30 min. The dorsoventral distance of the grafted epidermis doubles approximately as neurulation proceeds to stage 20. Bar: 1 mm.

Fig. 4. The embryo illustrated in Fig. 4A was exposed to nocodazole at stage 13–14. Neural fold movement stops in such embryos at stage 18. The control embryo (Fig. 4B) has reached stage 20. Embryos exposed to colchicine at the preneural fold stage 13–14 also show neurulation arrest at stage 18 (Fig. 4C). Bar: 1 mm.

Table 3. *Number of long filament bundles in superficial epidermis (lateral neural fold, anterior area, transverse sections)*

Stage of Neurula	Number of Cells Counted	Number of Bundles over 2·75 μm in length	Percent Cells with long Bundles
14	35	0	0%
15	37	8	21·6
16	35	12	35·0
18	42	21	50·0
20	38	25	65·0
24	48	13	27·0
nocodazole arrested	30	4	13·3*
colchicine arrested	55	6	10·9*

* Significantly different from control stage 18; $\chi^2 = 12{\cdot}48$, $P < 0{\cdot}01$.

indicates, embryos blocked for 3 h by colchicine are capable of resuming neurulation normally if the drug is washed out. In contrast to colchicine, nocodazole is capable of blocking neurulation (at stage 18) in embryos exposed to this drug after stage 15. Table 1 shows that the mean cell length of the superficial epidermis cells in drug-treated neurulae does not reach control values. This length is 30·65 in nocodazole-arrested neurulae and 28·62 in colchicine-blocked embryos.

The ultrastructure of the epidermis during neurulation

Figure 5A illustrates the typical ultrastructure of the superficial cell layer. Bundles of filaments are found in this layer throughout neurulation. The average diameter of the individual filaments is 9·5 ± 8 μm. The bundles run parallel and close to the cell membrane. They are frequently observed to be associated with desmosomes. We found such bundles at all stages of neurulation except at the preneural fold (stage 14). Table 3 lists the percentage of cells with long filament bundles. It increases from 0 % at at stage 14 to 65 % as neurulation proceeds to stage 20 (neural fold closure).

The percentage of epidermis cells with long filamentous bundles (> 2·75 μm) in neurulae exposed continuously to nocodazole or colchicine (beginning at stage 13) is lower than in controls. In nocodazole-exposed neurulae, we found 13·3 % of the cells containing such a bundle. For colchicine-exposed neurulae, this percentage is slightly lower: 10·9 %. Both figures are significantly lower ($P <$ 0·01) than the percentage found in stage-18 controls (50 %). This difference was found, however, only after bundle length was taken into account. It was not possible to find any differences in bundle frequency between drug-treated and control embryos if short bundles (similar to the ones in Fig. 5B) were also counted. The statistical analysis is given in Table 4.

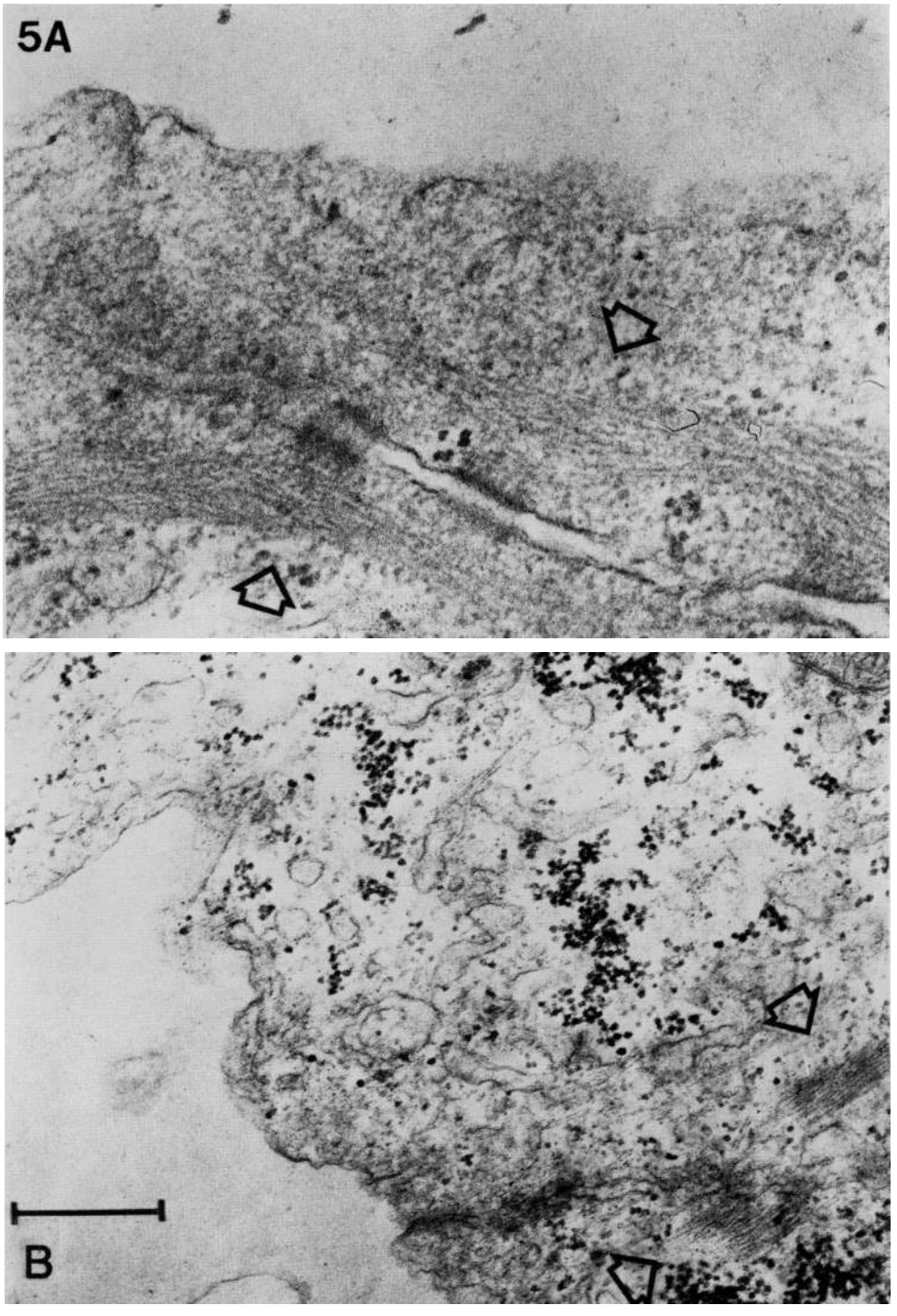


Fig. 5. TEM pictures of the superficial epidermis cells of a control (Fig. 5A), and from an embryo exposed to colchicine (or nocodazole) at stage 13–14 and arrested for 3 h at stage 18 (Fig. 5B). The controls show normal tonofilaments (arrows Fig. 5A) whereas colchicine-treated neurulae have only short fragments of such bundles (arrows Fig. 5B). Bar: 600 nm.

Neural plate excision in neurulae arrested due to continuous drug exposure

Table 1 indicates that the mean cell length of colchicine-arrested embryos is 28·62 whereas the value for nocodazole-blocked neurulae is higher (30·65). To test whether the different ratios reflect a physiological difference between the two classes of embryos, we excised the anterior neural plate (and underlying archenteron roof) from such drug-arrested neurulae. Table 5 lists the data obtained in this experiment. The results reveal a striking difference: following removal of the neural plate, the nocodazole-treated embryos are capable of closing their neural folds whereas the colchicine-arrested embryos are not.

Cell shape changes in the neural plate

Figure 6 summarizes the results obtained by measuring the distances from the neural plate surface to the nuclei in the first layer of the neural plate cells. The distance (depth) is plotted on the y axes, the position of the measured nuclei (in respect to the direction lateral fold to midplate) is recorded on the x axes. The dotted line represents the moving average calculated from the depths of five nuclei (see, Materials and Methods). We defined a constricted cell as a surface cell, the nucleus of which was at a distance of at least 50 μm from the neural plate surface line (see Fig. 2). The dotted lines (line of moving average) in the various

Table 4. *Intercorrelation of mean cell length, mean cell height, length/height ratio and % cells with long (>2·75 μm) filament bundles*

	Mean length	Mean height	l/h Ratio	% Tonofil.
Mean Length		−0·87	0·93	0·86
Mean Height			−0·98	−0·94
l/h Ratio				0·94
% Tonofil.				

n = 32, df = 5, $P<0·5 = 0·754$

Table 5. *Excision of the anterior neural plate (and underlying archenteron roof) following neurulation arrest of colchicine or nocodazole-exposed neurulae*

	Stage of Operation	Number of Embryos Operated	Stage Reached
Nocodazole	17–18	10	10 × 20
Colchicine	17–18	10	10 × 17–18
Control 1	17–18	10	10 × 20
Control 2*	15	6	5 × 19

* Wound closed with ventral tissue.

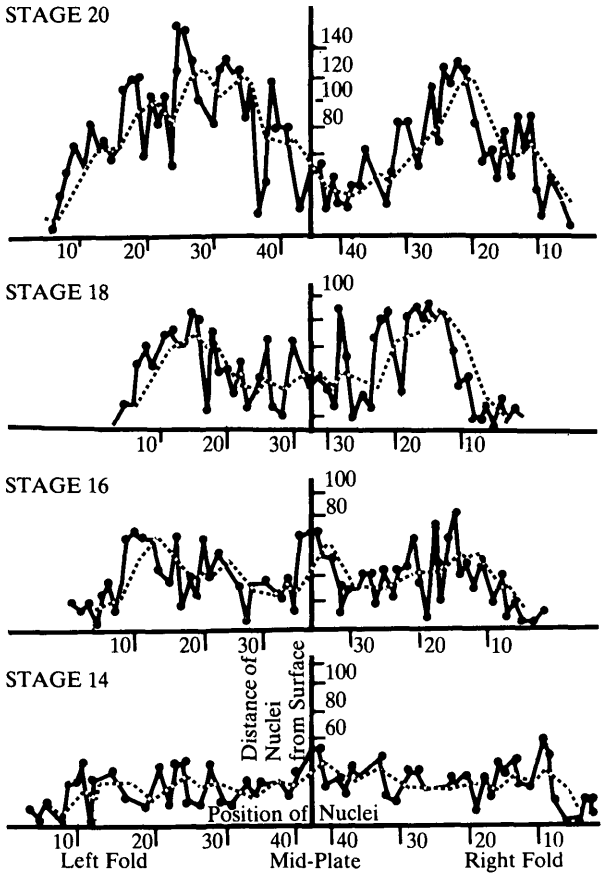


Fig. 6. The distance of the first layer of nuclei from the surface of the neural plate was determined according to Fig. 2. These distances were used for measuring the amount of apical constriction of the neural plate cells. In constricted cells, this distance is greater than in non-apically constricted cells. The moving average (dotted line) was calculated from successive stages of neurulation. Constriction of the neural plate cells starts in the lateral areas. As neurulation proceeds, cells positioned closer to the midneural plate also constrict.

stages is a mathematical expression of the amount of contraction in the neural plate. Figure 6 illustrates that at stage 14 this line does not reach the 50 μm mark on the y axes. According to the above mentioned definition there is no contraction in the neural plate at this stage. At stage 16 the dotted line surpasses the 50 μm mark in the area of the left and right neural fold. There is also some contraction in the midneural plate area. At stage 18, more cells have contracted. The cells added to the ones already constricted during prior stages, are located closer to the midneural plate. At stage 20 (Fig. 6) the moving average line shows the highest elevation as compared to earlier stages. Table 6 summarizes the data concerning the position and number of apically constricted cells throughout neurulation. The table shows a progressive increase of the

Table 6. *Position and number of constricted cells in cross sections through the neural plate*

Stage	Total number of cells	Total number of constricted cells	Percent of constricted cells	Position of constricted cells*		
				Left	Right	Midneural Plate
14	84	0	0	—	—	—
15	80	9	11·25	10–14	5–8	—
16	70	11	15·7	11–16	11–15	4
17	74	33	45·9	11–23	13–31	5
19	80	36	45	9–26	10–23	0
20	88	46	52	11–22	13–31	1

* The cells in position 1 on the left and right side of the neural plate are in contact with the epidermis.

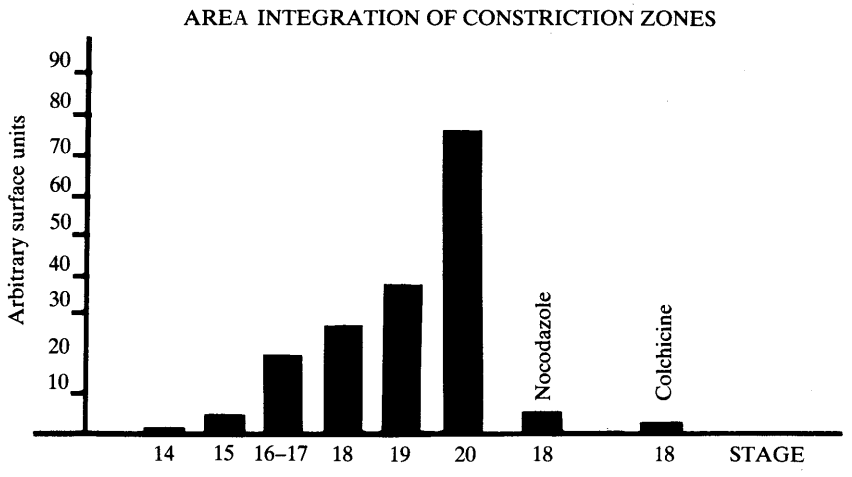


Fig. 7. The integration of the area between the line of the moving average and the line parallel to the x axes at the level of 50 μm shows that the constriction zones in normal neurulation increases approximately by a factor of 10. Although neural folds typical for stage 18 are present in drug-treated neurulae, constriction zones are absent in colchicine-exposed embryos (see Fig. 8).

number of wedge-shaped cells from 0 % (stage 14) to 52 % (stage 20). It further shows that the position of the constricted cells at early stages of neurulation is close to the border of the neural plate with the epidermis. During neurulation, cells constrict progressively closer to the midneural plate. Figure 7 provides quantitative data pertaining to this latero-median constriction wave. Using the moving average curves, we integrated the area of the increasing constriction zones. The zones were defined by the area under the moving average line limited by a line parallel to the x axes at the 50 μm, y point. Figure 7 shows that from stage 15 to stage 20 the area of the constriction zones increases approximately tenfold.

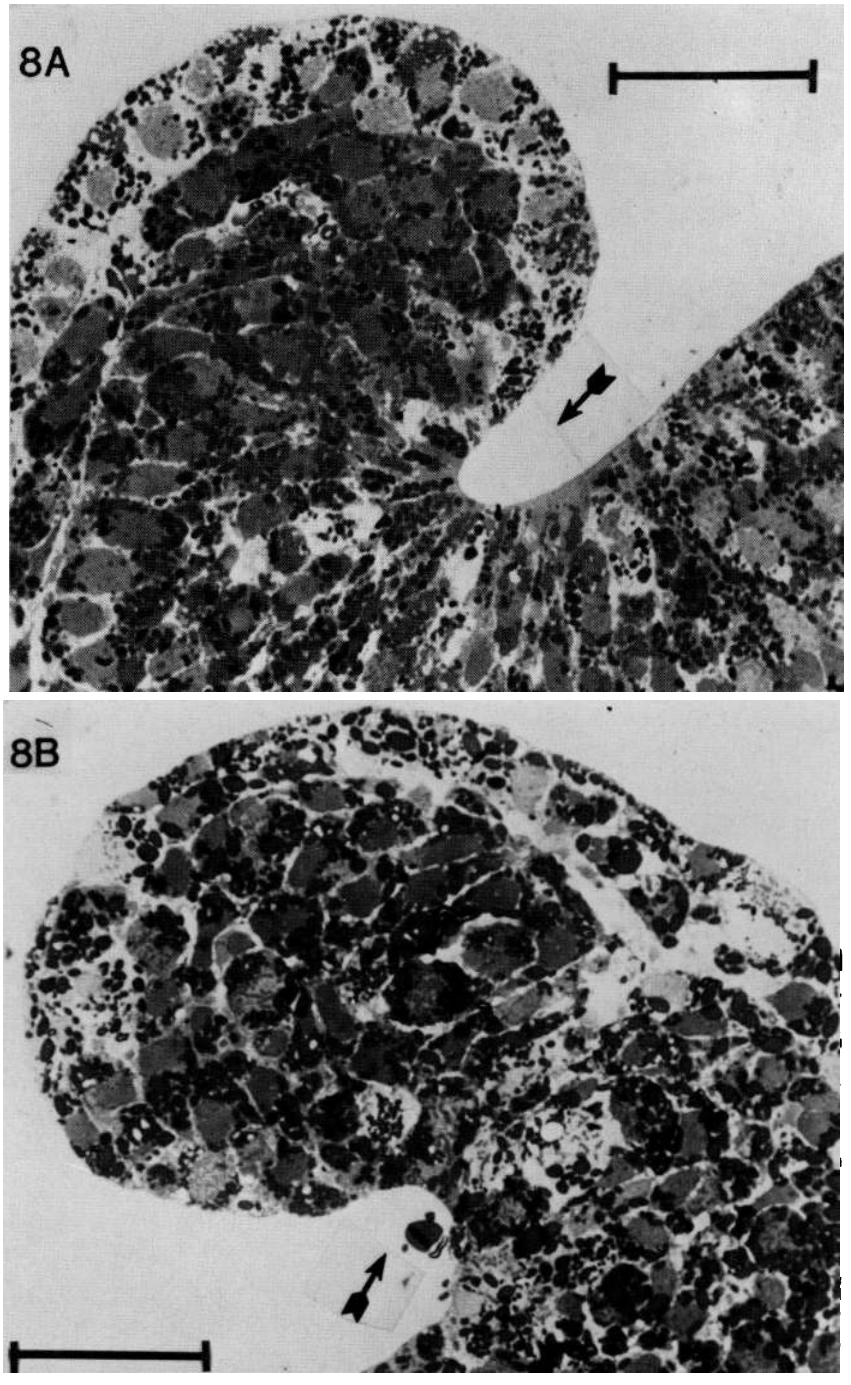


Fig. 8. (A) Normal constriction zone (arrow) in the anterior neural plate of a stage-18 control embryo. (B) This area is occupied by completely unconstricted cells in embryos exposed to colchicine at stage 13–14. In spite of the absence of constricted cells, neural folds are present. Bar: 0·2 mm.

The neural plate of embryos exposed to colchicine or nocodazole

Figure 8A represents a cross section through the anterior brain area of a normal (control) embryo at stage 18. Figure 8B shows the analogous area through a neurula blocked due to exposure to colchicine at stage 13–14. A large number of arrested (colchicine) mitoses can be found. There are no constriction zones present in colchicine-arrested neurulae (see also Fig. 7; colchicine). The neural plate cells are rounded up instead of being elongated as in the neural plates of controls. We were unable to detect microtubules in either colchicine- or nocodazole-exposed embryos. In control neurulae, microtubules were abundant in these regions.

DISCUSSION

Is the neural plate autonomous in forming the neural tube?

This irksome question has been asked over and over again. On the one hand, there are the reports that isolated neural plates are capable of forming the neural tube (for a review see Karfunkel, 1974). On the other hand, there is also the fact that the neural folds are capable of moving together following removal of the neural plate (Hoerstadius & Sellman, 1946; C. O. Jacobson, 1962; Jacobson & Jacobson, 1973). This latter observation clearly seems to indicate that the neural plate is not the only source of neurulation movements: neurulation continues without it. This well-known fact has however been interpreted as being a wound-healing phenomenon not related to neurulation (for a review and discussion see Karfunkel, 1974). In order to answer the question whether parts of the embryos other than the neural plate are actively contributing to neurulation, the neural plate has to be eliminated from participating in the process without causing wound-healing phenomena. In our opinion this can be accomplished by exposing embryos at the preneural fold stage to either nocodazole or colchicine. Figure 8A and 8B illustrate the typical cross sections through the neural plate of controls and colchicine-treated neurulae. Embryos exposed to colchicine at stage 13–14 produce neural folds. These folds move together completely in the midposterior areas but remain separated in the anterior regions of the prospective brain (Fig. 4). The important difference between embryos blocked this way by colchicine and controls (not colchicine exposed) is the total absence of wedge-shaped cells in the neural plate of drug-exposed embryos. The neural plate cells of such embryos round up. It seems to us unlikely that such cells are capable of producing neurulation force by apical constriction. The possibility that the wedge-shaped cells were present prior to neurulation arrest at stage 18, can be excluded. Colchicine is not capable of destroying the constriction zones once they are assembled in the neural plate (see Karfunkel, 1971; Burnside, 1973). Therefore, the observed neurulation block cannot be related to relaxation of constricted

neural plate cells under the influence of colchicine. How is it possible that neural folds form without constricted neural plate cells? In our opinion the answer to this question is that parts of the embryo other than the neural plate are also involved in neural fold formation.

The presence of neural folds in the absence of wedge-shaped neural plate cells has already been reported by C. O. Jacobson, 1970. He exposed embryos at stage 14 (ridges just appearing) to β-mercaptoethanol. He found that prominent neural folds formed, however they were incapable of closing into a neural tube. Brâchet & Délange-Cornil (1959) have also shown that no constricted cells could be found in such β-mercaptoethanol treated neurulae.

The suggestion that the epidermis produces neurulation force has been made by various investigators (e.g. Goette, 1874; His, 1894; Giersberg, 1924; Vogt, 1929; Selman, 1955, 1958; C. O. Jacobson, 1962; Schroeder, 1970; Jacobson & Jacobson, 1973). The extent of epidermal spreading during neurulation is illustrated in Fig. 9 (reproduced from Keller, 1976). However, there is the observation that the epidermis gaps open following slitting. This has been taken as evidence that the epidermis is under tension (caused by the constricting neural plate cells) rather than generating force. (Lewis, 1947; Karfunkel, 1974; Jacobson, 1970.) It is well known that the amphibian epidermis of early embryonic stages has a pronounced tendency to shrink following injury or isolation. This shrinkage appears to be a traumatic reaction of the wounded epidermis. In our opinion, gaping is due to this traumatic shrinkage of the epidermis on either side of the slit. This is consistent with the fact that gaping occurs following slitting of the epidermis in any direction. If gaping were due to the 'pulling' neural plate, it should occur only if the slit is cut in a direction more or less parallel to the neural folds. In addition, gaping of the epidermis following slitting also occurs long after the neural folds are closed. In our judgement, gaping therefore is not related to tensions caused by the closing neural plate, but to traumatic shrinkage of the epidermis following injury. We believe that this reinterpretation of epidermal gaping eliminates the main argument against active participation of the epidermis in producing neurulation force by active spreading (for data and

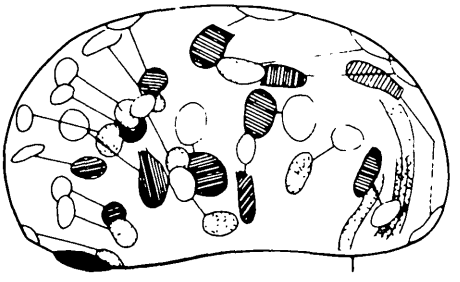


Fig. 9. Lateral view of stage-22 *Xenopus laevis* showing the separation of congruent marks placed in the mesodermal mantle and the overlaying ectoderm at stage 12·5–13. Note the displacement of the dye marks as the mesodermal and ectodermal layer moved relative to one another. This figure is reproduced from Keller, 1976.

discussion of the forces generating gastrulation movements in *Xenpopus laevis*, see Keller, 1980, 1978, 1976; Keller & Schoenwolf, 1977). Figure 10 illustrates our view of how epidermal flattening integrates into the overall process of neurulation.

This flattening process is proportionately related to the appearance of (roughly) 10 nm filament bundles. It has been observed frequently that cells involved in spreading contain such intermediate-sized filaments (for review, see Tucker, 1981). In the amphibian embryos, tonofilaments have been described in neurulae of *Xenopus laevis* by Schroeder (1970), and Karfunkel (1971). Burnside (1971) described these filament bundles in the newt *Taricha torosa*. Our data (Tables 3 & 4) show that the length of the tonofilaments increases with the lengthening of the superficial epidermis cells. This is also supported by the results obtained following exposure of neurulae to colchicine. In embryos exposed to this drug prior to neural fold formation, superficial cells do not flatten to the extent observed in controls (Table 1). The length/height ratio of epidermal cells from embryos exposed to colchicine is 1·26 as compared to 1·62 measured in controls at stage 20. Similarly, the frequency of long tonofilaments in colchicine-arrested neurulae is 10·9 % as compared to 65 % in (stage-20) controls (Table 3).

The data for nocodazole-exposed embryos are different. The length/height ratio measured in the superficial epidermis cells of these embryos is 1·52. This is rather close to the ratio of 1·62 obtained in controls. These ratios indicate that nocodazole-exposed superficial epidermis cells can proceed through the flattening process whereas colchicine-exposed neurulae cannot (see Beebe, Feagans, Blanchette-Mackie & Nau, 1979, for discussion of the effects of nocodazole on cellular elongation). How tonofilaments are involved in spreading is not known. Lengthening of the tonofilaments, however, does not seem to be the cause of cellular flattening: nocodazole-exposed embryos continue epidermal flattening without forming long tonofilaments (Fig. 5, Table 3). If the epidermis is producing neurulation force by means of spreading, neurulae from which the (anterior) neural plate has been excised should close in nocodazole but not in colchicine. This is what we found (Table 5).

At first sight, our data presented in Fig. 6 and Fig. 7 seem to fit well together with the computer model of neurulation suggested by Odell *et al.* 1981. The neural plate is shaped into the neural tube by a wave of apically constricting cells. More and more cells positioned medially are reached by this wave. We agree with Schroeder, 1970 (who analysed neurulation in *Xenopus laevis*) that this is not the only event leading to the elevation of the neural folds. Schroeder (1970) suggested that in addition to the epidermis, the elongating somites might also be involved in raising the neural folds. Lateromedial shrinkage of the archenteron roof probably further assists neurulation. We have (chemically) eliminated the constricting neural plate cell population without preventing the formation of the neural folds. In addition the neural folds were capable of moving together and establishing contact in the posterior areas of the embryo. In our judgement this

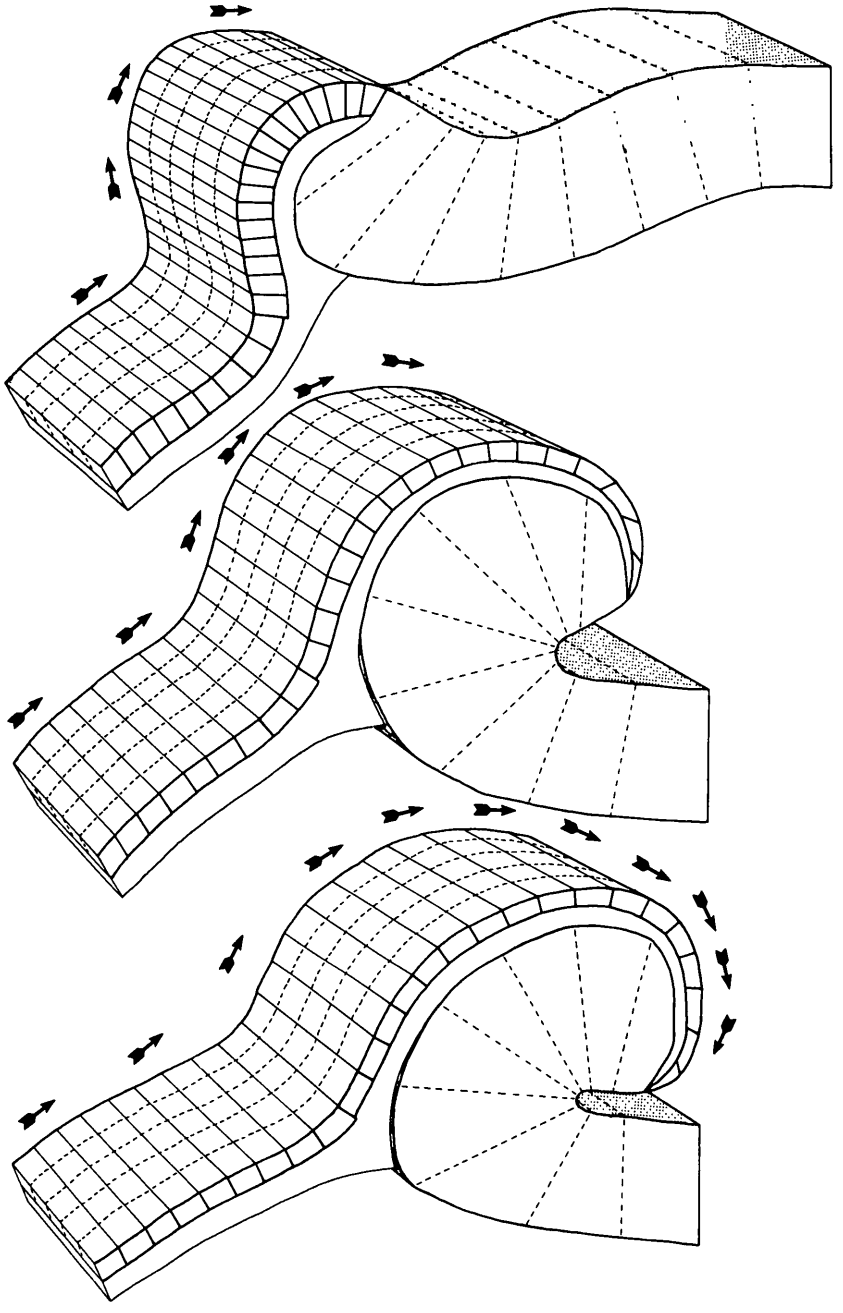


Fig. 10. The figure summarizes the cell shape changes occurring in the superficial epidermis during neurulation. The cell layer is columnar at the beginning of neural fold formation. As the folds are elevated the epidermis cells become more and more flattened. The height/width ratio changes from 0·57 at stage 14 to 1·62 at stage 20 (anterior region). The arrow indicates the direction of flattening of the superficial epidermis cells.

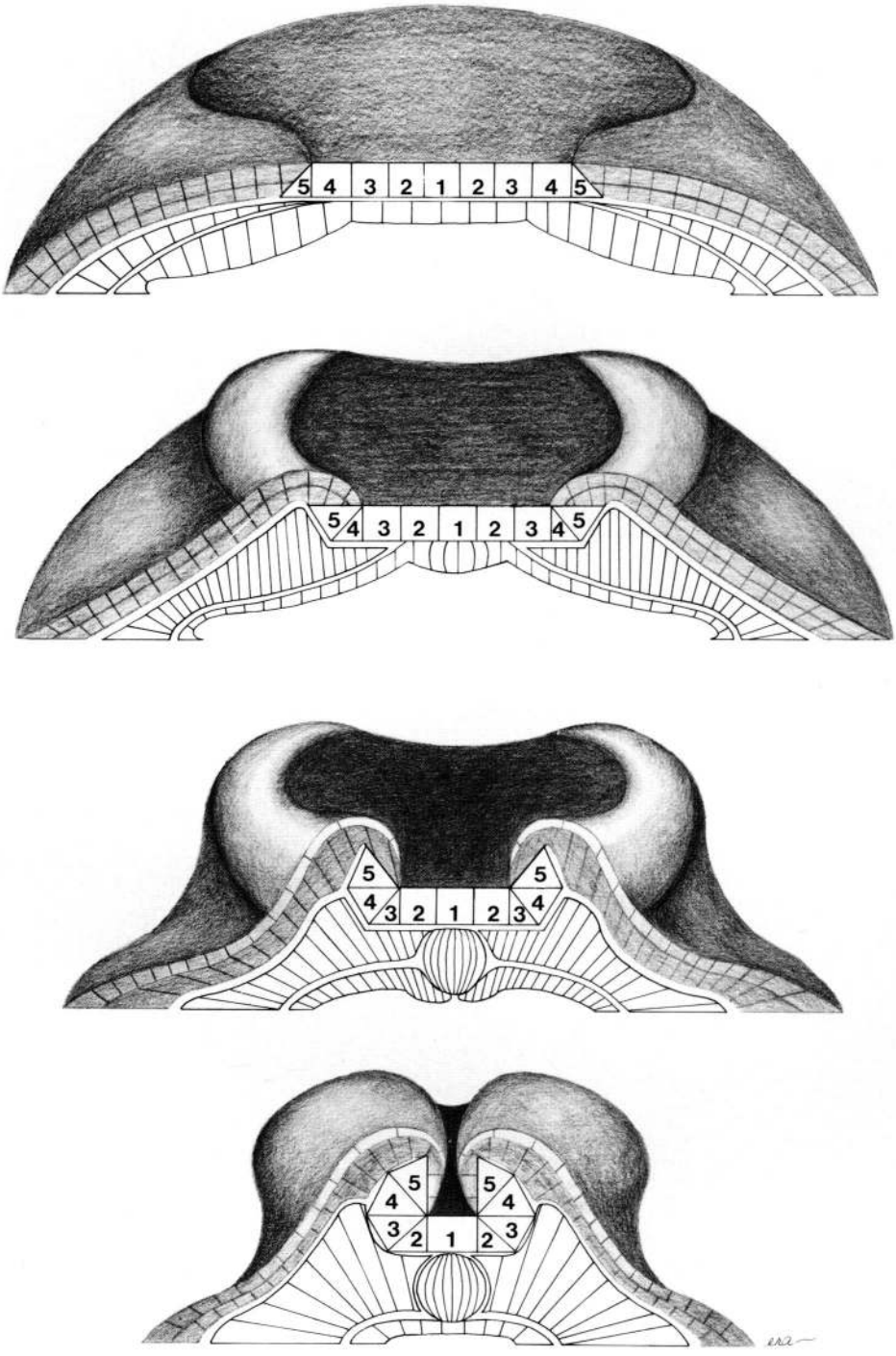


Fig. 11

demonstrates that neurulation is not exclusively driven by apically contracting neural plate cells as suggested in the computer model of Odell *et al.* 1981. We have also some doubts about the computer simulation of early neurulation performed by Jacobson & Gordon, 1976. If we correctly understand the model, it suggests that the neural folds are produced by two forces generated in the neural plate: apical constriction and stretching of the neural plate in the anteroposterior direction. The stretched elastic sheet model of neural tube formation (Jacobson, 1980) proposes that stretching is caused by cell convergence toward the midline of the neural plate. This cellular rearrangement, associated with the subsequent interdigitation of the neural plate cells in the midline of the plate, is believed to provide the driving force for neural tube narrowing and closure. In our judgement these events cannot take place in neurulae from which the neural plate has been excized. Closure of the folds however continues at almost normal speed (Hoerstadius & Sellman, 1946; C. O. Jacobson, 1962; Jacobson & Jacobson, 1973).

Figure 11 summarizes our view of the synergistic interplay of the various embryonic components involved in neurulation of the Mexican salamander.

We thank Dr. Donald F. Danserau for his advice on statistical evaluation of the data.

This research was supported by a TCU Bio-Medical Grant and by NSF Grant PCM 80003373 to R.B.

REFERENCES

BAKER, P. C. & SCHROEDER, T. E. (1967). Cytoplasmic filaments and movement in the amphibian neural tube. *Devl Biol.* **15**, 432–450.

BEEBE, D., FEAGANS, D. E., BLANCHETTE-MACKIE, E. J. & NAU, F. (1979). Lens epithelial cell elongation in the absence of microtubules: Evidence for a new effect of colchicine. *Science* **206**, 836–838.

BRÂCHET, J. & DÉLANGE-CORNIL, M. (1959). Recherches sur le rôle des groupes sulfhydril dans la morphogénèse. *Devl Biol.* **1**, 79–100.

BRUN, R. (1981). The movements of the prospective eye vesicles from the neural plate into the neural fold in *Ambystoma mexicanum* and *Xenopus laevis*. *Devl Biol.* **88**, 192–199.

BURNSIDE, B. (1971). Microtubules and microfilaments in newt neurulation. *Devl Biol.* **26**, 416–441.

BURNSIDE, B. (1973). Microtubules and microfilaments in amphibian neurulation. *Amer. Zool.* **13**, 989–1006.

BURNSIDE, M. B. & JACOBSON, A. G. (1968). Analysis of morphogenetic movements in the neural plate of the newt *Taricha torosa*. *Devl Biol.* **18**, 537–552.

Fig. 11. This figure summarizes our view in neurulation in the Axolotl. Simultaneously with the flattening of the epidermis, the cells at the edge of the neural plate become wedge-shaped, forming a constriction zone. More medial plate cells are sequentially incorporated into the constriction zones as neurulation proceeds. The neural plate is consequently capable of bending into a tube by means of the constriction zones moving towards each other. The epidermis forcibly contributes by flattening. The somites increase in height, whereas the archenteron shrinks dorsally to form the notochord.

GLERSBERG, H. (1924). Beitraege zur Entwicklungsphysiologie der Amphibien. *Arch. microsk. Anat. EntwMech.* **103**, 387–424.

GLASER, O. C. (1914). On the mechanisms of morphological differentiation in the nervous system. *Anat. Rec.* **8**, 525–551.

GOETTE, A. (1874). Ueber die Entwicklung des Zentralnervensystems der Teleostier. *Arch. microsk. Anat. EntwMech.* **15**, 139–200.

HIS, W. (1894). Ueber mechanische Grundvorgaenge thierischer Formbildung. *Arch. Anat. Physiol.* **1**, 1–80.

HOERSTADIUS, S. & SELLMAN, S. (1946). Experimentelle Untersuchung Ueber die Determination des knorpeligen Kopfskelettes bei Urodelen. *Nova Acta Regiae Soc. Sci. Upsallensis* **13**, 1–170.

JACOBSON, A. G. & GORDON, R. (1976). Changes in the shape of the developing vetebrate nervous system analyzed experimentally, mathematically and by computer simulation. *J. exp. Zool.* **197**, 191–246.

JACOBSON, A. G. (1980). Computer modelling of morphogenesis. *Amer. Zool.* **20**, 669–677.

JACOBSON, C. O. (1962). Cell migration in the neural plate and the process of neurulation in the Axolotl larva. *Zool. Bidrag* **35**, 433–449.

JACOBSON, C. O. (1970). Experiment on β-mercaptoethanol as an inhibitor of neurulation movements in amphibian larvae. *J. Embryol. exp. Morph.* **23**, 463–471.

JACOBSON, C. O. & JACOBSON, A. (1973). Studies on morphogenetic movements during neural tube closure in amphibia. *Zoon* **1**, 17–21.

KARFUNKEL, P. (1971). The role of microtubules and microfilaments in neurulation in *Xenopus laevis*. *Devl Biol.* **25**, 30–56.

KARFUNKEL, P. (1974). The mechanisms of neural tube formation. *Int. Rev. Cytol.* **38**, 245–272.

KELLER, E. R. (1975). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layers. *Devl Biol.* **42**, 222–241.

KELLER, E. R. (1976). Vital dye mapping of the gastrula and the neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movements of the deep layer. *Devl Biol.* **51**, 118–137.

KELLER, E. R. (1980). The cellular basis of epiboly: an SEM study of deep-cell rearrangement during gastrulation in *Xenopus laevis*. *J. Embryol. exp. Morph.* **60**, 201–234.

KELLER, E. R. & SCHOENWOLF, G. C. (1977). An SEM study of cellular morphology, contact, and arrangement, as related to gastrulation in *Xenopus laevis*. *Wilhelm Roux Arch. devl Biol.* **182**, 165–186.

LEWIS, W. H. (1947). Mechanics of invagination. *Anat. Rec.* **97**, 139–156.

ODELL, G. M., OSTER, G., ALBERCH, P. & BURNSIDE, B. (1981). The mechanical basis of morphogenesis. I. Epithelial folding and invagination. *Devl Biol.* **85**, 446–462.

ROUX, W. (1885). Zur Orientierung ueber einige Probleme der embryonalen Entwicklung. *Z. biol* **31**, 429–528.

SCHRECKENBERG, G. M. & JACOBSON, A. G. (1975). Normal stages of development of the Axolotl, *Ambystoma mexicanum*. *Devl Biol.* **42**, 391–400.

SCHROEDER, T. E. (1970). Neurulation in *Xenopus laevis*. An analysis and model based on light and electron microscopy. *J. Embryol. exp. Morph.* **23**, 427–462.

SELMAN, G. G. (1955). Studies on the forces producing neural closure in amphibia. *Proc. R. phys. Soc. Edinb.* **24**, 24–27.

SELMAN, G. G. (1958). The forces producing neural closure in amphibia. *J. Embryol. exp. Morph.* **6**, 448–465.

SOKAL, R. R. & ROHLF, F. J. (1969). *Biometry*. San Francisco: Freeman & Co.

SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**, 31–43.

TUCKER, J. B. (1981). Cytoskeletal coordination and intercellular signalling during metazoan embryogenesis. *J. Embryol. exp. Morph.* **65**, 1–25.

VOGT, W. (1929). Gestaltsanalyse am Amphibienkeim mit oertlicher vital Faerbung. II. Teil.

Gastrulation und Mesodermbidlung bei Urodelen und Anuren. *Wilhelm Roux Arch. EntwMech. Org.* **120**, 384–707.

(*Accepted 26 October 1982*)