In conclusion, YS erythropoiesis in the avian embryo is part of a complex scheme, involving several haemopoietic sites, several types of erythrocytes and a succession of overlapping Hbs. Morphological or biochemical study of normal development makes it possible to tie these factors together only partially. Our experimental approach, using quail chick 'YS chimaeras' yields a more precise picture of the contribution of the YS to the system. It demonstrates the importance of the intraembryonic blood islands, which participate in the sequential functioning of the mesodermal haemopoietic anlage. The first haemopoietic wave is extraembryonic, and occurs mainly in situ, without movements of SC. The 2nd wave is mainly intraembryonic; it involves very little in situ differentiation; the cells migrate to specific sites where they probably undergo commitment; one of these sites is the YS. The system is well adapted to ontogenic needs; it ensures rapid maximal production of erythrocytes at the early stage, and the diversification of cell lines at a later period.

33 T. Terni, Archo ital. Anat. Embriol. *21*, 533 (1924).
34 C. Martin, F. Dieterlen-Lièvre, O. Lassila and P. Toivanen, Folia biol. *23*, 373 (1977).
35 J. Samarut and V. Nigon, J. Embryol. exp. Morph. *36*, 247 (1976).
36 J. E. Till and E. A. McCulloch, Radiat. Res. *14*, 213 (1967).
37 D. Beaupain, C. Martin and F. Dieterlen-Lièvre, in preparation.
38 F. Jotereau and N. Le Douarin, Developmental Immunobiology. Ed. J. B. Solomon. Elsevier/North Holland Biomedical Press, Amsterdam 1978, in press.

Microtubules, interkinetic nuclear migration and neurulation*

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Summary. The hypotheses dealing with mechanisms of neurulation are reviewed briefly. The phenomenon of interkinetic nuclear migration is thought to be an important factor to be considered in the invagination of the neuroepithelium in the chick embryo. Evidence is presented that implicates cytoplasmic microtubules in this phenomenon. It is suggested that microtubules not only participate in cell elongation but also that they are involved, through interkinetic nuclear migration, in the broadening of the basal region of the cells; this widening progressively creates the strain that ensures the invagination of the neuroepithelium.

Little is known of the many processes involved during the invagination of epithelial tissues. Yet, in many organs, normal morphogenesis includes the transformation of relatively flat epithelial leaflets into curved structures. This process of invagination is observed, for instance, during neurulation in many species[1–3], in the forming pancreas of the mouse[4] and in the early stages of development of the lens[5,6], salivary glands[7] and tubular glands of the chick oviduct[8].

Neurulation, as it occurs in the chick embryo, is well suited for an analysis of the events undergone by an epithelium in the course of its morphogenetic invagination. In the chick, the neural plate, a flat pseudostratified epithelial leaflet, invaginates first to take the form of a U-shaped structure (as seen in transverse sections) and then adopts a O-shape, which marks the closure of the neural tube (figure 1). Baker and Schroeder[1] report that in *Xenopus* changes in the shape of the cells making up the epithelium are basic to the tissue movement that ensures the closure of the neural tube. During neurulation the general aspect of the neuroepithelium is modified, while changes are observed in the shape of the cells making up the tissue. Indeed, in an early chick embryo, the cells found in the primitive streak region are cuboidal, while those forming the neural plate are of the low columnar variety (figure 1). Later in development, as the neural plate begins to invaginate to form the neural groove, cells will acquire a highly columnar form. In 1966, Waddington and Perry[9], studying neurulation in amphibians, postulated that cytoplasmic microtubules might have something to do with these changes in cell shape. In 1969, it was shown[10] that microtubules are oriented at random in the cuboidal cells that characterize the neuroepithelium of the primitive streak region of early chick embryos. Further, as development proceeds, more and more microtubules come to lie in a direction parallel to the long axis of the columnar cells that are characteristic of the later stages. The following year, Schroeder[11] noted that microtubules, which progressively orient themselves parallel to the cell's long axis, were instrumental in the production of the highly columnar shape of these cells in *Xenopus*. Using monoiodoacetamide coupled with cold exposure, it was possible to correlate experimentally the presence and orientation of microtubules with the columnar shape of the cells in the neural tube of the chick[12].

Baker and Schroeder[1] and Schroeder[11] proposed that, once the cells acquire their columnar shape, an apical ring of microfilaments may, in a purse-like fashion, constrict the cellular apices, thereby a) inducing a new shape referred to as bottle or flask shape, and b) assuring neurulation (figure 1). The proposal was tested

experimentally. Thus Karfunkel[13], treating young neurulae of *Xenopus* with vinblastine, described a loss in the asymmetry of the so-called bottle-shaped cells with an ensuing arrest in neurulation. However, since vinblastine affects both microtubules and microfilaments, Karfunkel[14] completed his studies by treating neurulating chick embryos with colchicine and cytochalasin B. Both drugs, it was shown, block neurulation. Karfunkel reports that colchicine breaks down microtubules, thus inducing a change from columnar to round-shaped cells. Cytochalasin B, on the other hand, selectively disrupts the ring of microfilaments, thereby causing the loss of apical constriction and the resulting inhibition in neurulation. Burnside's[2] work on the newt *Taricha torosa* also stressed that microtubules are agents involved in cellular elongation and that microfilaments, through their sliding past each other, constrict the cellular apices, thus leading to invagination of the neuroepithelium.

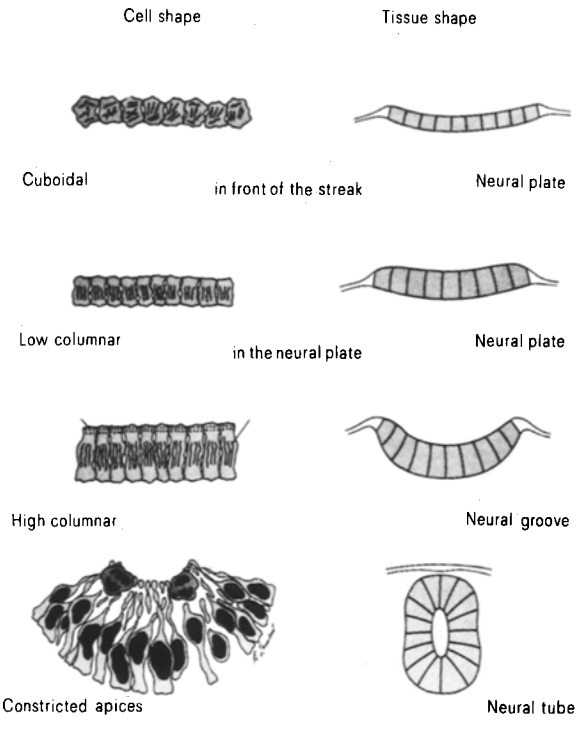


Fig. 1. Schematic illustration of the shapes of cells and tissues at different stages of development.

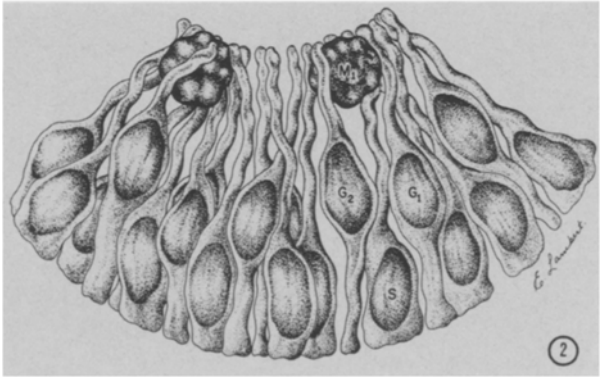


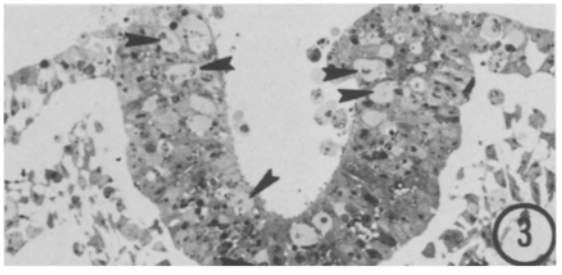
Fig. 2. Schematic illustration of the phenomenon of interkinetic nuclear migration. DNA synthesizing nuclei (S) are located in the outer zone of the epithelium; they occupy the base of the cells. In G_2 they migrate towards the apical pole, near the neurocoele, where they will undergo mitosis (M). Daughter nuclei migrate back to the base of the cell during the G_1 phase.

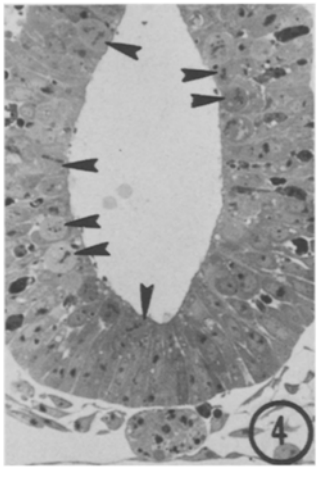
Now, none of the authors cited above have discussed, in their work dealing with the conversion of the neural plate into a neural tube, the phenomenon of interkinetic nuclear migration. Only Lofberg[3], in a study of neurulation in the axolotl *Ambystoma mexicanum*, alluded to a paper published earlier[16] and considered interkinetic nuclear migration as possibly instrumental in the invagination processes of the neural plate.

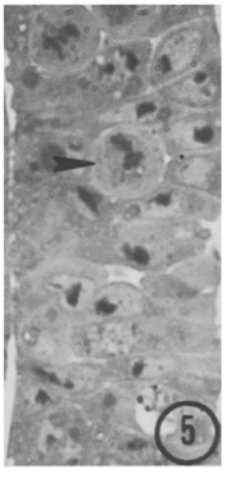
Nevertheless, it is well known that in many epithelia (the lens placodes, the nasal pits, the otic placodes, the thicker part of the coelomic walls, the mesonephric tubules, etc.[17–20]) nuclei undergo migratory movements. According to Sauer's original theory[17], formulated in a study dealing with mitosis in the neural tube of chick and pig embryos, 'cells in the epithelium when about to divide undergo a change in form by which the nucleus migrates to the lumen and the cytoplasm assumes a rounded form. After division the nucleus migrates away from the lumen'.

Work done with colchicine[21] offered the 1st experimental confirmation of the theory of interkinetic nuclear migration. The concept was further tested by measuring the DNA content of nuclei[22], and the use of autoradiography[23] gave evidence substantiating the occurrence of nuclear movements. It was shown that DNA synthesis occurs only in nuclei situated in the peripheral region of the wall of the neural tube. Finally, the phenomenon of interkinetic nuclear migration was definitively confirmed by Langman et al.[24]. These authors indicated that during DNA synthesis (S phase, approximately 5 h; figure 2) the nuclei are located at the base of the cells near the basement membrane of the epithelium. Subsequently nuclei migrate (G_2 phase, 2.5 h) to the lumen to undergo mitosis (M phase, 0.5 h) after which they return (G_1 phase) to the outer zone of the epithelium to start DNA synthesis for the next cell division. These authors found the cell generation time to be 8 h. According to Langman et al.[24], 97.5% of the newly formed cells in the neuroepithelium of the chick embryo remain firmly attached at their apex; they form the pseudostratified epithelial leaflet. On the other hand, only 2.5% of the young cells are free in the epithelium and may develop into neuroblasts. Moreover, in the pseudostratified layer of epithelial cells attached at their apex, the S phase inevitably causes an accumulation of nuclei at the base of the epithelium. Hence, the large proportion of wedge-shaped cells in the proliferating neuroepithelium would naturally result, it was felt[24], in the invagination and closure of the neural tube.

In 1971, we reported[25] that treatment of chick embryos with dithiodiglycol (an oxidizer of –SH groups) at concentrations (10^{-3} M) which inhibit neurulation had no effect on the cells' fine structure, with the exception of microtubules. Indeed, in cells of the neural tube which showed no sign of shrinkage, microtubules were highly sinuous, tortuous and rather crooked. Often the microtubular wall exhibited small characteristic notches. These tortuosities were quite different from the undulations to be found in microtubules of cells that have shrunken somewhat[26]. It was thought that dithiodiglycol might block neurulation through an interaction with thiol groups, which are a factor involved in the polymerization of microtubules[27].







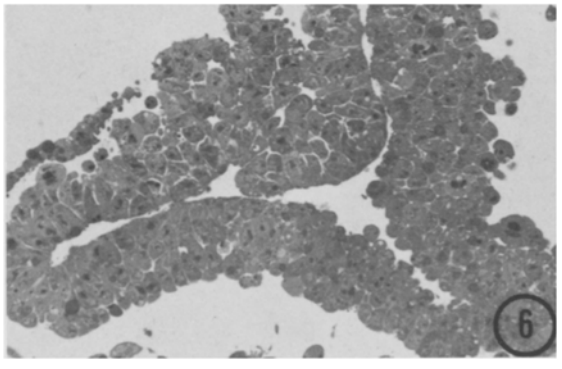


Fig. 3. Light micrograph of an embryo exposed to 2 °C for 3 h, treated with monoiodoacetamide (4×10^{-3} M) and brought back to 38 °C for 4 h. Many mitotic figures (arrows) are seen abnormally distributed within the thickness of the neural epithelium. ×375. Fig. 4. Transverse section of the neuroepithelium of an embryo exposed for 4 h to 0.25 M formamide. At least 13 mitotic figures (arrows) may be seen arrested in the metaphase stage near the neurocoele. ×600. Fig. 5. Light micrograph taken of an embryo exposed to a 0.31 M formamide. A mitotic figure (arrows) is found far from the neurocoele, within the thickness of epithelium. ×1500. Fig. 6. The characteristic shape of the neural tube is obliterated in this specimen treated with 0.43 M formamide. Cells are becoming spherical. ×600.

The following year it was shown[28] that, even when administered at concentrations higher than needed to inhibit neurulation, –SH reducing agents such as mercaptoethanol (10^{-1} M) and dithiotreitol (10^{-2} M), had no apparent effect on the morphology and distribution of microtubules, nor did these chemicals block interkinetic nuclear migration.

These results led us to a more detailed study to evaluate the possible role of microtubules in interkinetic nuclear migration. To start with, a system derived from Roth's[29] work on amoebae was devised, whereby microtubules were disrupted (cold exposure) and prevented from repolymerizing (exposure to monoiodoacetamide) during the period in which the specimens were brought back to physiological temperature[16].

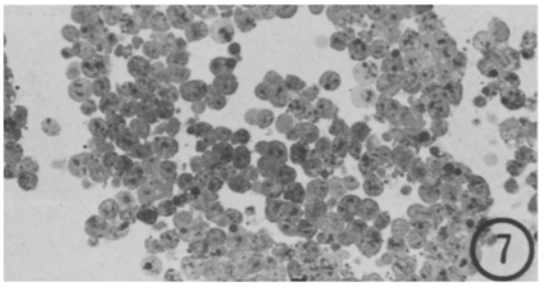
Thus, early chick embryos were exposed to temperatures of 2 °C for 3 h. Exposure to cold fully inhibited embryonic development. Low temperatures are known to induce depolymerization of microtubules and 4 °C-treatment of chick embryos has been used successfully by Handel and Roth[30] to deprive neural tube cells of their microtubules. Our 3-h treatment of embryos at 2 °C confirmed the observations of Handel and Roth: there was a reduction in the asymmetry of cell shape. Indeed, the normally highly columnar cells had lost their long cell neck and tended to be round in shape. In these cells microtubules not associated with cilia, centrioles or midbodies had disappeared. These effects were reversible. In fact, a 1-h recovery at 38 °C was sufficient to allow microtubules to reappear characteristically aligned parallel to the cell's long axis. In 1 h the normal cell asymmetry was restored.

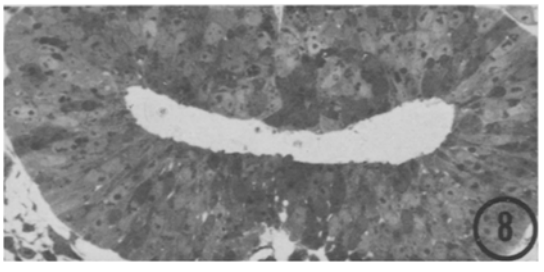
However, if specimens were a) exposed to cold, b) treated with monoiodoacetamide (a free –SH group inhibitor), and c) incubated at physiological temperature, then the forming neural tube cells lost their asymmetry and few or no microbtubules could be found. Also, in the 1st h that followed cold plus monoiodoacetamide exposure, there was a noticeable accumulation of mitotic figures near the lumen of the forming neural tube. After a 4-h period at 38 °C, many figures were located near the lumen but most were abnormally distributed throughout the thickness of the neural epithelium (figure 3).

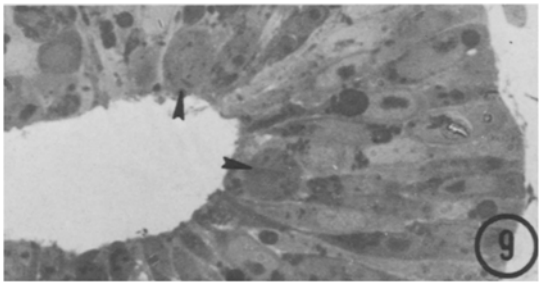
On the other hand, in embryos exposed to monoiodoacetamide while at 38 °C, no undue accumulation of mitotic figures was ever detected. Electron microscopy revealed that microtubules were present. Furthermore, when specimens were exposed to monoiodoacetamide while at their physiological temperature and then treated with vincristine (2.5×10^{-5} M), an accumulation of blocked metaphases occurred near the lumen, thus indicating that monoiodoacetamide did not in-

hibit nuclear migration, nor did it impede mitosis in nuclei situated near the lumen.

These results indicate that monoiodoacetamide, when administered to embryos following cold exposure, prevents repolymerization of microtubules and inhibits nuclear migration. This is evident since there is a lack of microtubules and an abundance of mitotic figures abnormally distributed *within* the thickness of the neural epithelium in those embryos that were exposed to cold, treated with monoiodoacetamide and brought back to their normal incubation temperature for 4 h.







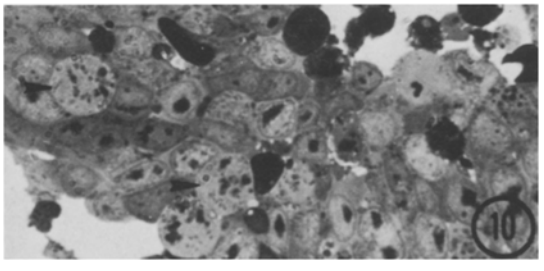


Fig. 7. Light micrograph of a section cut transversely through an embryo treated with 0.5 M formamide. All cells have become round. ×600. Fig. 8. The usually distorted neural tube observed after a 0.43 M formamide exposure regains its previous shape, following a 3-h incubation on a normal media. In such sections mitotic figures are seen only near the neurocoele. ×600. Fig. 9. Enlarged portion of figure 8. It is shown that cells tend to regain their asymmetric shape. Interkinetic nuclear migration has been resumed, as is evident by nuclei dividing near the apex (arrows). ×1500. Fig. 10. Cytochalasin B inhibits interkinetic nuclear migration. Indeed mitotic figures are seen far away from the neurocoele, abnormally situated within the thickness of the epithelium (arrows). ×1500.

It appears that monoiodoacetamide, which does not affect formed cytoplasmic microtubules, prevents their repolymerization when they are disrupted by cold treatment. The chemical, a free –SH group inhibitor, has in fact been shown to impair normal polymerization of microtubules. Roth[29] obtained similar results with urea, which impedes normal hydrogen bonding and weakens salt linkages. He found that urea, when given at culture temperature, had no effect on the microtubules of the mitotic apparatus of amoebae. However, it prevented their reappearance once the specimens had been exposed to low temperature and treated with the drug.

Our conclusion, which relates microtubules to nuclear migration, is based on the fact that cells lacking normal microtubules (treatment with 4×10^{-3} M monoiodoacetamide after cold exposure) do not show nuclear migration; indeed mitotic figures are found throughout the thickness of the epithelium. No other cell organelle was seen to respond to our experimentation as did microtubules. Surely monoiodoacetamide affects many cellular processes but, at 38 °C and used at the concentration quoted above, it has been shown to be ineffective in blocking nuclear movements (mitotic figures accumulated only near the neurocoele under monoiodoacetamide plus vincristine treatments) and also ineffective in disturbing microtubules if they had not already been disrupted by exposure to cold.

Once we have evaluated the effects of a substance that interferes with –SH groups, it was decided to turn to a chemical that would impair normal hydrogen bonding and to use it, if all at possible, in a simpler approach. Formamide was selected[31]. Here we have a product which, like other amides, modifies the conformation of proteins, and which biochemists use to solubilize protein structures. Nevo et al.[32] showed that formamide disrupts the mitotic apparatus and alters chromosomes in the sea urchin eggs. Their results indicate that formamide disturbed microtubular structure.

Table 1. Effects of varied concentrations of formamide on various aspects of the chick neuroepithelium

Concentration	Arrested mitosis	Nuclear migration	Cell asymmetry	Altered neuroepithelium	Cytoplasmic microtubules
0.10 M	–	+	+++	–	+++
0.25 M	++	+	+++	–	+++
0.31 M	++	–	++	–	++
0.37 M	++	–	+–	+	+–
0.43 M	NA	NA	–	++	–
0.50 M	NA	NA	–	+++	–

A 0.25 M concentration affects only mitosis. A small increment (0.31 M) reduces the amount of cytoplasmic microtubules, blocks interkinetic nuclear migration and influences cell asymmetry. At 0.37 M only a few microtubules are observed, cell asymmetry is further affected and signs of altered epithelium appear. Reaching 0.43 M most cells are spherical and the pseudostratified nature of the epithelium is lost. At 0.50 M all cells are spherical. Note that a progressive loss of cytoplasmic microtubules first affects interkinetic nuclear migration and then leads to a gradual deterioration of cell asymmetry. NA stands for not applicable.

Our experimentation showed that the effects of formamide on our specimens were graded (table 1). After a 6-h treatment the chemical, at a 0.1 M concentration, had no detectable effect. A slight increase to 0.25 M became very effective in blocking mitosis, but it did not affect nuclear migration nor the cells' asymmetry (figure 4). With an additional increment in concentration (0.31 M), there appeared the first signs of an inhibition of interkinetic nuclear migration (figure 5). However, the molarity had to be raised to 0.37 M before the cells began to lose some of their asymmetry. At 0.43 M formamide, most cells were spherical (figure 6) and devoid of microtubules, whereas at 0.5 M all cells were completely spherical (figure 7). However, this last concentration did not produce pycnotic nuclei nor necrosis after a 6-h exposure, an indication that the cells manage to resist the toxicity of the product. At this point, tests were made to see whether the effects of formamide were reversible. First, embryos ranging from 1 to 3 pairs of somites were laid on media containing formamide at 0.37 M. Following a 3-h incubation period they were transferred to control media for an additional 3 h. These embryos, rather young at the beginning of the treatment, were studied macroscopically after their 3-h stay on the normal media. Half showed signs of abnormal morphogenesis; neurulation was inhibited and somitogenesis did not proceed normally. Yet, on the control media, mitosis was resumed partially and all embryos gained at least 2 pairs of somites in a 3-h period.

Since a 0.37 M concentration only partially depleted the cells of their microtubules (table 1), it was decided further to test reversibility by using the next concentration (0.43 M), which leaves the cells completely devoid of microtubules. Here we used embryos at 6 pairs of somites. It was found that the collapsed and distorted neural tube, observed after exposure to 0.43 M formamide (figure 6), is partly reshaped following a 3-h incubation on a normal medium (figure 8). The neuroepithelium resumes its normal architecture, that is, a pseudostratified epithelium. The cytological effects we have been concerned with were fully reversible, an indication that the chemical did not interfere with the process of differentiation but suppressed the manifestation of differentiated functions. Neuroepithelial cells returned to their typically asymmetric shape. Mitosis was no longer arrested, interkinetic nuclear migration occurred normally and mitotic figures were observed only near the neurocoele (figures 8 and 9). Electron microscopy showed that these cells contained as many microtubules as did normal embryos.

If formamide is kept at concentrations equal to or lower than 0.25 M, it will not prevent cells from entering into mitosis and will not disturb interkinetic nuclear migration. However, it must be kept in mind that such concentrations, though they are relatively low compared to what is used in biochemical practice, are definitely high from a biological standpoint and may affect many cellular processes. With these points in mind, it was decided to test whether formamide could directly affect microtubules.

The experimental procedure referred to above, that of coupling cold exposure with chemical treatment, was used again. Some embryos explanted on 0.1 M or 0.25 M formamide media were first exposed for 1.5 h at 38 °C, then, to 2 °C for 3 h and finally incubated for 1.5 h at 38 °C on their original formamide media. Using this procedure and a 0.1 M concentration, it was found that microtubules were not affected in any detectable manner. However, when 0.25 M formamide was used, microtubules could no longer be found in the neuroepithelial cells of the embryos when brought back to physiological temperature. Moreover, under these conditions, interkinetic nuclear migration was inhibited – a situation which is never encountered when formamide is used alone and at concentrations not exceeding 0.25 M. Control specimens, exposed to cold as above and brought back to 38 °C for 1.5 h on normal media,

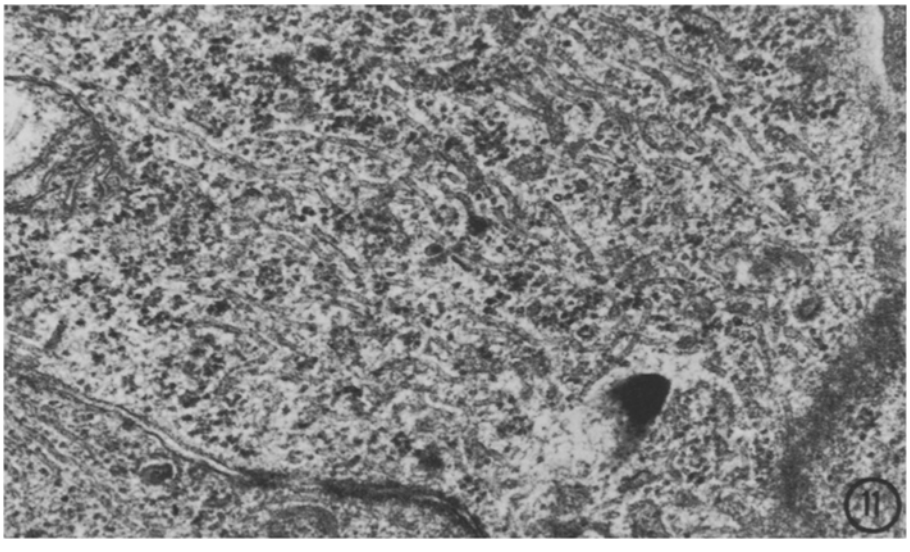
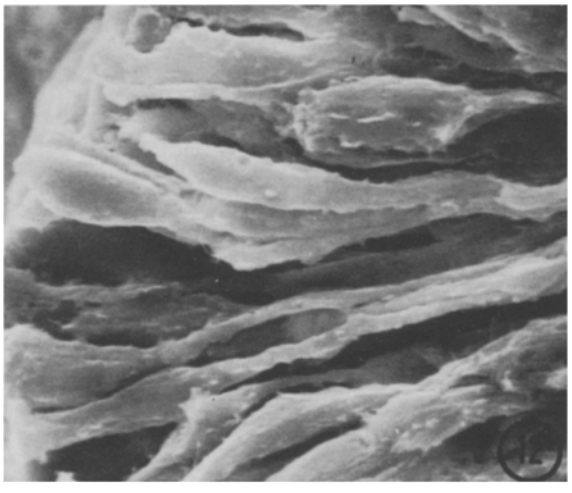


Fig. 11. Transmission electron micrograph of portions of neuroepithelial cells from an embryo exposed to db-cAMP (25 mM) for 4 h. Note abundance of microtubules. × 30,000.

always contained numerous microtubules and exhibited undisturbed interkinetic nuclear migration. These results support the idea that formamide, although it probably affects many cellular processes, does interact directly with microtubular components. Furthermore, our data suggest that microtubules are part of the active forces involved in the displacement of nuclei observed during interkinetic nuclear migration. It must be emphasized that microtubules might not be the only agent involved in nuclear migration. Indeed we recently showed that cytochalasin B (5 µg/ml) is also capable of blocking interkinetic nuclear migration[33]. The fungus metabolite cytochalasin B was first reported to inhibit cell motility and cytokinesis in fibroblasts[34] and to this day its primary site of action is still uncertain[35]. On the one hand, there is a group of results revolving around data showing a loss of contractile function linked to the disruption of microfilaments[36,37] and, on the other hand, there exists compelling evidence that the drug interacts with the surface membrane[38–43].



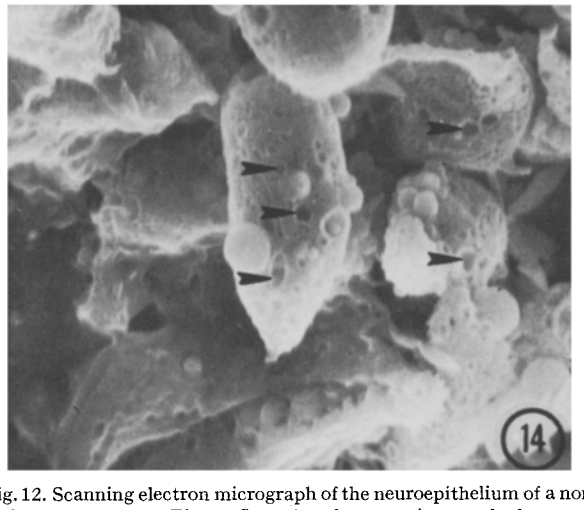


Fig. 12. Scanning electron micrograph of the neuroepithelium of a normal embryo. × 2000. Fig. 13. Scanning electron micrograph of a neuroepithelial cell from an embryo treated with cytochalasin B. Note the numerous cavities (arrow) appearing on the surface membrane near the basal pole. × 3000. Fig. 14. Spherical cells of a cytochalasin-treated embryo seen in scanning electron microscopy. Note the many cavities (arrows) on the surface membrane of the cells. × 3000.

In our material, it was difficult to interpret the response of microfilaments to exposure to cytochalasin B. After treatment, the arrangement of the apically situated ring of microfilaments was disturbed, although the filaments themselves were not entirely disrupted. One result was most apparent, however: cytochalasin B fully inhibited interkinetic nuclear migration (figure 10). Considering the arrangement of the microfilaments, their alignment perpendicular to the direction of movements and knowing that cytochalasin B has no stabilizing effect (much to the contrary) on these filamentous structures, we find it difficult to envisage how they could be related to nuclear migration. With this in mind, and considering the evidence pointing to microtubules as an active force in interkinetic nuclear migration, a process effectively inhibited by cytochalasin, it was found necessary to ascertain experimentally if the drug could affect microtubules[44].

Again we reverted to our procedure of cold exposure followed by chemical treatments. Cytochalasin was used at concentrations (5 µg/ml) sufficient to inhibit interkinetic nuclear migration and neurulation. Experiments were designed to provide answers to the following questions: a) Is cytochalasin a stabilizing chemical, does it protect microtubules against cold disruption? b) Can the drug prevent the repolymerization of microtubules already disrupted by cold expoure? c) Can a stabilizing or protective agent for microtubules (i.e. one that prevents their degradation at 2 °C) block the inhibitory action of cytochalasin on interkinetic nuclear migration?

Dibutyryl cyclic AMP (N^6, O^2-dibutyryl adenosine 3′, 5′· cyclic monophosphate, db-cAMP) was used as a protective agent. Indeed db-cAMP (0.5 mM) was found to protect microtubules of the neural tube cells of chick embryos against cold disruption. These results are concordant with those observed by Kirkland and Burton[45] in mouse neuroblastoma cells. We also felt that after treatment with db-cAMP, there were more microtubules in the neuroepithelial cells of our embryos (figure 11). This suggestion is in agreement with the demonstration of Roisen et al.[46] that db-cAMP induces the assembly of microtubules from a pool of subunits. However, despite the protective effect of db-cAMP on the microtubules of our specimens, cytochalasin B totally inhibited interkinetic nuclear migration. It was found also that cytochalasin did not prevent the disruption of microtubules after a 3-h stay at 2 °C, nor did it interfere with repolymerisation of microtubules once they had been disrupted by cold

(table 2). Finally, in some specimens treated concomitantly with both cytochalasin B and db-cAMP (and which showed inhibition of interkinetic nuclear migration), the associations or bands of microfilaments and, in fact, microfilaments themselves were at time hardly disturbed.

It is safe to conclude then that the inhibitory effect of cytochalasin B on interkinetic nuclear migration cannot be ascribed to a direct effect on microtubules. Logically it should now be verified whether the drug has any detectable effect on the plasma membrane of the neuroepithelial cells. This we attempted using the scanning electron microscope[47]. Recently, others have used this instrument to describe the morphological aspects of the surface membrane of cells exposed to cytochalasins. Jones[41] reported 'little significant change' in the surface morphology of neural retinal cells treated with cytochalasin B, whereas Everhart and Rubin[40] showed that chinese hamster ovary cells treated with the drug 'assumed a smooth or gently convoluted surface'. Finally, examining VeRo and HeLa cells with the scanning electron microscope, Miranda et al.[48] have shown that cytochalasin D induces the production of small zeiotic protuberances.

We have found the surface topography of neuroepithelial cells in the young chick embryo to be modified by exposure to cytochalasin B. Cells lose their slender finger-like projections, the surface of the membrane abandons much of its ruffling and acquires cavities of varied shape and diameter. These cavities are observed on the surface of the spherical cells, produced in great number by cytochalasin. They are found as well on elongated cells, where they usually decorate the basal region of the cell. In the neuroepithelial cells of the young chick embryo, microfilaments are grouped together at the cellular apex where they form a thin circular band oriented perpendicular to the cell's long axis. The localization of these microfilaments, and the fact that cavities occur at the base of the cells, indicates that the alterations in surface membrane topography probably do not result from an effect of cytochalasin B on microfilaments. On the contrary, our observations suggest that, in our material, the drug directly attacks the plasma membrane (compare figures 12–14). However, our demonstration of a direct effect of the drug at the cell surface, provides no information about the manner in which it inhibits nuclear migration. It remains to be determined how the morphological alterations observed here reflect changes in the properties of the membrane or lead to changes in the state of some cellular component that would ultimately prevent the normal occurrence of interkinetic nuclear migration.

Table 2. Treatments used to test the effects of cytochalasin B on microtubules

Treatments	Interkinetic nuclear migration	Microtubules
1. N at 38 °C	+	+
2. N at 38 °C, then 2 °C (3 h)	—	—
3. CB at 38 °C (2 h), then at 2 °C (3 h)	—	—
4. CB at 38 °C (2 h), then at 2 °C (3 h), and return to 38 °C (1 h)	—	+
5. db-cAMP at 38 °C (4 h)	+	+
6. db-cAMP at 38 °C (1 h), then at 2 °C (3 h)	—	+
7. CB + db-cAMP at 38 °C (3 h)	—	+

Embryos were incubated on unmodified Spratt (1950) (N) media or on cytochalasin B (CB) enriched ones, or on media containing db-cAMP alone or with cytochalasin B[44]. Cytochalasin, which does not prevent microtubules from being cold-disrupted (3.), nor from repolymerizing after cold exposure (4.), inhibits interkinetic nuclear migration even if db-cAMP, which is a protective agent (6.), is present in the media (7.).

Over the past 50 years many hypotheses have dealt with the mechanism of the invagination of the neural plate. As early as 1924, Giersberg[49] dismissed the possibility that tissues neighbouring on the neural plate would intervene mechanically to force the closure of the neural tube. However, on that account, a more recent study[11] proposed that the myotomes and the epidermal ectoderm can build up pressure, which in concert with other forces, favors neurulation in *Xenopus*. On the other hand, concepts based on differential water uptake[50] and mitotic activity[51] have been discarded. In fact, in recent years, investigators have turned to the cell itself in their search for the motive force in the invagination of the neural plate[15]. It is to be expected that the agents which are capable of inducing the cellular deformation that leads to neurulation, lie within the cell itself[15].

Sauer[52], as early as 1937, found it 'necessary to invoke forces within the cell itself to account for most cases of elongation'. Two sets of observations may be given as examples in support of the idea that existing internal forces within the neuroepithelial cells determine the capacity of the cell to elongate, to modify its form. First, Holtfreter[53], using cells isolated from the neural plate of salamanders, showed that in culture they retain their columnar shape and even continue to elongate. Secondly, Adler[54] demonstrated that dissociated cells from the optic lobe of 4-day-old chick embryos form aggregates with a placode-like structure which will eventually invaginate to give a tube-like structure. During invagination the cells undergo structural changes: they become wedge-shaped. This occurrence of structural changes in the cells of an invaginating tissue, reconstituted from a suspension of dissociated cells, suggests that the capacity of cells to change their shape might very well be intrinsic to the cells themselves.

One interesting issue to pursue would be to search for cytoplasmic agents capable of inducing cell deformations as observed during neurulation. Many investigators[10,11,13,15] are of the opinion that microtubules force, or maintain, cellular elongation, whereas an apical ring of microfilaments[1,13,15] would constrict the cell apices, thereby producing the typical wedge- or

bottle-shape observed in neuroepithelial cells. There does exist a correlation between the presence of microtubules and the elongated shape of the cells making up the neuroepithelium[12], just as such a correlation exists in other cell types[55,56]. Still, it is not well understood how microtubules function mechanically in elongating cells. On this point, Burnside[15] examines 3 hypotheses, favoring the one whereby microtubules would 'extend the basal end of the cells by a basally directed transport of cytoplasmic elements'. Interkinetic nuclear migration, which causes an accumulation of basally located nuclei may, as proposed by Langman et al.[24], induce the cellular deformations, which lead to the invagination of the neuroepithelium. These nuclear movements are related to the presence of microtubules. Burnside's favored hypothesis on the mechanism of microtubular activity is compatible with such migratory movements. Moreover, Zwaan and Hendrix[57] and Hendrix and Zwaan[58], in their studies of lens development in the chick, stress the role of interkinetic nuclear migration as a force that induces the cell deformations which lead to the invagination of this epithelium.

To summarize, it is felt that the invagination of the neural plate in the chick embryo could result from the broadening of the basal region of the cells, which is induced by nuclei accumulating in the outer zone of the neuroepithelium. This accumulation of nuclei derives directly from interkinetic nuclear migration, a phenomenon linked to the presence of microtubules. The view that microtubules are involved in broadening the base of the cells does not minimize the role allotted by others to microfilaments in neurulation. These microfilaments whether they contract or not, or whether they only mechanically maintain cell apices in a constricted state, appear to be well localized in order to favor the induction and maintenance of the slender cell necks. This allows the microtubules to move the nuclei basally. The increase in volume of the basal pole of juxtaposed cells, in a situation where the volume of apices remains relatively constant, progressively creates the strain that forces the invagination of the neuroepithelium. Also, our considerations do not exclude the possible involvement of other cellular features. For instance, endocytosis, or the resorption of membrane at the cellular apex would also increase cellular asymmetry[3] and the attachment devices, which guarantee cohesion in the epithelium, could be actively involved in morphogenesis. The role of intercellular adhesiveness in morphogenesis has been discussed by Weiss[59] and by Gustafson and Wolpert[60], who stress its significance in invaginating epithelia, and by Adler[54], who shows experimentally the importance of cell contracts in the invagination of neural tissue.

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1 P. C. Baker and T. E. Schroeder, Devl Biol. *15*, 432 (1967).
2 B. Burnside, Devl Biol. *26*, 416 (1971).
3 J. Lofberg, Acta Univ. upsal. *319*, 1 (1974).
4 N. K. Wessells and J. Evans, Devl Biol. *17*, 413 (1968).
5 T. L. Pearce and J. Zwaan, J. Embryol. exp. Morph. *23*, 491 (1970).
6 J. T. Wrenn and M. K. Wessells, J. exp. Zool. *171*, 359 (1969).
7 B. S. Spooner and N. K. Wessells, Proc. nat. Acad. Sci., USA *66*, 360 (1970).
8 J. T. Wrenn, Devl Biol. *26*, 400 (1971).
9 C. H. Waddington and M. M. Perry, Expl Cell Res. *41*, 691 (1966).
10 P. E. Messier, J. Embryol. exp. Morph. *21*, 309 (1969).
11 T. E. Schroeder, J. Embryol. exp. Morph. *23*, 427 (1970).
12 C. Auclair and P. E. Messier, Rev. Can. Biol. *33*, 33 (1974).
13 P. Karfunkel, Devl Biol. *25*, 30 (1971).
14 P. Karfunkel, J. exp. Zool. *181*, 289 (1972).
15 B. Burnside, Am. Zool. *13*, 989 (1973).
16 P. E. Messier and C. Auclair, J. Embryol. exp. Morph. *30*, 661 (1973).
17 F. C. Sauer, J. comp. Neurol. *62*, 377 (1935).
18 F. C. Sauer, J. comp. Neurol. *63*, 13 (1935).
19 F. C. Sauer, J. Morph. *60*, 1 (1936).
20 S. Fujita, Nature *185*, 702 (1960).
21 R. L. Watterson, P. Veneziano and A. Bartha, Anat. Rec. *124*, 379 (1955).
22 M. E. Sauer and A. C. Chittenden, Expl Cell Res. *16*, 1 (1959).
23 M. E. Sauer and B. E. Walker, Proc. Soc. exp. Biol. Med. *101*, 557 (1959).
24 J. Langman, R. L. Guerrant and B. G. Freeman, J. comp. Neurol. *127*, 399 (1966).
25 P. E. Messier, Experientia *27*, 304 (1971).
26 C. Jensen and A. Bajer, J. Ultrastruct. Res. *26*, 367 (1969).
27 D. Mazia, Symp. int. Soc. Cell Biol. *6*, 39 (1967).
28 P. E. Messier, J. Embryol. exp. Morph. *27*, 577 (1972).
29 L. E. Roth, J. Cell Biol. *34*, 47 (1967).
30 M. A. Handel and L. E. Roth, Devl Biol. *25*, 78 (1971).
31 P. E. Messier, J. Embryol. exp. Morph. *35*, 197 (1976).
32 A. C. Nevo, D. Mazia and P. J. Harris, Expl Cell Res. *62*, 173 (1970).
33 P. E. Messier and C. Auclair, Devl Biol. *36*, 218 (1974).
34 S. B. Carter, Nature *213*, 261 (1967).
35 H. Holtzer and J. Sanger, Devl Biol. *27*, 443 (1972).
36 N. K. Wessells, B. S. Spooner, J. F. Ash, M. O. Bradley, M. A. Luduena, E. L. Taylor, J. T. Wrenn and K. M. Yamada, Science *171*, 135 (1971).
37 R. A. Cloney, Z. Zellforsch. mikrosk. Anat. *132*, 167 (1972).
38 J. G. Bluemink, Z. Zellforsch. mikrosk. Anat. *121*, 102 (1971).
39 L. P. Everhart and R. W. Rubin, J. Cell Biol. *60*, 434 (1974).
40 L. B. Everhart and R. W. Rubin, J. Cell Biol. *60*, 442 (1974).
41 G. E. Jones, J. Cell Sci. *17*, 371 (1975).
42 L. Weiss, Expl Cell Res. *74*, 21 (1972).
43 N. K. Wessells, B. S. Spooner and M. A. Luduena, Ciba Foundation Symposium *14*, 53 (1973).
44 P. E. Messier and C. Auclair, J. Embryol. exp. Morph. *34*, 339 (1975).
45 W. L. Kirkland and P. R. Burton, Nature *240*, 205 (1972).
46 F. J. Roisen, R. A. Murphy and W. G. Braden, Science *177*, 809 (1972).
47 P. E. Messier, Micron *7*, 1 (1976).
48 A. F. Miranda, G. C. Godman, A. D. Deitch and S. W. Tanenbaum, J. Cell Biol. *61*, 481 (1974).
49 H. Giersberg, Wilhelm Roux Arch. EntwMech Org. *103*, 387 (1924).
50 M. G. Brown, V. Hamberger and F. O. Schmitt, J. exp. Zool. *88*, 353 (1941).
51 R. Gillette, J. exp. Zool. *96*, 201 (1944).
52 R. C. Sauer, J. Morph. *61*, 563 (1937).
53 J. Holtfreter, J. Morph. *80*, 25 (1947).
54 R. Adler, Expl Cell Res. *68*, 395 (1971).
55 B. Byers and K. R. Porter, Proc. nat. Acad. Sci., USA *52*, 1091 (1964).
56 L. G. Tilney, Devl Biol., suppl. *2*, 63 (1968).
57 J. Zwaan and R. W. Hendrix, Am. Zool. *13*, 1039 (1973).
58 R. W. Hendrix and J. Zwaan, Nature *247*, 145 (1974).
59 P. Weiss, Expl Cell Res., suppl. *8*, 260 (1961).
60 T. Gustafson and L. Wolpert, Int. Rev. Cytol. *15*, 139 (1963).