Retinal glial cells in alcohol-treated chick embryos

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SUMMARY

In the present study we used electron microscope techniques to analyze the effect of ethanol on the inner layers of chick embryo retinas. We show that these layers are very strongly affected by alcohol during development, with a delay in differentiation, a considerable degree of destructuring of the inner layers, accompanied by a loss of the inner prolongations of Müller cells, and the glial cells' location at the retinal inner layers (inner plexiform layer and optic nerve fiber layer) replacing these degenerated inner prolongations. We performed a count and statistical study of the axonal loss from the optic nerve fiber layer of the retinas of ethanol-treated embryos and observed a loss of total axons, mostly of myelinic axons.

Key Words: Ethanol – Chick retina – Glial cells – Myelin

INTRODUCTION

Alcohol can produce defects in the central nervous system (CNS) (Abel, 1998). In the retina, the alterations reported include hypoplasia, atrophy, and changes in the course of the optic nerve (Beattle et al., 1983; Strömland, 1981a and b; 1985; Chan et al., 1991; Chmielewski et al., 1997), together with alterations in the synaptogenesis of the inner plexiform layer (IPL) (Chmielewski et al. 1997).

The glial cells of the chick retina can be classified in three types: 1) Müller cells (MCs) - glia able to develop the functions of astrocytes, oligodendrocytes, ependymocytes, and microglial cells that, during development, behave as radial glial cells to direct neuronal migration and help in retinal lamination (Prada et al., 1989; 1998; Willbold et al., 1998); 2) oligodendrocytes, which penetrate the retina from the optic nerve and are found in its peripapillary regions (Ono et al., 1998), containing a myelin-specific protein (MOSP) (Prada et al., in preparation); and 3) pericytes, which arise from the vascular structures of the pecten root. These migrate into the retina and are distributed in the innermost layers; when differentiated, they presumably act as microglia and macrophages (Prada et al., in preparation).

Here, using optical and electron microscope techniques and axonal counts, we analyzed the effect of alcohol on glial cell populations in the retina of the chick embryo.

MATERIAL AND METHODS

We used 25 fertile eggs of White Leghorn chickens incubated at 37° C and a humidity of 80-90%. The embryos were injected at E-6 with 100 µl of ethanol (50% v/v) in physiological saline directly into the vitelline sac (Means et al., 1988). The embryos were left to incubate until sacrifice at E-9, E-12, or E-18. The stages were as in the tables of Hamburguer and Hamilton (1951). Some embryos were studied as controls, injecting them under the same experimental conditions with 100 µl of a sterile saline solution. The animal care protocols used in our laboratory and University vivaria conform to the current national legislation (decree 223/1988. BOE no. 67) and

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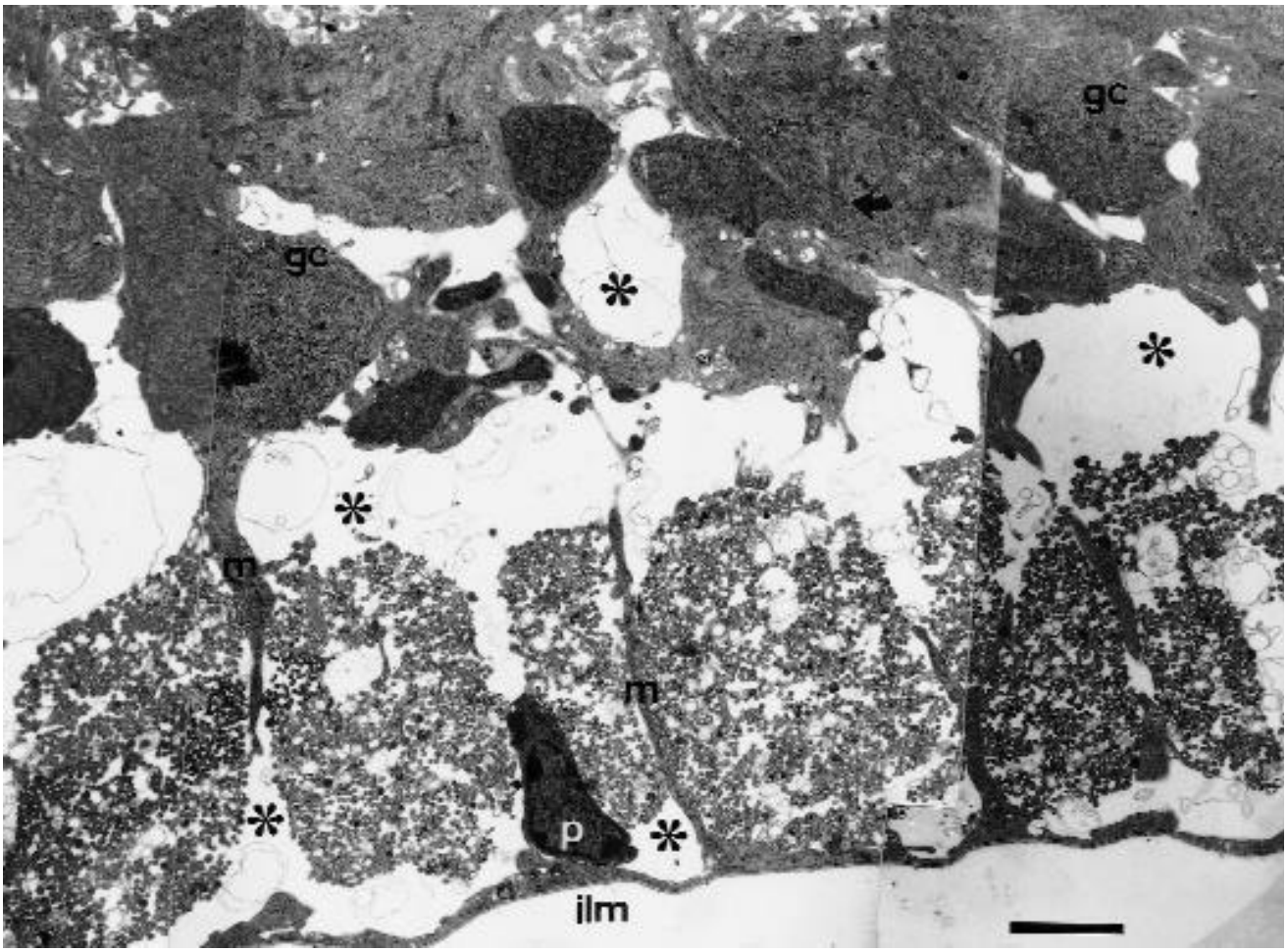


Fig. 1.- Ultrathin section of the GCL and ONFL of an embryo at E-18 exposed to 100 µl of ethanol (50% v:v) at E-6. Asterisks: increased intercellular spaces; gc: ganglion cells; m: inner prolongations of Müller cells; p: differentiated glial cell from pericytes arising from the vascular zone of the pectin; ilm: inner limiting membrane. Scale bar: 10 µm.

guidelines from the Council of the European Communities (86/609/CEE).

Fifteen of the embryos were sacrificed by immersion in a 0.9% sodium chloride solution. The eyes were enucleated in the same solution, and the retinas extracted complete. These were then mounted flat on gelatinized slides and left to dry until they adhered. The retinas were then stained with quick-acting Panoptic method. Observations were performed using an axioplan Zeiss microscope fitted with a photographic camera; the photos were taken on Ilford 400 ASA film.

The remaining 10 embryos were sacrificed at E-18, at 4° C for study of the retinas by electron microscopy using the method described by Palay and Chan Palay (1974) for the CNS. Ultrathin sections at 600-800 Å were obtained using an Ultracut ultramicrotome with a diamond blade and stained with uranyl acetate and lead citrate (Richardson, 1960). They were then examined under a Jeol 100C transmission electron microscope at 80 KV.

The counting of myelinic and amyelinic axons was performed in the retinas processed for electron microscope study.

RESULTS

We observed that alcohol administration produces a general delay in the differentiation of retinal cells. This is seen above all in the ganglion cell layer (GCL) (Fig. 2a, arrow) and in the optic nerve fiber layer (ONFL) (Figs. 1 and 2) where, at E-18, there were still abundant growth cones (Fig. 2a, cc).

In alcohol-treated embryos, the GCL elicits a considerable degree of destructuring, with increased intercellular spaces (Figs 1 and 2, *) and reduced cell body size. This reduction in GC size is essentially due to a loss of cytoplasmic mass, with a loss of organelles and mitochondrial degeneration (Fig. 1, arrow), leaving the GC reduced practically to the size of its nucleus (compare Figures 1 and 2, gc – experimental retinas – with Figure 3, gc – a control retina).

The ONFL undergoes a degeneration and destructuring of the inner prolongations of the MCs (compare Fig. 3, m – a control retina – with Figs. 1 and 2, m – experimental retinas). However, the inner limiting membrane, formed by the inner feet of the MCs, remains unaffected

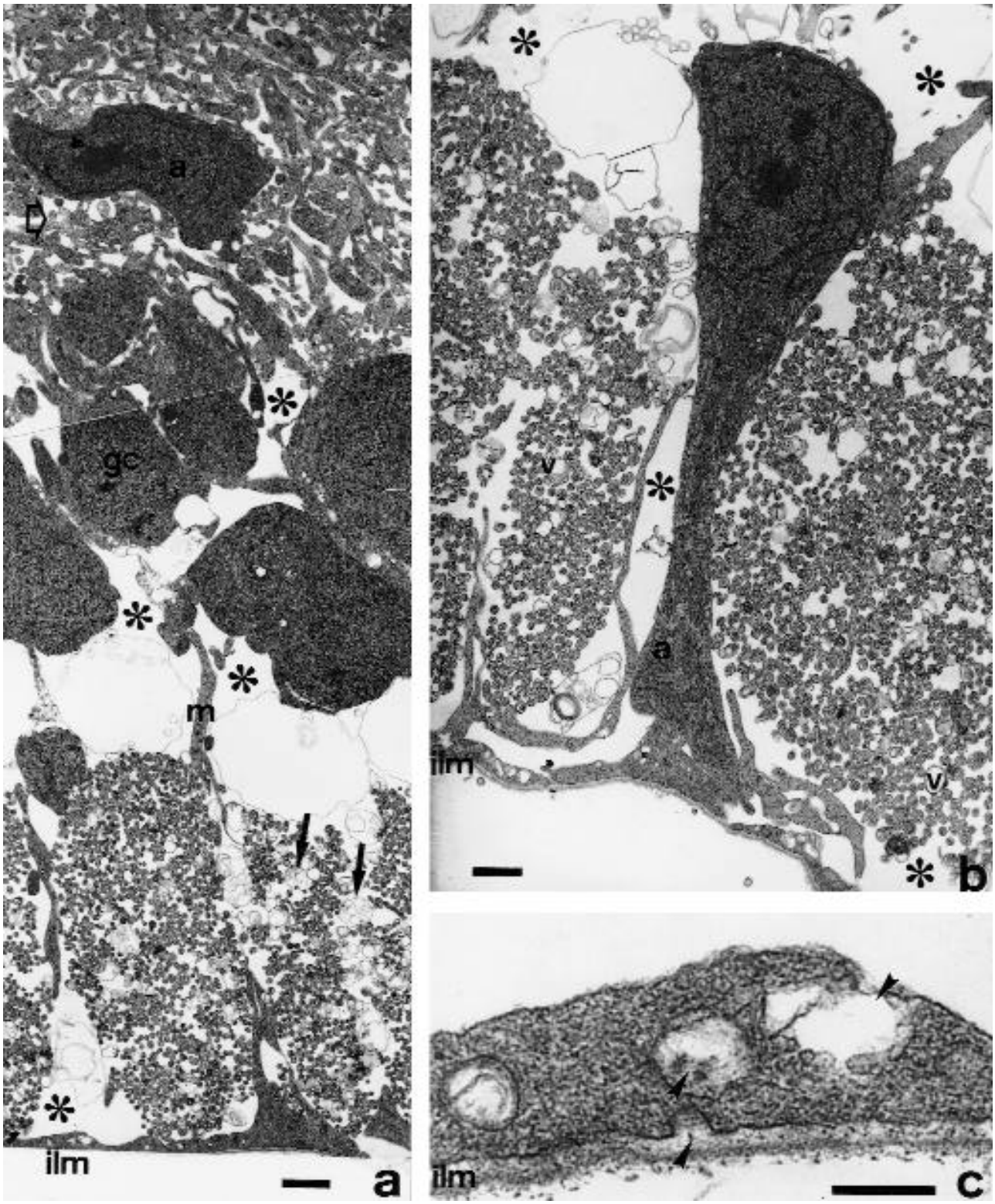
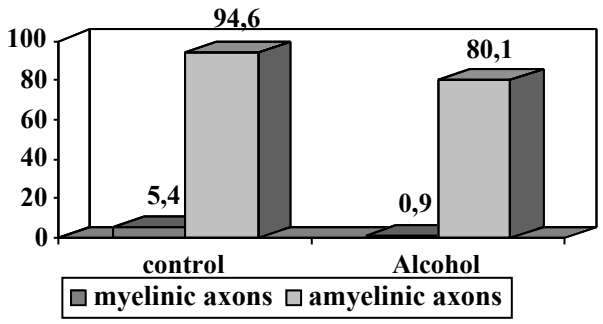


Fig. 2.- Ultrathin sections of the GCL and ONFL of an embryo at E-18 exposed to 100 µl of ethanol (50% v:v) at E-6. Asterisks: increased intercellular spaces; a: astrocyte; gc: ganglion cells; m: inner prolongations of Müller cells; hollow arrow: growth cone in the GCL; arrows: growth cones in the ONFL; v: vacuoles; arrowheads: vesicles; ilm: inner limiting membrane. Scale bars: 1 µm.

(Figs. 1 and 2, m, ilm). It also includes vesicles in different phases (Fig. 2c, curved arrows) compatible with endocytotic vesicles.

The increase in intercellular spaces mentioned above in connection with the GCL is more evident and pronounced in the ONFL (Figs. 1 and 2, *). Numerous vacuoles are seen (Fig. 2, v), possibly resulting from the degeneration of myelinic axons (compare Figs. 1 and 2 – experimental, where these axons are not seen – with Fig. 3, ma – control).

The Graphic 1 shows a comparative study of the number of myelinic and amyelinic axons in alcohol-treated embryos and control embryos. A



Graphic 1.- Percentaje of myelinic and amyelinic axons in the ONFL of control embryos and embryos treated with 100 µl of ethanol (50% v:v) at E-6.

decrease of approximately 20% can be seen in the total number of axons in the ONFL of the experimental embryos compared with the controls. In the ONFL of the treated embryos, only 0.9% of the myelinic axons remain, as compared with 80.1% of the amyelinic ones, while in the ONFL of the control embryos, myelinic axons constitute 5.4% of the total, and amyelinic ones 94.6%.

Despite the destructuring and degeneration of the inner prolongations of the MCs and the loss of axons, the ONFL is seen to retain its typical packing (Figs. 1 and 2). This can be explained partly by the presence of astrocytes, which adopt the position of the inner prolongations of the MCs in the ONFL (Fig. 2b, a). In the inner plexiform layer (IPL), the normal position of these astrocytes remains unaltered (Fig. 2a, a).

The pericytes that migrate through the ONFL during development are unaltered in the experimental retinas. This is seen in Fig. 4, showing pericytes in the process of migration during stages E-9, E-12, and E-18 in control retinas (photographs a, b, and d, respectively) and

Fig. 3.- Ultrathin section of the GCL and ONFL of a control embryo at E-18. gc: ganglion cells; m: inner prolongations of Müller cells; ma: myelinic axons. Scale bar: 1 µm.

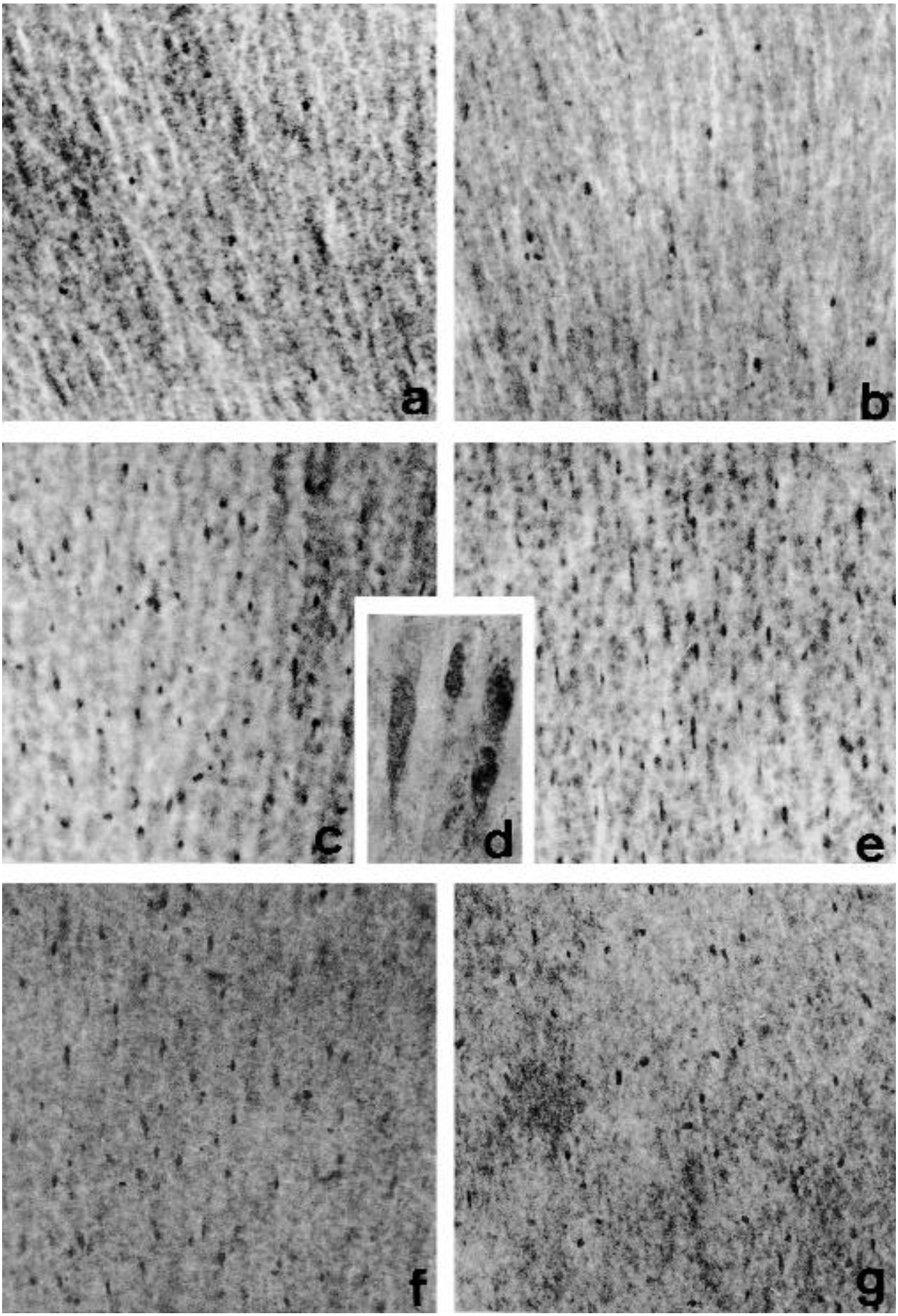


Fig. 4.- Flat mount of the ONFL of retinas of chick embryo, showing glial cells migrating through this layer. **a** (E-9), **c** (E-12) and **f** (E-18): retinas of control embryos; **b** (E-9), **d** (E-12), **e** (E-12) and **g** (E-18): retinas of embryos treated at E-6 with 100 µl of ethanol (50% v:v). a, b, c, e, f and g: x 200; d: x 1000.

experimental retinas (photographs e, f, and g, respectively). The count of this cell population shows that no appreciable difference occurs during development in adopting their migratory positions through the inner layers of the retina (Fig. 1, p).

Discussion

It is currently accepted that alcohol causes a delay in development (Miller, 1986; Quesada et al., 1990; Chmielewski et al., 1997). In the retinas of alcohol-treated embryos, this is seen in the existence at E-18 of numerous growth cones in the GCL (Fig. 2a, hollow arrows) and ONFL (Fig. 2a, arrows). The same is not seen in the retinas of control embryos (Fig. 3). The present findings are in agreement with those of Chmielewski et al. (1997) in that the retinal layers most affected by alcohol are the innermost ones, above all the GCL and ONFL. Moreover, a decrease of some 20% occurs in the total number of axons in the ONFL in experimental embryos compared with controls; this loss is 83.4% of myelinic axons versus 15.4% of amyelinic ones. This confirms the axonal loss in the optic nerve, producing its hypoplasia, described by Strömland and Pinazo-Durán (1994) in the rat, and by Strömland (1985) and Chan et al. (1991) in children with FAS.

In humans and higher vertebrates, lesions to the nervous system (NS) (medulla and optic nerve) have been reported to elicit a failure of the nerve fibers to reach the target cells and make functional contact (Berry, 1979; Reier, 1979; Reier et al., 1983; Wujek et al., 1984). In lower vertebrates, however, the opposite effect has been demonstrated: the nerve fibers are functionally regenerated (Gaze, 1959; 1960; Attardi et al., 1963). In all cases of lesioned zones of the NS, "reactive astrocytes" have been found. In higher vertebrates, these act as an obstacle to neuronal growth (Berry, 1979; Reier et al., 1983; Wujek et al., 1984), whereas in fishes (Wolburg, 1981) and amphibians (Reier, 1979; Reier et al., 1983; Stensaas et al., 1977) they provide an appropriate medium for cell regeneration. We found that in the retina of the alcohol-treated chick embryo the glial cells of the retinal inner layers can carry out the function of these "reactive astrocytes", since the astrocytes are located in the ONFL, adopting the position of the inner prolongations of the MCs that have degenerated (Fig. 2b, a). Moreover, the glial cells arising from pericytes that migrate from the vascular zone of the pecten are situated in the innermost part of the ONFL, presumably increasing their probable function as microglia and macrophages, and substituting the inner prolongations of the MCs.

Acknowledgements

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