Inhibition of nuclear migration in the absence of microtubules in the chick embryo

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SUMMARY

Interkinetic nuclear migration was studied in cells of the forming neural tube of cultured chick embryos aged from 1 to 7 pairs of somites. In specimens that were first exposed to 2°C for 3 h, then treated with $4 \times 10^{-3}$ M monoiodoacetamide and finally transferred to new media to be reincubated for 1–2.5 h, it was found that microtubules were absent and nuclear movements blocked. These findings point to microtubules as essential structures for interkinetic nuclear migration. Their relation to the problem of neurulation is discussed.

INTRODUCTION

Histological studies dating back to 1936 (Sauer, 1936) indicated that nuclei migrate in the cells of the early neural tube. Work done with colchicine (Watterson, Veneziano & Bartha, 1956; Woodward & Estes, 1944) offered the first experimental confirmation of this concept and the use of autoradiography (Sauer & Walker, 1959) gave evidence substantiating the occurrence of interkinetic nuclear migration; namely it was shown that DNA synthesis occurs only in nuclei situated in the peripheral region of the wall of the neural tube. Moreover, in their study of the behaviour of neuro-epithelial cells during the closure of the neural tube, Langman, Guerrant & Freeman (1966) indicated that during DNA synthesis the nuclei are located near the basement membrane of the tube. Subsequently nuclei migrate to the lumen to undergo mitosis, after which they return to the outer zone to start DNA synthesis for the next cell division. These authors found the cell generation time to be 8 h.

Many reports suggest that there is a correlation between microtubular integrity and nuclear movements (Woodward & Estes, 1944; Watterson, 1965) or organelle orientation (Dahlstrom, 1968; Holmes & Choppin, 1968). Along this line Pearce & Zwaan (1970), treating chick embryos in vitro with colcemid, showed a steady accumulation of dividing nuclei at the luminal surface of lens placodes and an almost total absence of microtubules in these cells, thus confirming the existence of interkinetic nuclear migration in lens placodes and suggesting the involvement of microtubules in nuclear movements. We have obtained similar results in the neural tube of chick embryos exposed to colchicine in vitro (Messier, 1972).

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Recent work done with cytochalasin B (Karfunkel, 1972) has implicated apically situated microfilaments as the main force involved in the cell deformations that ensure the closure of the neural tube as first proposed by Baker & Schroeder (1967). Microtubules here appear to be essentially structures involved in the maintenance of cell shape, as they were shown to be in numerous other systems (see reviews by Porter, 1966; Tilney, 1968). However, should microtubules be motive agents in nuclear migration, the localized tissue expansion they would induce would be as important to neurulation as the contraction of apically located microfilaments. Thus both structures (microfilaments and microtubules) would have to be considered essential to the morphogenetic movements of neurulation.

With these issues in mind we have further investigated the phenomenon of interkinetic nuclear migration in cells of the forming neural tube of chick embryos. We devised a system whereby microtubules are disrupted (cold exposure) and prevented from repolymerizing (exposure to monoiodoacetamide) during the time specimens are brought back to physiological temperature.

**Materials and Methods**

**Specimens**

All embryos, of the chicken *Gallus domesticus*, were explanted at stages 1-7 pairs of somites (stages 7-9; Hamburger & Hamilton, 1951) and cultivated on Spratt's culture medium (1950) at 38 °C for up to 6 h. Since in our experimental design many treated embryos had to be transferred from one culture medium to another, control embryos were first incubated at 38 °C for 3 h on a normal Spratt's medium and later transferred to a similar medium to check if the actual manipulation affected the embryos.

**Treatments**

(1) Embryos to be cold-treated were first incubated at 38 °C for 3 h on their culture media and then brought into a cold room at 2 °C and kept there for 3 h. They were fixed in the cold.

(2) Some embryos, cold-treated as above (1), were brought back to their normal 38 °C incubation temperature for periods varying from 1 to 6 h.

(3) Other cold-exposed embryos, following their 3 h treatment were covered with, and left in contact with $4 \times 10^{-3}$ M monoiodoacetamide (MIA) for 15 min in the cold room. They were then thoroughly rinsed in a chick Ringer solution, transferred to a new Spratt medium and reincubated at 38 °C for periods ranging from 1 to 4 h. Other concentrations, covering the $10^{-2}$ to $10^{-3}$ M range, were also experimented with but were found less useful (see Discussion). Only MIA at $4 \times 10^{-3}$ M was used extensively and this is the only concentration referred to throughout the present work.

(4) Some embryos, cultured normally for 3 h at 38 °C, were covered with, and
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left in contact with $4 \times 10^{-3}$ M MIA for 15 min. They were then rinsed and transferred to new media and reincubated at 38°C for 1–2.5 h.

(5) Some embryos exposed to MIA as above (4) were washed, transferred to Spratt’s media enriched with $2.5 \times 10^{-5}$ M vincristine sulfate and incubated at 38°C for 1 and 2.5 h.

Microscopy

All embryos were fixed for 60 min in 1.25% phosphate-buffered glutaraldehyde and post-fixed for 1 h in 1% phosphate-buffered osmium tetroxide. After dehydration in ethanol solutions, the specimens were flat embedded in Epon. The thin sections, obtained with an LKB ultrotome, were stained at room temperature, first with 1% aqueous uranyl acetate for 20 min and then with lead citrate according to Reynolds (1963) for the same period of time. The grids were examined with a Siemens IA electron microscope. Sections 1 μm thick, stained with a 1% aqueous solution of toluidine blue saturated with sodium borate, were used for light microscopy.

Observations

Untreated embryos

Embryos first incubated for 3 h at 38°C on a normal Spratt culture medium, then transferred to another similar medium and reincubated for 4 more h, grew normally and at the expected rate of 1 somite pair per h.

In accordance with what is well known in normal embryos, mitotic figures were observed only near the neurocoele – that is, along the inner surface of the forming neural tube (Fig. 1A). Since the ultrastructure of such material has been described elsewhere (Messier, 1969), it will not be presented here. It is sufficient to recall that the forming neural tube cells (1) are highly asymmetric, with their long cell neck extending to the lumen and their interphase nucleus situated in the peripheral region of the tube wall, (2) contain numerous microtubules oriented in a direction parallel to that of the cell’s long axis.

Exposed at 2°C

Exposure to cold fully inhibited embryonic development. Low temperatures are known to induce depolymerization of microtubules and 4°C treatments of chick embryos have been used successfully by Handel & Roth (1971) to rid neural tube cells of their microtubules. Three-hour treatments of embryos at 2°C confirmed Handel & Roth’s observations: there was a reduction in the asymmetry of cell shape, intercellular spaces expanded considerably, long microvillus-like extensions appeared along the lateral and apical surfaces of cells, ribosome crystals formed and all microtubules not associated with cilia, centrioles or midbodies disappeared. Furthermore, we noted that nuclei tended to become spherical but that their mitotic index and distribution within the thickness of the neural epithelium was not disturbed (Fig. 1B, C).
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Exposure at 2°C and recovery at 38°C

Embryos exhibiting a flat neural plate following the 3 h cold exposure showed a closed neural tube after a 6 h recovery at 38°C. Most embryos, however, were incubated only for 4 h at 38°C following the cold treatment. They grew normally and gained the expected four somite pairs. In these embryos normal asymmetric cell shape was observed, the intercellular spaces disappeared together with the microvillus-like extensions and ribosome crystals (Fig. 2E). It was further noted that a 1 h recovery at 38°C was sufficient to allow microtubules to be seen characteristically aligned parallel to the cell's long axis. In 1 h the normal cell asymmetry and the elongated nuclei were restored.

Exposure at 2°C, MIA treatment and recovery at 38°C

Specimens incubated at 38°C for 2-5 h following cold and MIA treatments showed reduced intercellular spaces, no microvillus-like extensions and no ribosome crystals. In the very first hour that followed cold plus MIA exposures there was a noticeable accumulation of mitotic figures along the inner surface of the forming neural tube (Fig. 2A). After a 4 h period at 38°C many mitotic figures were located near the neurocoele but most were abnormally distributed throughout the thickness of the neural epithelium (Fig. 2B).

The forming neural tube cells of embryos submitted to these treatments lost their asymmetry and their nuclei tended to become spherical. Few or no microtubules could be found.

MIA treatment, and MIA followed by vincristine treatment

Embryos exposed to MIA while at 38°C and later transferred to normal media and incubated for 2.5 h at 38°C always showed considerable cytolysis in the basal region of the forming neural tube. Yet no undue accumulation of mitotic figures was ever detected in the neural epithelium of these embryos (Fig. 2C). Electron microscopy revealed that microtubules were present (Fig. 3A). However, when specimens were exposed to MIA while at their physiological temperature and then placed for 2 h on vincristine enriched media, an accumulation of blocked metaphases along the inner surface of the forming neural tube

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**Figure 1**

The scale line represents 10 μm in A and B, 1 μm in C.

(A) Light micrograph of a cross-section in the neural tissue of a normal embryo at stage 8. Mitotic figures (arrows) are observed near the neurocoele only. × 700.

(B) This specimen, at stage 8, was exposed for 3 h to 2°C. Note the tendency for a loss in asymmetric cell shape and also large intercellular spaces. × 625.

(C) Electron micrograph of neural tube cells of an embryo kept for 3 h at 2°C. Large intercellular spaces populated with microvillus-like extensions are seen. × 3500.
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occurred, thus indicating that MIA did not prevent nuclear migration nor did it prevent the nuclei situated near the neurocoele from entering into mitosis (Fig. 2D). After such a brief treatment cytoplasmic microtubules, comparable to those found in the untreated embryos, could still be observed (Fig. 3B).

DISCUSSION

In accordance with Handel & Roth (1971) we found that the neural cells of early chick embryos exposed to low temperatures are devoid of microtubules. We found further that a period of recovery as brief as 1 h at 38°C is sufficient to allow microtubules to reappear.

In order to find conditions in which microtubules disrupted by cold treatment would be prevented from repolymerizing, a series of MIA concentrations combined with varying durations of exposure were tested. The chemical was either added to the media or the embryos were covered with it. Best results were obtained by covering the embryos with the chemical, provided they were rinsed thoroughly and transferred to new media. To begin with, MIA at a given concentration and for a given period of time was tested on embryos exposed to 2°C. If the treatment blocked microtubular repolymerization it was then tried on other embryos at 38°C and the degree of cytolysis was then noted. In this manner it was found that concentrations in the $10^{-2}$ M range, which hindered repolymerization of microtubules, were completely destroying cells when tested in brief exposure at 38°C. On the other hand, MIA at $10^{-3}$ M, which had no

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**Figure 2**

The scale line represents 10 μm in A–D, 1 μm in E.

(A) Light micrograph of an embryo exposed to 2°C, treated with MIA and brought back to 38°C for 1 h. More than a dozen mitotic figures (arrows) can be counted along the inner surface of the forming neural tube. × 800.

(B) Embryo treated as in (A) above but brought back at 38°C for 4 h. Many mitotic figures (arrows) are seen abnormally distributed within the thickness of the neural epithelium. × 500.

(C) Light micrograph of a transverse section of the first somite region of an embryo exposed to MIA at 38°C and transferred to a normal media for 2.5 h. Considerable cytolysis is observed at the basal region of the forming neural tube. However the *lamina basa* is not affected (arrow). There is no undue accumulation of mitotic figures. × 800.

(D) Light micrograph of a transverse section cut high in the cephalic region of a malformed embryo exposed to MIA at 38°C and treated with vincristine for 2 h. Numerous mitotic figures (arrows) are seen accumulated along the inner surface of the neural tube. × 700.

(E) Neural tube cells of an embryo exposed to 2°C for 5 h and brought back to 38°C for 3 h. The asymmetric cell shape has reappeared as evidenced by the long narrow cell necks extending to the lumen on the right. Inter cellular spaces assume normal proportions and are free of microvillus-like extensions. Microtubules (arrows) are present. × 24000.
cytolytic effect at 38°C, was ineffective, even in long exposures, in blocking microtubular repolymerization. Finally it was found that 4 \times 10^{-3} \, \text{M} was effective in preventing repolymerization of microtubules disrupted by cold exposure while it produced a minimum of cytolysis when administered at 38°C. Therefore only MIA at 4 \times 10^{-3} \, \text{M} was used extensively and this is the only concentration referred to throughout this work.

Monoiodoacetamide given at 38°C and at a 4 \times 10^{-3} \, \text{M} concentration, even for a brief exposure, was found to be fatal only to the cells of the basal region of the forming neural tube. The localized cytolysis induced was repeatedly observed from the first somite pair area down to the caudal region of the embryo. Such cellular destruction does not seem to be due to purely mechanical stress (e.g. tearing, collapsing) since the slender and delicate lamina basa was always present and undisturbed. This reaction to the drug is all the more surprising when we consider that all embryos were fully covered with MIA during treatment and incubated with their ectoderm facing the culture media. At present, we are unable to determine whether the destroyed cells took up more MIA or whether they were more sensitive to the drug than the rest of the cells of the neural epithelium.

Vincristine, an antimitotic agent, has been shown by Langman et al. (1966) to be ineffective in preventing nuclei from ascending to the inner surface of the neuro-epithelial cells in the chick embryo. After vincristine treatment mitotic figures accumulated along the inner surface of the neural tube. In preliminary experiments with vincristine we confirmed these observations and further noted that the drug did not affect cytoplasmic microtubules. In embryos exposed to MIA at 38°C then treated with vincristine we found an accumulation of arrested metaphases which were situated only along the inner surface of the forming neural tube, as though MIA had not been used at all. It seems therefore that MIA, which, we agree, affects many cellular processes, at least does not prevent these cells from entering into mitosis nor does it disturb interkinetic nuclear migrations when the chemical is administered at 38°C. Furthermore, in embryos treated with MIA and vincristine at 38°C, morphologically intact microtubules were always present, thus indicating that neither MIA nor vincristine, as used here, affected these organelles.

Our results indicate that MIA, when administered to embryos following cold exposure, prevents repolymerization of microtubules and inhibits nuclear migra-
tions. This is evident as 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 MIA and brought back to their normal incubation temperature for 4 h. It appears that MIA, which does not affect ready formed cytoplasmic microtubules, prevents their repolymerization when they are disrupted by cold treatment. The chemical, a free SH group inhibitor, can indeed impair normal polymerization of microtubules. Roth (1967) obtained similar results with urea which impairs 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 amebae whereas it prevented their reappearance once the specimens had been exposed to low temperature and treated with the drug.

Pearce & Zwann (1970) raised the issue that morphogenetic movements in lens placodes and the neural plate might not be strictly comparable. They have shown that the former will invaginate even when most microtubules are absent from the epithelial cells whereas Langman et al. (1966) concluded that nuclear migration is necessary for the normal invagination of the neural plate. We agree that nuclear migration does induce localized tissue expansion at the base of the neural epithelium so as to permit neurulation to occur.

Now, our present work indicates that microtubules are essential for the occurrence of nuclear migration and therefore the tubular organelles should be considered as important to the closure of the neural plate as microfilaments are thought to be (Baker & Schroeder, 1967; Karfunkel, 1972). Our conclusion, which relates microtubules to nuclear migration, is based on the fact that cells kept from having normal microtubules through $4 \times 10^{-3}$ M MIA treatment (following cold exposure) do not show nuclear migration; indeed mitotic figures are found throughout the thickness of the epithelium. No other cell structure was seen to respond to our experimentation as microtubules did. Surely MIA affects many cellular processes, but at $38^\circ$C and used at the same concentration as above it was shown to be ineffective in blocking nuclear movements (mitotic figures accumulated only near the neurocoele under MIA + vincristine treatments) and also ineffective in disturbing microtubules if they had not already been disrupted by cold exposure.

RÉSUMÉ

L’inhibition des mouvements nucléaires en l’absence des microtubules chez l’embryo de poulet.

Chez des embryons de poulet ayant atteint les stades de 1 à 7 paires de somites, les cellules du tube neural ont servi à l’étude des mouvements nucléaires intercinétiques. Chez des spécimens qui sont à la fois (a) maintenus à 2°C pendant 3 h, (b) mis en contact avec du monoiodoacétamide à la concentration de $4 \times 10^{-3}$ M, (c) explantés sur un nouveau milieu et réincubés à 38°C pour 1 à 2.5 h; on a noté l’absence de microtubules et l’inhibition des mouvements nucléaires. Ces résultats donnent à penser que les microtubules sont indispensables aux mouvements nucléaires intercinétiques et sont discutés dans le cadre des phénomènes de la neurulation.
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REFERENCES


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