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The occurrence of nuclear migration under thiol treatment effective in inhibiting neurulation

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SUMMARY

Early chick embryos were explanted on culture media containing mercaptoethanol and dithiothreitol in concentrations reaching 10^{-1} M and 10^{-2} M respectively. Nuclear migration was found to occur normally even under such thiol concentration, which is more than required to inhibit neurulation. Electron miscroscopy revealed that microtubules were not affected in their number and morphology. However, nuclear migration was blocked with $2{\cdot}5 \times 10^{-5}$ M colchicine; such treated embryos showed a loss of their microtubules. It is concluded that the two thiols do not act primarily on microtubules when they cause inhibition of neurulation.

INTRODUCTION

The search for a morphologic expression of the motive force in neurulation is centring around two structures: (1) cytoplasmic filaments, as seen by Baker & Schroeder (1967); (2) microtubules, as advocated by Waddington & Perry (1966). Both structures have been thought to be pertinent to cell deformation in a variety of systems (see Discussion).

Our recent study of the neural plate cells of the normal chick embryo (Messier, 1969) showed that filaments are quite scarce as compared, for instance, to what is seen in *Xenopus* (Baker & Schroeder, 1967). Microtubules, however, were abundant and we have shown them to be the only organelles morphologically altered when embryos were treated with dithiodiglycol (Messier, 1971).

Taking into consideration (1) the importance of –SH groups in morphogenesis (Rapkine & Brachet, 1951; Brachet & Delange-Cornil, 1959; Brachet, 1959), (2) the fact that both dithiodiglycol and mercaptoethanol inhibit neurulation in the chick embryo (Pohl & Brachet, 1962), (3) the indication by Wade & Satir (1968) that mercaptoethanol could react with –SH groups, thus preventing microtubular polymerization, we decided to investigate the effect of two thiols (mercaptoethanol and dithiothreitol) on microtubules.

To test this point specifically we followed nuclear migration. Indeed it is known that in many epithelia the nucleus migrates to the cell's free surface for division, thereafter returning to the basal region where DNA synthesis takes place.

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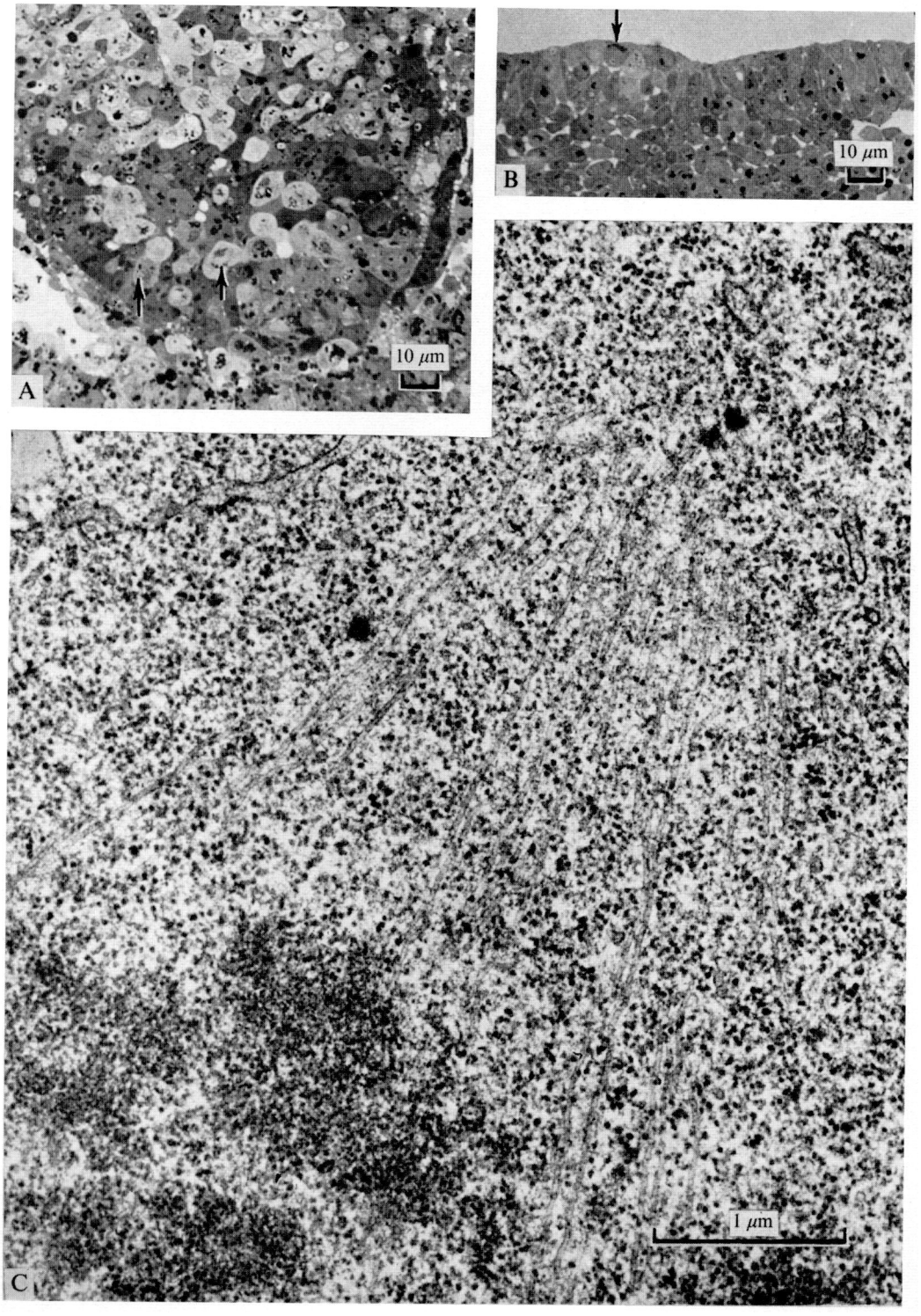
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Sauer in 1936 indicated that such migrations occur in the neural tube of the chick. This was confirmed by many authors and in 1966 by Langman, Guerrant & Freeman who evaluated the generation time of neuro-epithelial cells of the young chick embryo to be of 8 h.

Many evidences point to a correlation between microtubular integrity and nuclear movements (Woodard & Estes, 1944; Watterson, 1965) or organelle orientation (Dahlstrom, 1968; Holmes & Choppin, 1968) and recently Pearce & Zwaan (1970), treating chick embryos with colcemid, showed a direct relationship between the disappearance of microtubules and the arrest of migration in lens placodes.

Together with the electron microscope we use here nuclear migration as a test for microtubular integrity in experiments where chick embryos are subjected to mercaptoethanol or dithiothreitol – Cleland's reagent – at concentrations higher than those needed to inhibit neurulation. As a control of interkinetic nuclear migration in the neural tube, other embryos were treated with colchicine, in much the same way as colcemid was used in lens placodes by Pearce & Zwaan (1970).

MATERIALS AND METHODS

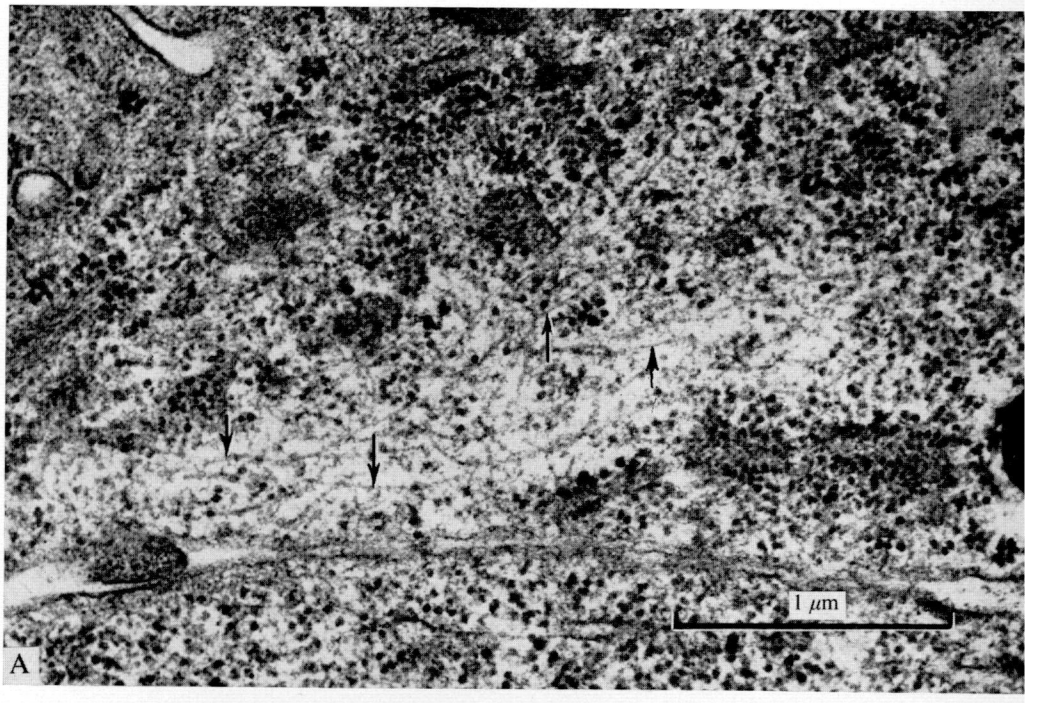
All embryos, of the chicken *Gallus domesticus*, were explanted at stages 2–11 somites (stages 7+ to 10+: Hamburger & Hamilton, 1951) and cultivated on Spratt's medium (1950) at 38 °C for periods of 6 and 8 h. For normal development, embryos were laid on 3 ml of this medium poured into a watch-glass which was seated on damp cotton-wool in a Petri dish.

Embryos to be treated were similarly cultivated on Spratt's medium to which was added mercaptoethanol in concentrations reaching 10^{-1} M or dithiothreitol in concentrations reaching 10^{-2} M. In the same way, $2{\cdot}5 \times 10^{-5}$ M colchicine was also used.

Embryos were fixed for 90 min in 1·25 % phosphate-buffered glutaraldehyde. They were post-fixed for 1 h in 2 % 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 0·5 % uranyl acetate in 50 % ethanol for 1 h and then with lead citrate according to Reynolds (1963) for 20 min. They were examined with a Siemens 1 A electron microscope.

FIGURE 1

(A) Light micrograph of part of a cross-section of a colchicine-treated embryo showing mitotic figures (arrows) in the outer edge of the neural tube. × 500.
(B) Light micrograph of a 10^{-2} M dithiothreitol-treated embryo. The neural plate is flat. There is no undue accumulation of dividing cells. Mitosis (arrow) is observed near the neurocoele only. × 500.
(C) Microtubules are seen as part of the mitotic apparatus in a dividing cell of a 10^{-1} M mercaptoethanol-treated embryo. × 28000.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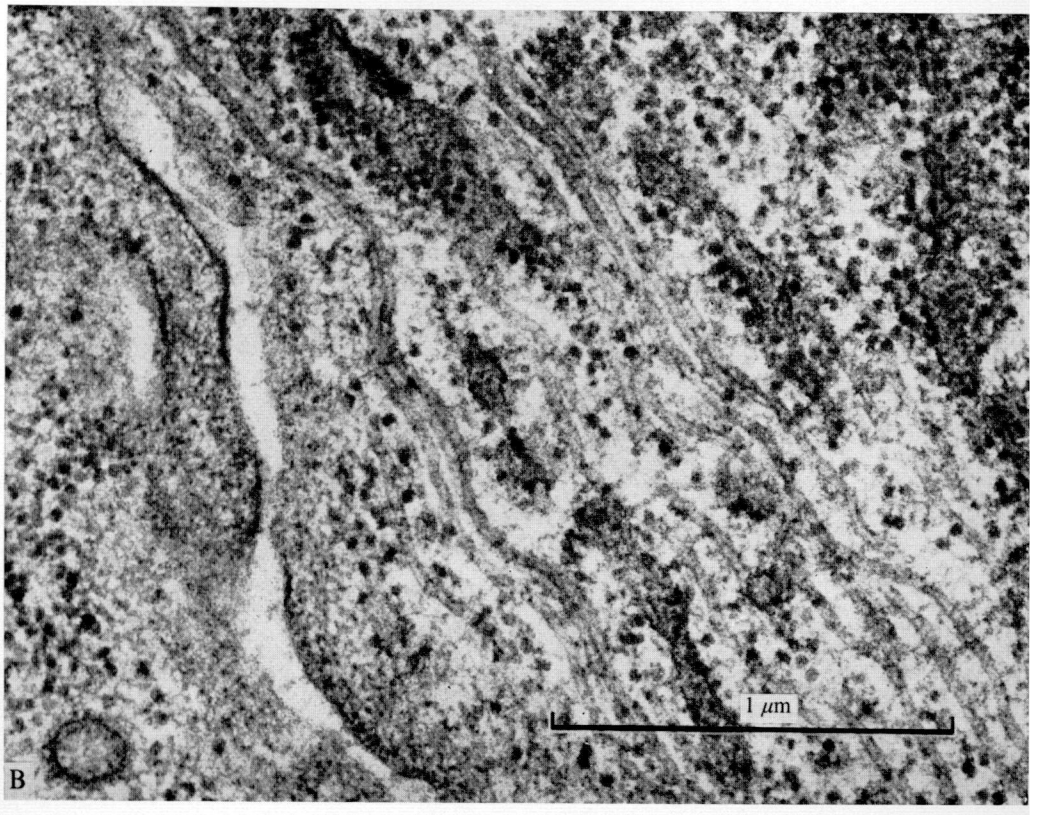
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Sections 1 μm thick, stained with a 1 % aqueous solution of toluidine blue saturated with sodium borate, were used for light microscopy.

OBSERVATIONS

Mercaptoethanol

In accordance with Pohl & Brachet (1962), we found that all embryos that had reached at least three somites when treatment began with mercaptoethanol would not neurulate, provided the concentration of the sulphydril reagent reached 10^{-2} M. As the ultrastructural aspects of such embryos have been described earlier (Messier, 1969), we shall mention here only that which is pertinent to the present title.

In the present work most of the embryos used were of 9–11 somites and they were subjected to 10^{-1} M mercaptoethanol, a concentration well over that sufficient to inhibit neurulation. This treatment only slightly retarded the segmentation of mesoderm into somites. Furthermore, in such embryos all stages of mitosis could be seen. Dividing cells were always observed near the neurocoele.

The electron microscope revealed that microtubules were abundant and apparently undisturbed by the treatment in both dividing (Fig. 1C) and non-dividing cells (Fig. 2B).

Dithiothreitol

Dithiothreitol was tried because it is known to be a reagent more effective than mercaptoethanol (Cleland, 1964). Its effect on the neural plate cells paralleled that of mercaptoethanol. Following a 6 h exposure to a 10^{-3} M concentration embryos did not seem to be affected and neurulation proceeded normally. Dithiothreitol at a 10^{-2} M concentration is fully effective in inhibiting neurulation whereas a 10^{-1} M concentration prevents the mesoderm from segmenting into somites.

Light-microscope analysis of embryos treated with 10^{-2} M showed that mitosis occurred and that interkinetic nuclear migration was allowed to proceed normally. This is evidenced by the fact that (1) dividing cells were not more numerous than would be expected from a consideration of the number of cells entering mitosis during the time period that the treatment lasted, (2) dividing cells were only observed near the neurocoele (Fig. 1B).

FIGURE 2

(A) Electron micrograph of a colchicine-treated embryo. Microtubules have disappeared and cytoplasmic filaments (arrows) accumulate. × 35000.
(B) Microtubules in part of a non-dividing cell of 10^{-1} M mercaptoethanol-treated embryo. × 52000.

Colchicine

As a control of nuclear migration embryos were treated for 6 h with $2{\cdot}5 \times 10^{-5}$ M colchicine. In such embryos the gross morphology of the neural tube was greatly altered. As recently shown by Handel & Roth (1971), colchicine treatment induced a rounding and a bulging of cells into the lumen together with an accumulation of cells in mitotic arrest. In our material some mitotic figures were seen in the outer edge of the neural tube, suggesting that premitotic nuclear migration had been blocked (Fig. 1A).

Electron microscopy confirmed the disappearance of microtubules and showed the accumulation of cytoplasmic filaments in regions normally occupied by microtubules (Fig. 2A).

DISCUSSION

A series of experiments on the role of sulphydril groups in morphogenesis led Brachet to the hypothesis that cell deformation, such as observed during the closure of the neural groove, is the result of reversible chemical alterations in a structure protein.

Brachet (1944) and Rapkine & Brachet (1951) suggested that oxidation of –SH groups into –S–S groups would transform a fibrous macromolecule, and as a result, the shape of cells would be changed. Mercaptoethanol, a strongly reducing sulphydril reagent, would prevent this normally occurring mechanism and indeed it has been shown to inhibit neurulation both in amphibians (Brachet, 1963; Brachet *et al.* 1961; Malpoix, Quertier & Brachet, 1963; Pohl & Quertier, 1963) and chick embryos (Pohl & Brachet, 1962; Pohl & Quertier, 1963).

As a result of work done with the electron microscope, Baker & Schroeder (1967) proposed that in amphibians the change in cell shape observed at neurulation is brought about through the contraction of a circular band of filaments located beneath the apical surface of the neural groove cells. Waddington & Perry (1966) added that microtubules might also be an active force in the cell deformation that ensures neurulation in amphibians. Recently, Karfunkel (1971) tested these proposals experimentally and found causal involvement of microtubules and microfilaments in neurulation.

In the past years a number of authors suggested that filaments (Cloney, 1966; Karfunkel, 1971; Baker & Schroeder, 1967) and/or microtubules (Tilney, 1971) may in some way influence cell shape. For instance such has been proposed for lens ectodermal cells of the chick (Byers & Porter, 1964), blastoporal cells of the newt (Perry & Waddington, 1966), primary mesenchymal cells of the sea urchin (Gibbins, Tilney & Porter, 1969) and primitive streak cells of the chick (Granholm & Baker, 1970).

Although we have experimented with numerous types of fixatives, buffer (pH and osmolarity), dehydration, different duration and temperature of fixation, etc., we have always observed this lack of filaments. Yet we cannot rule out that

the scarcity of filaments in our material is due to an inadequate technical procedure.

As for microtubules, they did not seem to be affected by our treatment. Mercaptoethanol and dithiothreitol, as they were used here, did not interfere with mitosis in the neural tube cells. Since the concentrations used were well over those needed to inhibit neurulation and noting that the slender tubules are present and apparently functional (as evidenced by nuclear migration), it is thought that microtubules might not be primarily involved when neurulation is inhibited following mercaptoethanol and dithiothreitol treatment.

Our study, though not excluding microtubules as a possible active force in neurulation, clearly indicates that they are not primarily affected when neurulation is inhibited following exposure to mercaptoethanol and dithiothreitol.

RÉSUMÉ

Migration nucléaire chez des embryons traités par des thiols en concentrations suffisantes pour inhiber la neurulation

De jeunes embryons de poulet ont été explantés sur des milieux de culture enrichis de mercaptoéthanol et de dithiothreitol à des concentrations atteignant respectivement 10^{-1} M et 10^{-2} M. De telles concentrations, supérieures à celles utilisées pour inhiber la neurulation, n'ont pas empêché les mouvements nucléaires de se produire. De plus, les traitements sont restés sans effet sur le nombre et la morphologie des microtubules. Il semble que les deux thiols utilisés n'agissent pas sur les microtubules lorsqu'ils sont employés comme inhibiteur de la neurulation.

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