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Alkaline phosphatase activity of the IVth ventricular choroidal epithelium of rats during embryonic and neonatal development

Takafumi Yoshioka[1], Osamu Tanaka[1], Hiroki Otani[1], Haruo Shinohara[1] and Kenichirou Inomata[2]

[1]Department of Anatomy, Shimane Medical University, Izumo 693 and [2]Department of Anatomy, Faculty of Medicine, Toho University, Tokyo 143, Japan

Summary. The cytochemical localization of alkaline phosphatase (AlPase) activity in the developing IVth ventricular choroidal epithelium was investigated in embryonic and neonatal rats. During the initial development of the choroidal primodium the flattened and/or cuboidal epithelial cells of the ventricular roof were changed to columnar cells with well-developed microvilli and apical tight junctions. When compared to AlPase activity on the lateral plasma membranes of the surrounding ependymal cells, these columnar cells of the choroidal primodium revealed activity on the lateral and luminal plasma membranes, but no activity was found on the basal surface of these cells. On the other hand, the epithelial cells in the neonatal choroid plexus showed a continuous morphological alteration from columnar cells with short microvilli to mature cuboidal cells with numerous long microvilli. AlPase activity in immature columnar cells was observed on all plasma membranes, except for the apical junctional area of the lateral surface. With maturing of the choroidal epithelial cells, the activity appeared to be eliminated from the lateral and luminal plasma membranes of the cuboidal cells, and mature choroidal epithelial cells showed activity on the basal surface only. These findings suggest that AlPase may play an important role in the membrane activity of epithelial cells differentiating between the primitive epithelial cells of the ventricular roof and the mature choroidal epithelial cells.

Key words: Alkaline phosphatase, Cytochemistry, Choroidal epithelium, Development, Rats

Introduction

The choroid plexus contains unique surface aspects in order to play a role in the production of cerebrospinal fluid (CSF) (Milhorat and Hammock, 1983). The choroidal epithelium serves as a barrier between CSF and interstitial fluid in production of CSF and in the absorption activity of various materials from the CSF (see reviews by Brightman, 1975; Van Deurs, 1980).

The origin of the choroidal epithelium is ontogenetically identical to that of the ventricular ependyma. The formation of the choroid plexus is initiated at the embryonic stage and is continued throughout brain development. The IVth ventricular choroid plexus is first formed by the invagination of the thin roof of the epithelium, accompanied with the lining interstitial cells and capillaries, into the IVth ventricular lumen (Netsky and Shuangshoti, 1975). During its development, the epithelial cells proliferate and change in shape to cuboidal cells containing numerous microvilli seen in the mature plexus (Tennyson, 1975; Sturrock, 1979; el-Gammal, 1981). This morphological development also involves the effective establishment of apical tight junctions, suggesting that the choroidal epithelium acquires the function as a barrier during fetal life (Dermietzel et al., 1977; Wakai and Hirokawa, 1981; Tauc et al., 1984).

The present study was performed to elucidate some of the metabolic activities, particularly the membrane activity of the choroidal epithelium converting immature morphological forms into mature morphological forms during prenatal and postnatal development, using the cytochemical technique for membrane-bound alkaline phosphatase (AlPase) activity.

Materials and methods

Albino rats of Jcl: Wistar were used in the present study. The day of positive vaginal plug was designated as embryonic day (E) 0. Embryos from E12 to E14 obtained by Caesarian section from pregnant animals were immediately fixed with a cold mixture of 2% glutaraldehyde (GLA) and 2% paraformaldehyde (PFA) in a 30mM PIPES buffer, pH 7.3, for 60 min. On

Offprint requests to: T. Yoshioka, Department of Anatomy, Shimane Medical University, Izumo 693, Japan

the other hand, neonatal rats of 0,3,5,7 and 10 days postnatal, anesthetized with sodium pentobarbital, were perfused with physiological saline through the left cardiac ventricle and followed by a cold fixative of 1% GLA and 2% PFA. After perfusion for 15 min, the hindbrain was rapidly removed and immersed in the same fixative for an additional 15 min. The embryonic and neonatal hindbrains containing the developing IVth ventricular choroid plexus were successively rinsed with a 30mM PIPES-buffered solution, pH 7.3, and cut into 40-60 μm transverse sections for enzymatic reaction, using a Microslicer. These sections were then incubated in the medium for AlPase activity (Mayahara et al., 1967) for 30 min at 37°C. As a control experiment, a medium containing 5mM levamisole, an inhibitor for non-specific AlPase activity, was designed. After incubation, specimens for electron microscopy were postfixed with PIPES-buffered 2% osmium tetroxide for 40 min, dehydrated in a series of graded ethanol solutions, treated with propylene oxide, and embedded in epoxy resin. Thin sections for electron microscopy were obtained using a Reichert-Jung OmU_4 ultramicrotome. These thin sections were unstained or stained with uranyl acetate only, and examined under a Hitachi HS-9 (75kV) electron microscope.

For light-microscopic examination, some thick sections of approximately 1μm in thickness were obtained from the epoxy resin-embedded materials and treated with yellow ammonium sulphide solution. These specimens were briefly counterstained with toluidine blue and mounted in gelatin.

Results

1. Localization of AlPase activity in embryonic choroidal epithelium

The initial development of the choroidal primodium in the IVth ventricular roof was investigated from the 12th to the 14th embryonic day.

The IVth ventricular roof of E12 embryo consisted of a single layer of flattened and/or cuboidal cells with smooth luminal and basal surfaces. At E13, the cuboidal cells containing more cytoplasmic projections at the luminal surface appeared in the lateral regions of the roof and these cells also exhibited irregular holdings in the basal surface (Fig. 1a). Electron-microscopically, AlPase activity in the epithelial cells of the ventricular roof until E13 was localized on the lateral plasma membranes and on some pinocytotic vesicles associated to the lateral plasma membranes, while no reaction product was found on the luminal and basal plasma membranes of the cells (Fig. 1a and 1b). The initial invaginations of the choroidal primodium appeared in the bilateral regions of the roof at E14. The choroidal primodium consisted of a single layer of tall columnar epithelial cells (Fig. 1c). The cells, located at the base of this primodium, bulged at the luminal surface and luminal microvilli were observed on the edges of the surface (Fig. 1d). On the other hand, the peripheral cells contained numerous microvilli (Fig. 1e). These columnar cells in the primodium also showed AlPase activity on the luminal plasma membranes, in addition to the lateral plasma membranes (Figs 1c, 1d and 1e). However, reaction products were eliminated from small areas of the lateral plasma membrane at the apical sides (Figs. 1d and 1e). The basal surface of the choroidal epithelial cells until E14 did not appear to contain AlPase activity (Fig. 1e).

2. Localization of AlPase activity in the neonatal choroidal epithelium

Figure 2 shows a light-microscopic view of AlPase activity in the neonatal choroid plexus at postnatal day 3. In addition to the mature choroidal epithelial cells of the peripheral loops, columnar cells were also observed in the base of the plexus. The activity in the peripheral choroidal loops was mainly observed in the interstitial elements, whereas the columnar cells in the base contained the activity throughout the entire cell surface and was particularly intense at the luminal surface. The ependymal linings adjacent to the choroid plexus appeared to be less positive for AlPase activity.

Electron-microscopically, the border between the ventricular ependyma and the choroid plexus was distinguishable by the following structures at the rear. The choroidal epithelium consisted of a single layer of columnar cells lined by basement membrane, whereas the ependyma cells extended their cytoplasmic processes into the neuropil. Reaction products for AlPase activity in the ependyma cells were localized on the plasma membranes of lateral surfaces and their processes extended into the neuropil, whereas no activity was found on the luminal plasma membrane (Fig. 3a). On the other hand, the columnar cells at the base of the plexus commonly contained the activity on the entire plasma membranes, including the luminal microvilli (Fig. 3c). Reaction product on the lateral surfaces was not observed in the junctional regions at the apical side (Fig. 3d). At the transitory region from the ependymal to the choroidal epithelium, the choroidal epithelial cells possessed the complicated holdings of basal surface, containing AlPase activity (Fig. 3b). In the peripheral choroidal loops, the columnar cells were changed to cuboidal cells containing abundant cytoplasmic organelles and well-developed microvilli. Among these cuboidal epithelial cells the proximal cells showed the activity on the luminal and basal plasma membranes, but no reaction product was found on the lateral plasma membrane (Fig. 4a). And finally, the activity was localized on the basal plasma membranes of mature choroidal epithelial cells in the peripheral loops (Fig. 4b). Some of the intracytoplasmic vacuoles in these mature cells also contained AlPase activy (Fig. 4c).

In control experiments, levamisole, added into the incubation medium, completely inhibited AlPase activity in embryonic and neonatal materials.

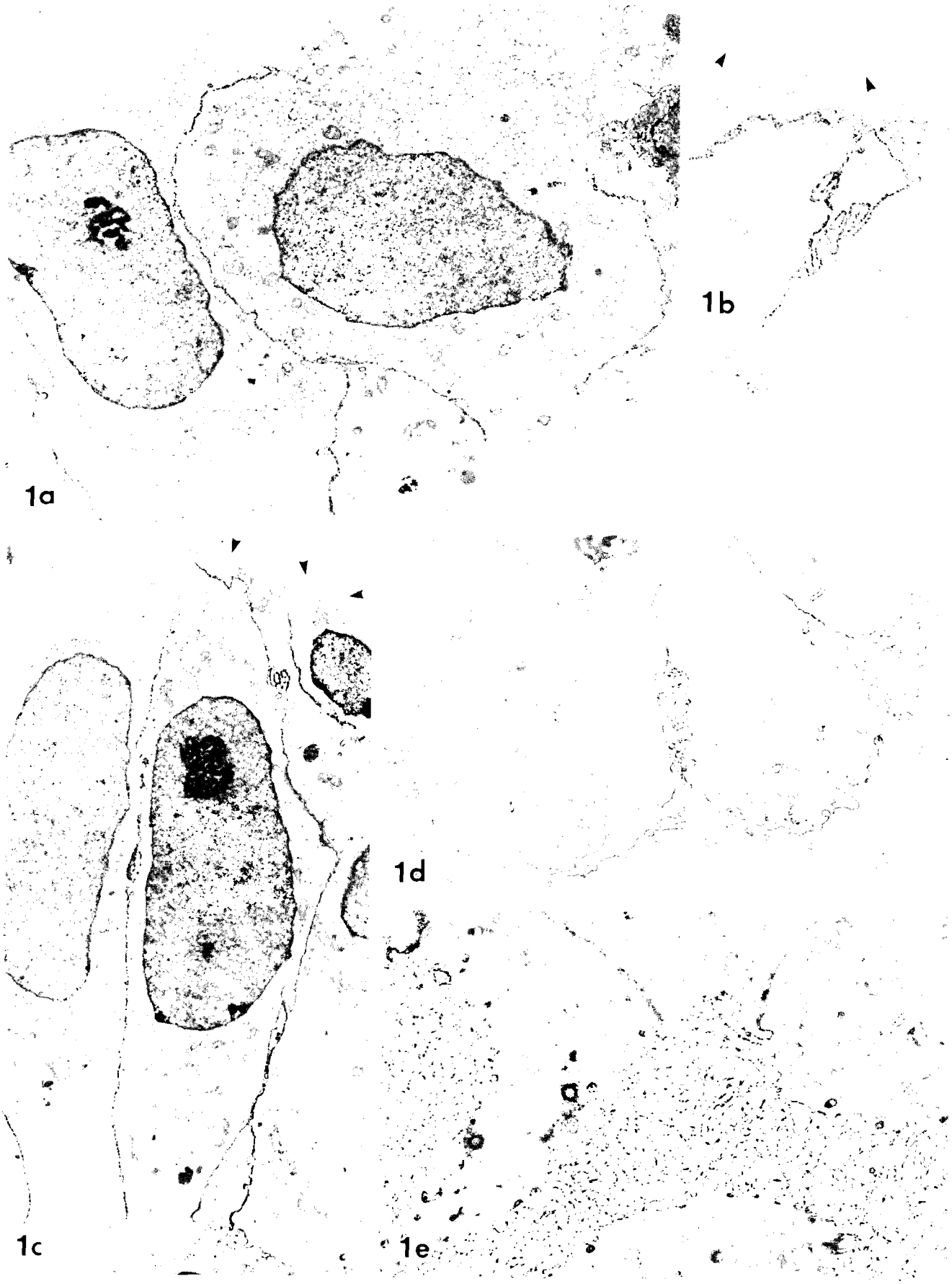

1a
1b
1c
1d
1e

Fig. 1. AlPase activity in the embryonic choroidal epithelium. **a.** Cuboidal epithelial cells appeared in bilateral regions of ventricular roof at E13. Reaction products were localized on the lateral plasma membrane, whereas no activity was found on the luminal and basal surface. × 8,500. **b.** Absence of AlPase activity on the basal plasma membrane (arrowheads) of cells. E13. Unstained. × 14,000. **c.** Columnar epithelial cells in the basis of choroidal primodium at E14. In addition to lateral plasma membrane, the activity was seen on the luminal surface of these cells. But, reaction product was not observed on the basal surface (arrowheads). × 6,000. **d.** Activity on the luminal surface of primodial epithelium. The luminal surface was bulged, and microvilli had developed on the edges of the surface. The apical junctional regions (J) did not contain reaction product. E14. × 16,500. **e.** Activity on the plasma membrane of well-developed microvilli. Many pinocytotic vesicles, associated to the luminal plasma membrane, also contained the activity. E14. × 17,500

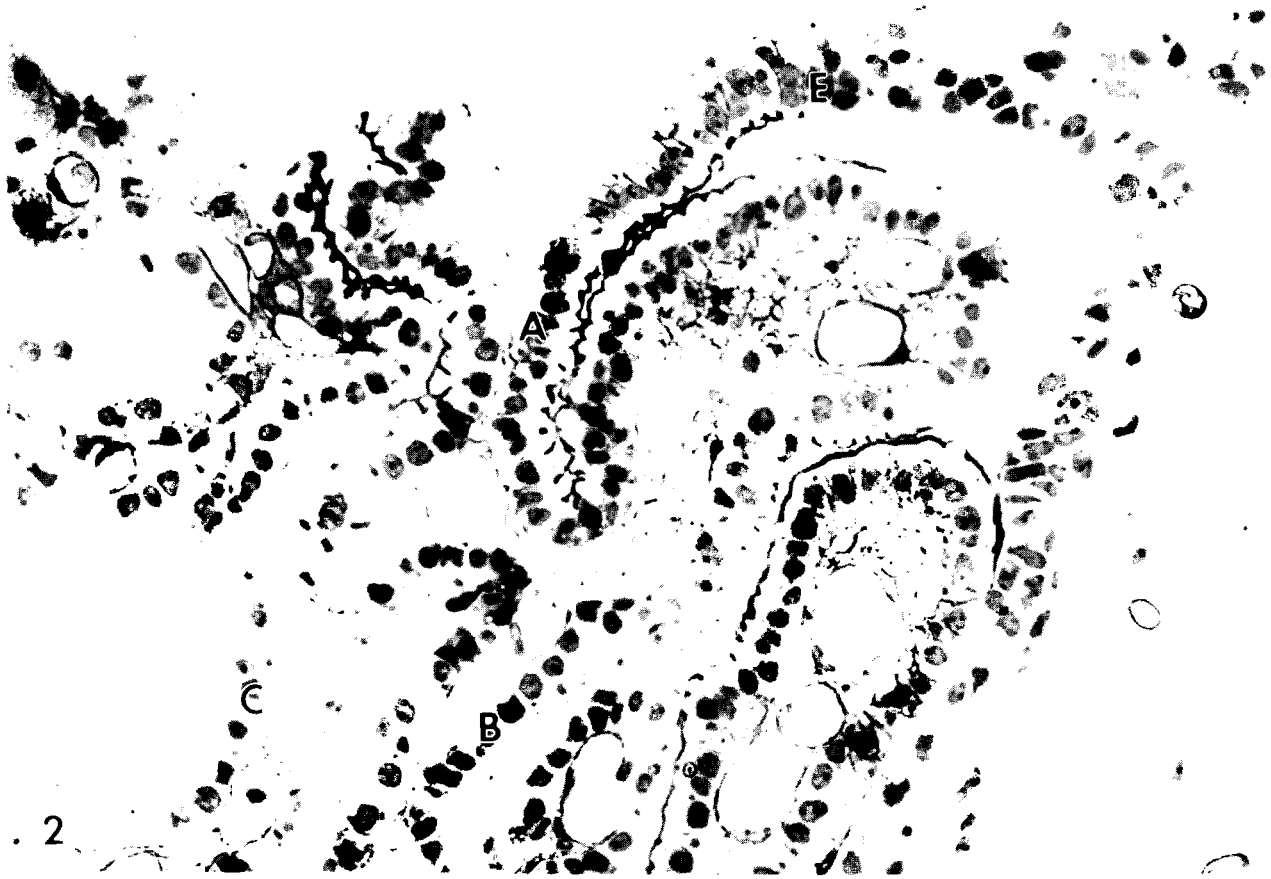
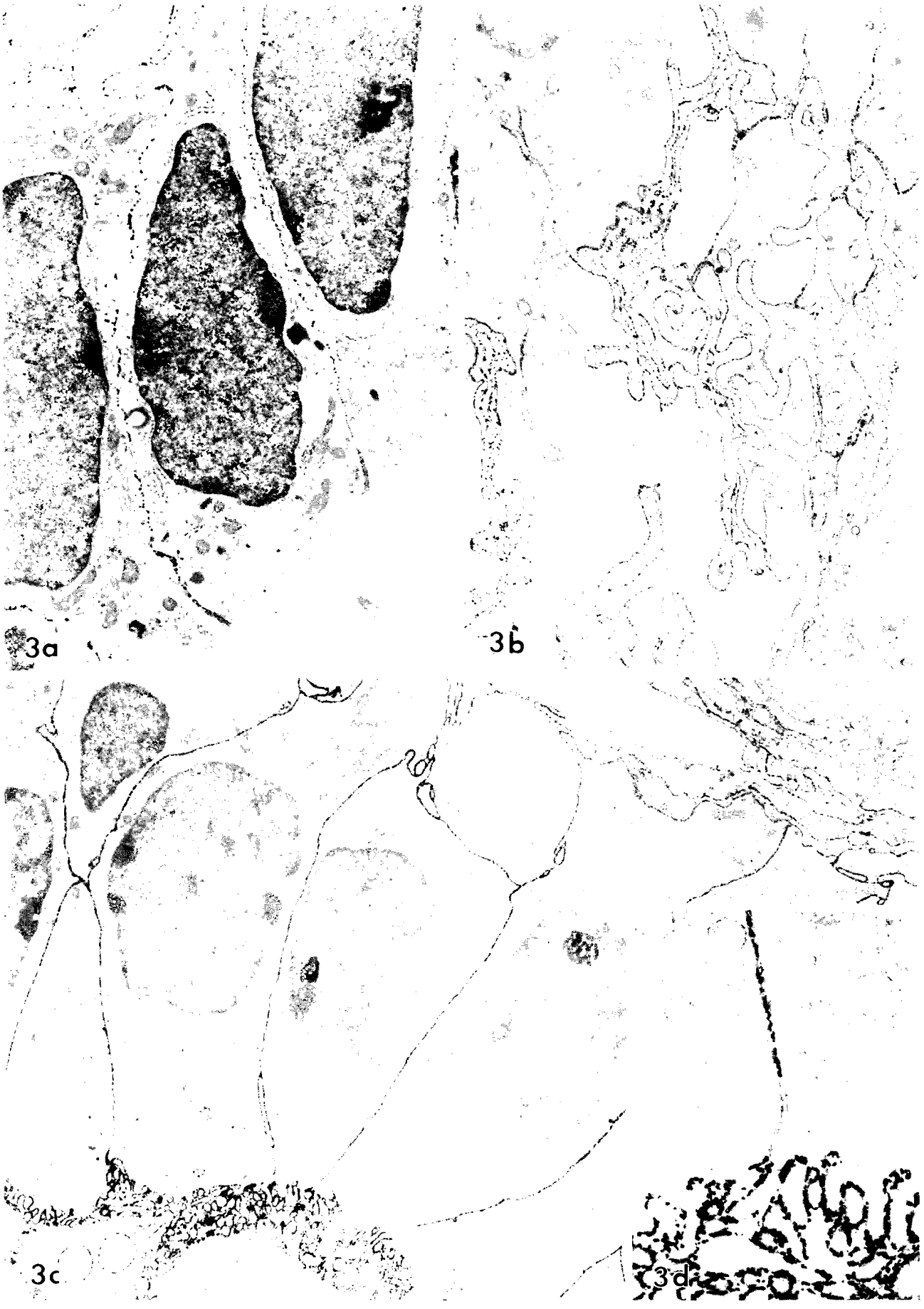


Fig. 2. Light micrograph of AlPase activity in the developing choroid plexus of 3-day-old rat, using a 1μm-thick section of resin-embedded material. Electron-microscopic localization of AlPase activity in regions indicated as A, B, C and E in this figure is represented in later photo-plates, though postnatal age is different. × 530.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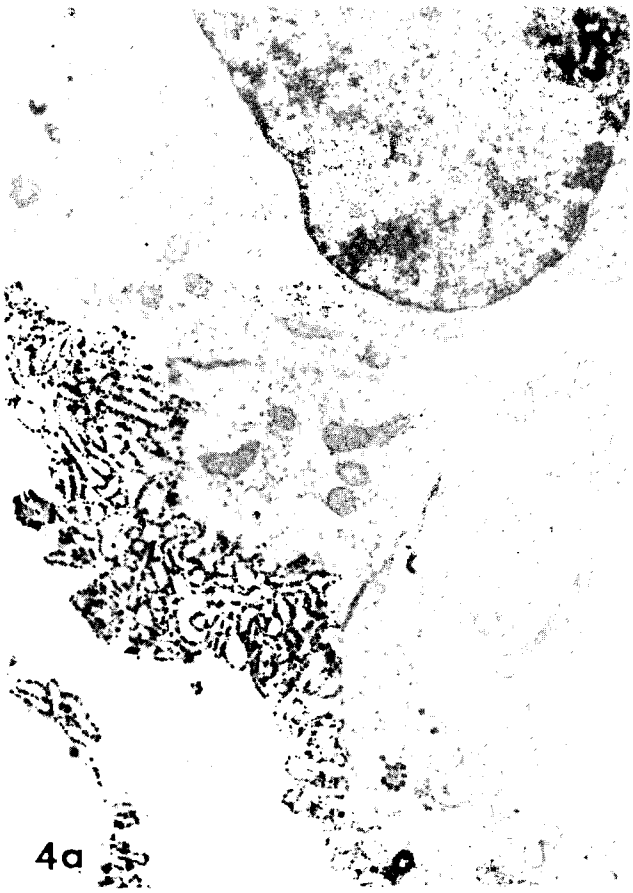

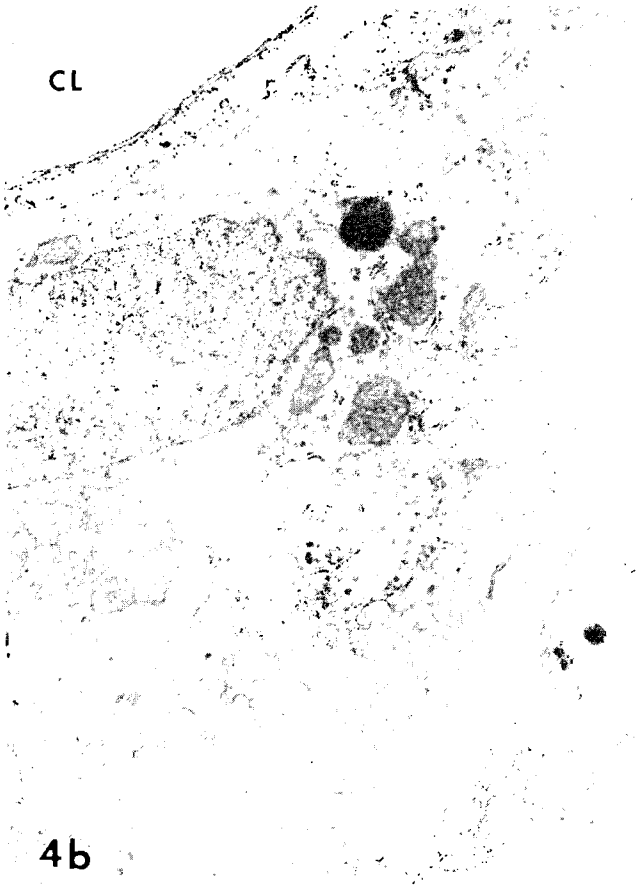

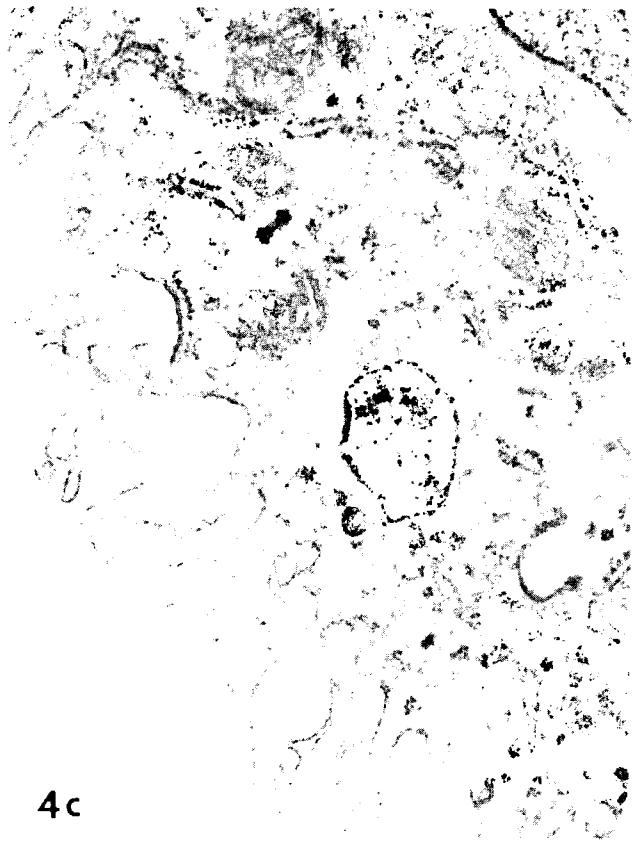

Fig. 3. AlPase activity in the neonatal choroid plexus.
a. Ependymal cells adjacent to choroidal epithelium (indicated as E in figure 2). Reaction products were mainly localized on the plasma membranes of lateral surface and cytoplasmic processes. 10-day-old rat. × 14,500.
b. Activity in the basal holdings of choroidal epithelial cells near the border region between ependymal and choroidal epithelium. 0-day-old rat. × 9,500.
c. Columnar epithelial cells at the basis of plexus, indicated as A in figure 2. Activity was observed on all cell surfaces of these epithelial cells. 3-day-old rat. × 6,500.
d. Absence of the AlPase activity in the apical junctional region of columnar choroidal epithelial cell. 3-day-old rat. × 27,000

Fig. 4. AlPase activity in the neonatal choroid plexus.
a. Activity in the luminal surface of differentiated choroidal epithelial cells seen in the peripheral choroidal loop (indicated as B in figure 2). Note the absence of reaction product on the lateral plasma membranes. 10-day-old rat. × 10,500.
b. Mature choroidal epithelial cells in the peripheral choroidal loop (indicated as C in figure 2). Reaction products were localized on the basal plasma membrane of cell (arrowheads). CL indicates the capillary lumen. 10-day-old rat. × 13,500.
c. Activity in the intracytoplasmic vacuole of mature choroidal epithelial cells. 10-day-old rat. × 18,500

Discussion

In the mature choroid plexus, AlPase is a dominant enzyme in the interstitial elements including the capillaries (Shimizu, 1950; Leduc and Wislocki, 1952; Becker and Sutton, 1975; Masuzawa and Sato, 1983). Masuzawa et al. (1980) showed that the choroidal epithelial cells contain this activity on the basal surface only, and indicated that AlPase may also be involved in the polarized permeability of materials across the choroidal epithelium. On the other hand, an earlier study of Kaluza et al. (1964) had reported that, in contrast to an increase of various kinds of enzyme activity with increasing age, AlPase activity was absent in developing chick choroidal epithelial cells. The present study, however, demonstrates that the immature epithelial cells of rat choroid plexus during embryonic and neonatal development contain an elevated activity of AlPase on their cell surface, and that this activity is eliminated with morphological maturity from the luminal and lateral surfaces. These findings suggest that the immature choroidal epithelial cells at the proliferative and/or differentiative phase contain a different membrane activity from the mature epithelial cells.

The immature choroidal epithelial cells in neonatal life exhibited AlPase activity on all plasma membranes, except for the apical junctional areas of the lateral surface. Absence of the activity in the junctional area was also observed in the primodial cells at embryonic stage, suggesting the establishment of effective tight junctions during embryonic development. The strict elimination of AlPase activity from the apical junctional areas distinguishes the choridal epithelial cells from the ependymal cells (Yoshioka and Inomata, 1983; Yoshioka et al., 1985). On the other hand, in contrast to the common appearance of AlPase activity on the basal surface of the neonatal choroidal epithelium, the apparent activity was not demonstrable on the basal surface of primodial choroidal epithelial cells. This may suggest that the choroidal epithelium in the early embryonic stage possesses a different transporting activity to that of the mature choroidal epithelium, because the foetal CSF contains a somewhat different composition to that of the mature animal (Woodbury, 1968; Birge and Haywood, 1971).

AlPase is distributed in various tissues of animals, but however, its physiological function remains vague (Kaplan, 1972; Borgers and Verheyen, 1983). It has been considered that AlPase in the absorptive epithelia such as the small intestine and kidney might play an important role in the transport of substances across the cell membranes (Mayahara et al., 1967; Hugon and Borgers, 1968; Healy and Dinsdale, 1979). On the other hand, some kinds of proliferative cells also possess intense AlPase activity (Berstine et al., 1973; Karasaki, 1975; Johnson et al., 1977; Kwong and Tam, 1984). Such intense AlPase activity may reflect an elevated membrane activity in the various metabolic states of the cells.

The changes in the surface localization of AlPase activity may reflect a metabolic conversion of choroidal epithelial cells in their membrane activity. This is closely combined with the morphology of choroidal epithelial cells. During cellular differentiation, AlPase may be inserted in the cell membranes through the intracellular pathway (Tokomitsu and Fishman, 1983); however, the mechanisms regulating the enzyme distribution on the cell membrane are not clear. The tight apical junctions between adjacent epithelial cells may function as barriers separating surface domains within cell membranes (Gumbiner and Louvard, 1985; Miller and Baldridge, 1985). But, such a hindrance between the lateral and basal plasma membranes of choroidal epithelial cells has not been identified. It remains to be determined how the distribution of AlPase on the plasma membranes is regulated during the differentiation of the choroidal epithelial cells.

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