A stereoscan analysis of cell surface characteristics during the interkinetic nuclear migration in normal and colchicine-treated developing chick retina

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Summary. The cell surface and shape changes taking place during the interkinetic nuclear migration are reported in normal neuroepithelial cells of chick retina, and after metaphase-arrest induced by colchicine. Persistence of the apical junctions and loss of basal connections during the preparation for mitosis have been demonstrated in these cells.

As in other embryonic pseudostratified epithelia[1-3], the neuroepithelium (NE) of the developing retina[4] undergoes interkinetic nuclear migration (INM). Several shape and surface-cell modifications are associated with INM[1,5], however, some of these features, such as the fate of the basal connections of the dividing cells, are still controversial[6]. Metaphase-arrest agents have been usefully employed to demonstrate INM and their effects have been studied by light microscopy[2,7,8] and transmission electron microscopy[8,9]. In order to analyze the shape and surface-cell changes related to INM we have observed the cell surface characters of the developing chick neural retina, and the effects induced by colchicine administration by scanning electron microscopy.

Materials and methods. Chick embryos ranging from stages[10] 14 to 16 were fixed for 6 h in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3. Embryos of the same stages were treated in ovo with 0.1 ml. of 5×10^{-6} M colchicine solution dissolved in saline. After 1, 4 and 6 h the embryos were removed and fixed in a similar way. Both were transferred to a buffer solution, where the eye rudiment was carefully frontally sectioned under a binocular microscope. The specimens were then dehydrated in a series of acetones, dried by the critical point method, coated with gold and examined with a Philips SEM-501.

Results and discussion. Normal embryos (figure 1). Both elongated and rounding cells can be observed in the inner wall of the developing optic cup. Elongated cells appear as columnar bipolar cells displaying a bulging nuclear region and 2 cell processes. The basal process lies over the basal lamina and the apical process reaches the luminal surface. Several types of rounding cells can be identified.

Some of them are degenerating cells[11] and appear to be located randomly scattered within the NE-cells. The others are located close to the lumen and represent different phases of dividing cells. As described in the cerebral vesicles of the rat[5], these rounding mitotic cells can appear pyriform, conical or globular in shape. Pyriform cells show a short, thin basal process, which we have never seen reaching the basal lamina. Conical cells display several filopodium-like basal processes which are thinner and scarce in globular cells. These processes are always lost, after a short traject, among the neighbouring cells. Except for the early pyriform cells numerous microvilli are observed over the cell surface of the mitotic cells.

Embryos treated with colchicine. Metaphase-blocked rounded cells are numerous at the luminal zone of the NE 1 h after treatment[2] (figure 2). They are globular in shape but do not display either thin basal filopodia or microvilli. Since superficial microvilli are related to active mitotic

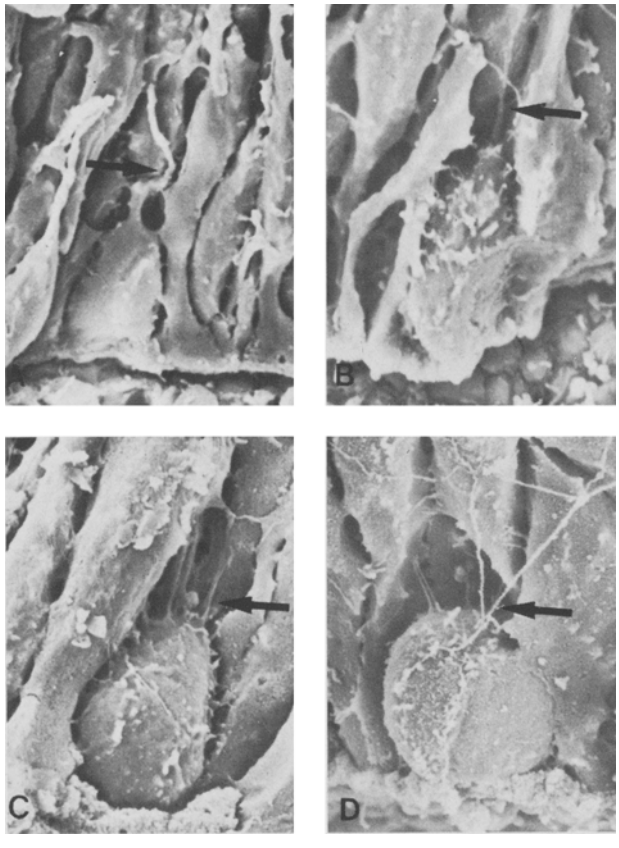

Fig. 1. Photographic composition showing differents stages of the rounding neuroepithelial cells of the embryonic retina. *A* Early pyriform cell displaying a thin basal process (arrow). *B* Late pyriform cell showing a thinner and shorter basal process (arrow). *C* Conical cell showing several basal filopodia (arrow). *D* Typical globular cell which still retains a few thin filopodia (arrow). Note the presence of microvilli and cell junctions within these mitotic cells. Stage 15. *A* × 2050. *B*, *C* and *D* × 3075.

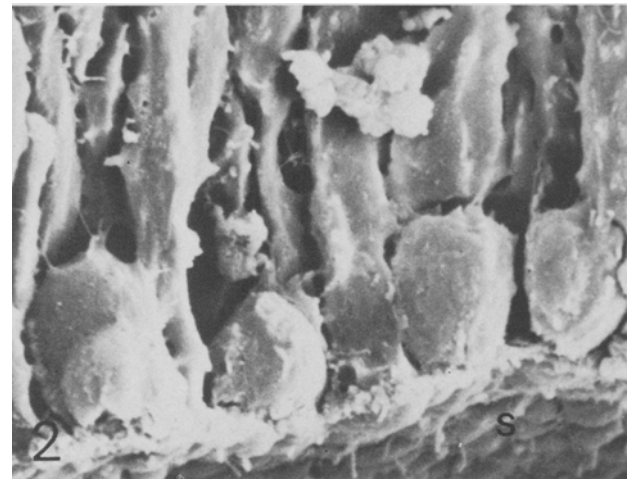

Fig. 2. SEM micrograph of the NE-retina 1 h after colchicine administration. Note the presence of several mitotic-arrested globular cells lacking both microvilli and basal filopodia. Cell junctions are not altered by the treatment. Intraretinal space (S). × 2590.

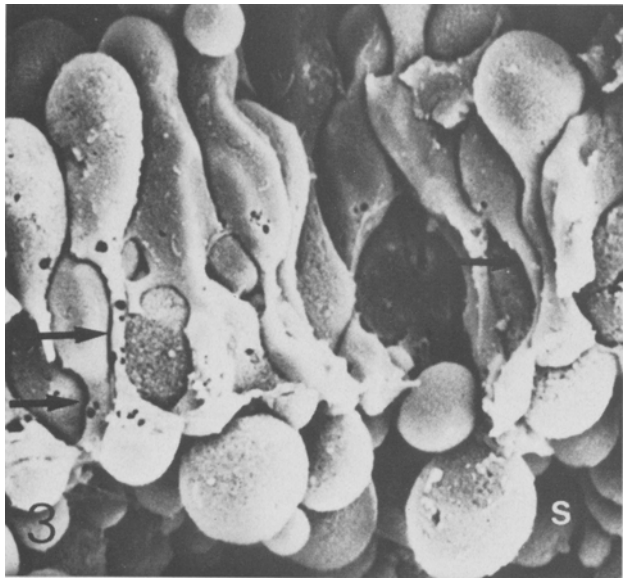

Fig. 3. SEM micrograph of the NE-retina 4 h after colchicine administration. Some globular cells are protruding towards the intraretinal space (S). The wall of the NE appears to be formed mostly by long cells lacking basal connections and displaying a conspicuous apical process (arrows). × 2590.

changes[12], it might be inferred that arrest of mitosis prevents their formation. After 4 h of colchicine exposure (figure 3) very conspicuous changes are observed in the NE. In addition to the luminal globular cells, cells displaying a very peculiar shape are now observed. These cells are not in contact with the basal lamina and show an apical process of variable length and a bulging region pointing towards the basal surface. The apical process very often shows a dilated spheric prominence protruding into the lumen. 6 h after exposure, these structural alterations are more intense (figure 4). Most of the cells are now presumably blocked in metaphase forming a 3- or 4-layered epithelium[2,7]. The cells are now rather rounded, but, with the exception of the ones in the luminal layer, they retain a thin apical process which is often observed to reach the luminal surface. The intercellular spaces appear to be reduced and cell surface alterations, consisting of the formation of flattened faces transforming the rounded cell profile into a rather polygonal shape, are also observed. These shape alterations can be related to disruptions of the cytoplasmic microtubules[13] and/or to cell membrane alterations[14]. In all of the experimental conditions some of the most luminal metaphase-arrested cells show holes in their surfaces, suggesting degenerative changes similar to those observed in dying interphasic cells[15]. The amount of these degenerating cells increases with progressive colchicine exposure; they appear in some instances to be detached into the lumen[8].

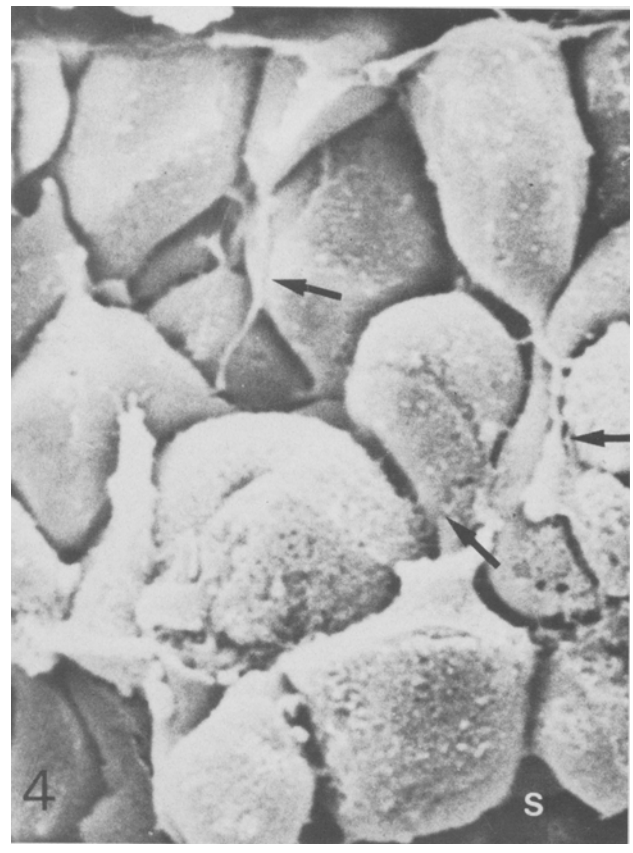

Fig. 4. NE-retina 6 h after colchicine administration. NE appears now as a polystratified epithelium formed by rounding mitotic arrested epithelial cells. Apical processes can, however, be recognized (arrows). Note that some cells are becoming polygonal in shape. Cell junctions can be also observed. Intraretinal space (S). × 5180.

Our observations of a loss of the basal connections during the preparation for mitosis are in agreement with previous similar studies carried out in other NE-tissues[5,16]. But it should be noted that the usual technical procedure employed for scanning electron microscopy observation cannot rule out possible artifacts due to the required fracture of the tissue. However, the observations under the experimental conditions employed in this study allow us to discard that possible interpretation.

An interesting feature observed in this study is that the metaphase-arrested cells, in spite of being arranged in succesive apposed layers, always retain a thin apical process which reaches the luminal surface. This fact supports the view that the persistence of the apical connections in dividing NE-cells[16,17] is not due to their normal apical arrangement but that it is a character which is retained even if the cells are induced to divide in an anomalous position.

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