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A comparison of the processes of ventricular coarctation and choroid and ependymal fusion in the mouse brain

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INTRODUCTION

Coarctation or fusion of adjacent walls of the lateral ventricles has been described in a number of species including man (Davidoff, 1946; Knudsen, 1958; Costoulas, 1958); the cat (Epstein, 1953; Fleischhauer, 1960) and the mouse, rat, hamster, guinea-pig and rabbit (Westergaard, 1970). The most detailed and extensive study has been that of Westergaard who not only studied a variety of species but also examined animals of different ages both by light and electron microscopy; he was, however, unable to explain the mechanism by which coarctation occurred. It is hoped that the present findings may help to elucidate the mechanism of coarctation, and also the fusions which occur between parts of the choroid plexus and ependyma of the lateral ventricles.

MATERIALS AND METHODS

Mouse embryos killed at daily intervals from 11 days post-conception (pc) up to 18 days pc, newborn mice, and mice aged 5, 10, 15, 25 and 90 days postnatum, were used in this study. The pregnant females were anaesthetised with an intraperitoneal injection of Nembutal and the embryos were removed by Caesarean section. Embryos and postnatal animals were killed by intracardiac perfusion with a solution of 2 % paraformaldehyde and 2 % glutaraldehyde in a 0·05 M phosphate buffer, each 100 ml of which contained 1·2 ml of dimethylsulphoxide and 0·3 ml of 10 % $CaCl_2$. The postnatal animals were anaesthetised with intraperitoneal Nembutal.

The embryonic brains were bisected in the coronal plane just rostral to the optic chiasm and each half was placed in 0·1 M cacodylate buffer. The postnatal brains were cut into 1–2 mm coronal slices from the rostral pole to the optic chiasm. Each slice was bisected in the mid-line and trimmed to leave a block of tissue containing the lateral ventricle, including any coarcted part, and placed in a 0·1 M cacodylate buffer. The embryo half-brains and the postnatal blocks were rinsed for 1 hour in buffer then placed in 1 % osmium tetroxide in a 0·1 M phosphate buffer for 2 hours, dehydrated in graded alcohols and embedded in Spurr's resin.

Semithin sections (1 μm) were stained with toluidine blue and examined at $\times 1000$ under oil immersion. The blocks were further trimmed to find areas of (1) apposition of ventricular walls, (2) apposition of choroid plexus and ventricular wall or roof, and (3) ventricular coarctation. Ultrathin sections were obtained, stained with uranyl acetate and lead citrate, and examined in an AEI 801 electron microsope.

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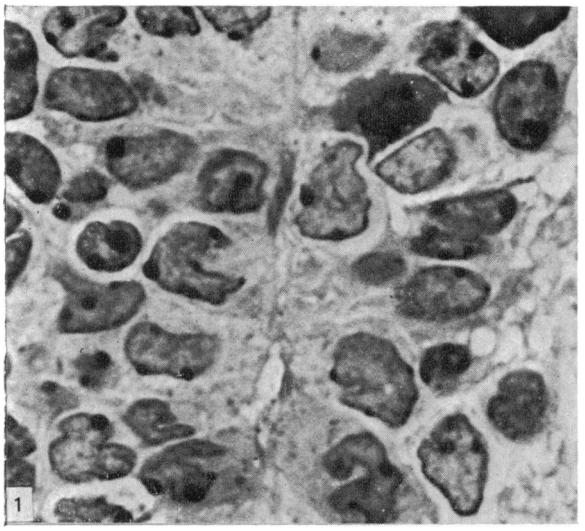

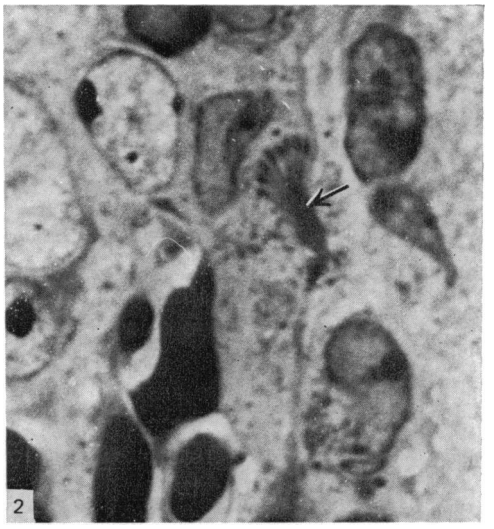

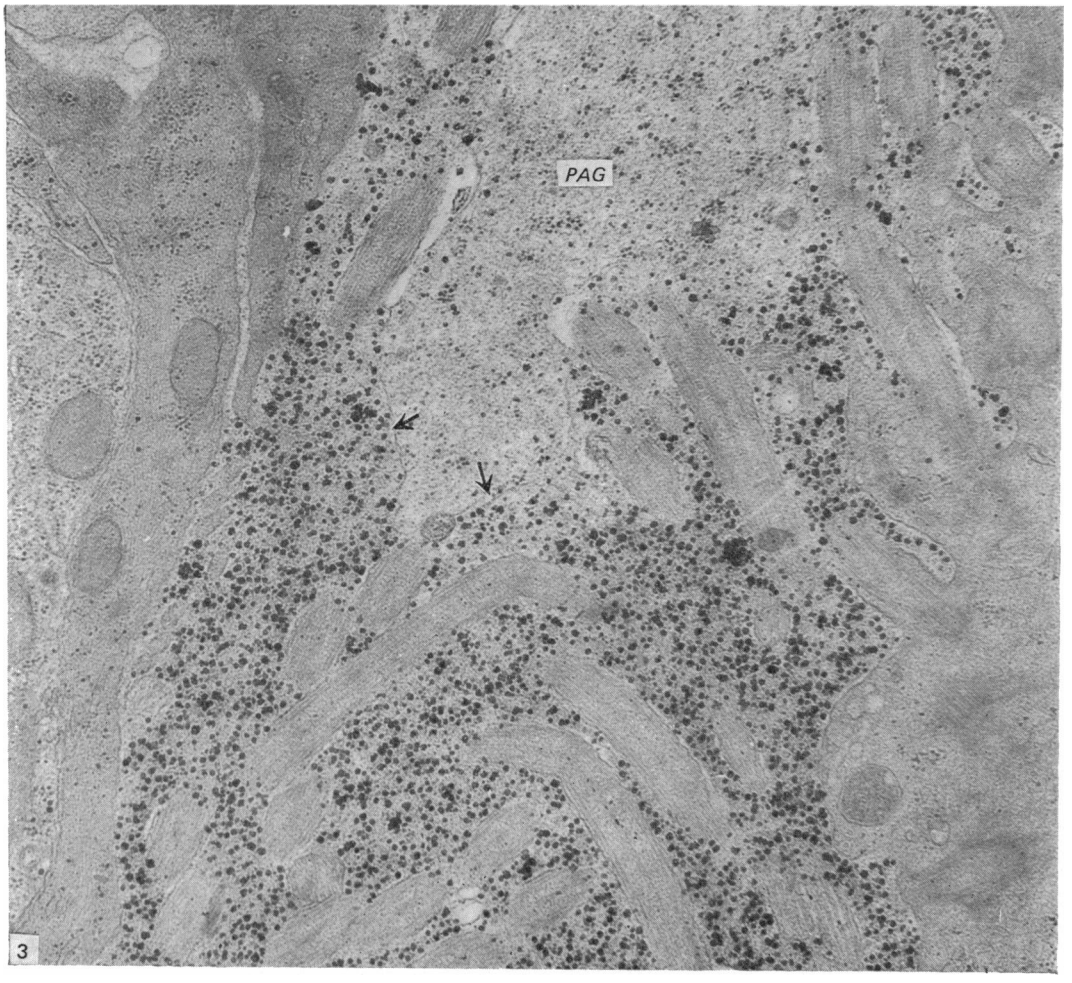
PAG

OBSERVATIONS

In embryos, the ventral portions of the walls of the lateral ventricle are frequently in apposition (Fig. 1). These apposed walls, however, seemingly do not fuse because examination of older animals demonstrates a complete lumen throughout the lateral ventricles. Comparison of sections from similar regions identified by such anatomical landmarks as the rostral part of the anterior limb of the anterior commissure shows that, while the walls of the ventral third of the lateral ventricle are usually apposed at 15, 16, 17 days pc, in newborn animals the corresponding part contains a lumen. Indeed, when fusion or coarctation of the ventricular walls does occur the most ventral part invariably remains patent. This was also observed by Westergaard (1970).

In semithin sections the most striking feature of areas of the ventricular walls in the process of fusing was the frequent presence of clumps of darkly stained cilia with prominent basal bodies (Fig. 2). Electron microscopically, however, the most striking feature was the large amount of glycogen in the extracellular space between the cilia. Figure 3 shows an area in the dorsal part of the fusing ventricular walls of a 15 days postnatum mouse. Most dorsally the space between the two walls contains an amorphous ground substance with glycogen scattered sparsely throughout it. The remnants of what appears to be a membrane can be seen at the lower edge of it. In the lower part of Figure 3 tangled cilia from both walls can be observed, with the spaces between them filled with large masses of glycogen in a moderately dense, amorphous ground substance.

As one moves ventrally from the dorsal pole of fusion (Fig. 4), cilia cut in different planes and embedded in a glycogen-rich, moderately dense, amorphous ground substance remain evident until one reaches areas where the cilia are more densely packed and only sparse clumps of glycogen remain (Figs. 5, 7). Fusion appears to be complete in areas where the cilia between the two walls disappear and ependymal cells from adjacent sides are joined together by maculae adherentes (Fig. 6).

A similar picture of extracellular glycogen and tangled cilia is also found in regions where the choroid plexus fuses with the ependymal wall. Figure 8 shows an edge of an area where the choroid plexus and ependyma seem to be fusing together. Glycogen can be seen in the ventricle between the choroid plexus and the mural ependyma, but the amorphous ground substance seen between the apposed ventricular walls appears to be absent. Glycogen is also present as one moves towards the areas of closest fusion (Fig. 9). In these areas, however, although extracellular glycogen is absent, the microvilli of the choroid plexus and the ependymal cilia are still present

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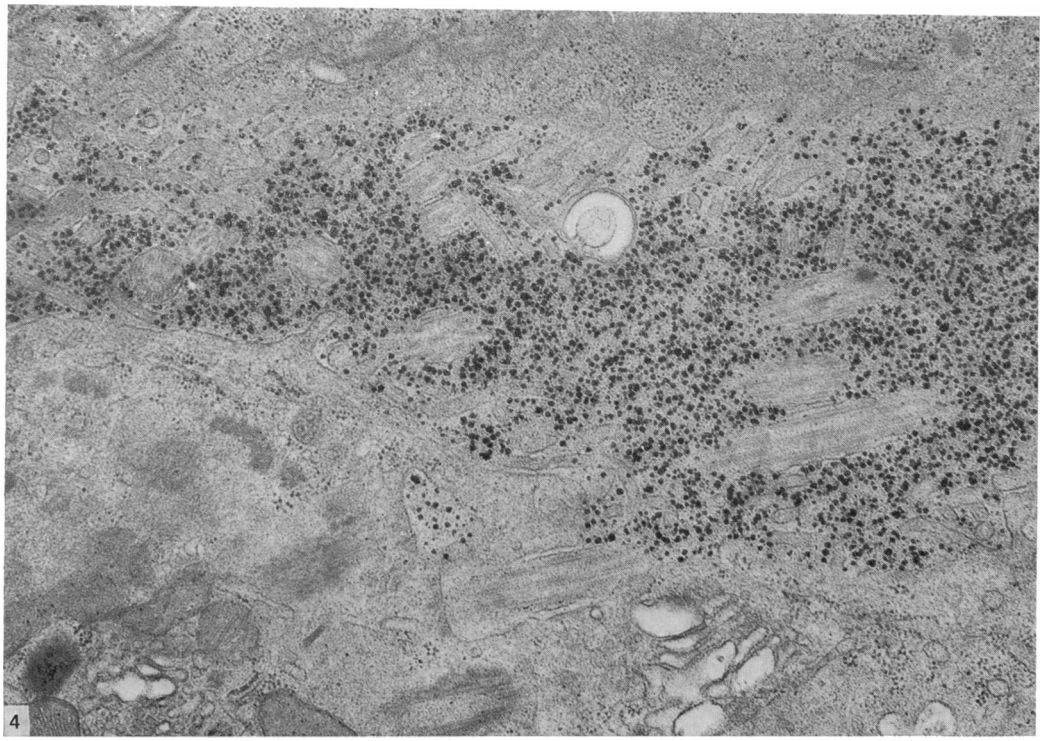
Fig. 1. Toluidine blue-stained semithin section of lateral ventricle shows medial and lateral walls in apposition, but not fused. Mouse. 16 days post-conception. × 1500.

Fig. 2. Toluidine blue-stained semithin section shows two walls of the lateral ventricle in the process of fusion. The most striking feature is the leash of cilia (arrow) with the basal bodies clearly in evidence. Mouse. 15 days postnatum. × 2400.

Fig. 3. The two walls of the lateral ventricles at the beginning of fusion. The upper space between the two walls is filled with a fairly pale amorphous ground substance (*PAG*) containing sparsely scattered glycogen granules. Parts of an incomplete membrane can be seen (arrows). Below this area numerous cilia cut in various planes are evident, and the spaces between them are filled with a glycogen-rich, darkly staining ground substance. Mouse. 15 days postpartum. × 19200.



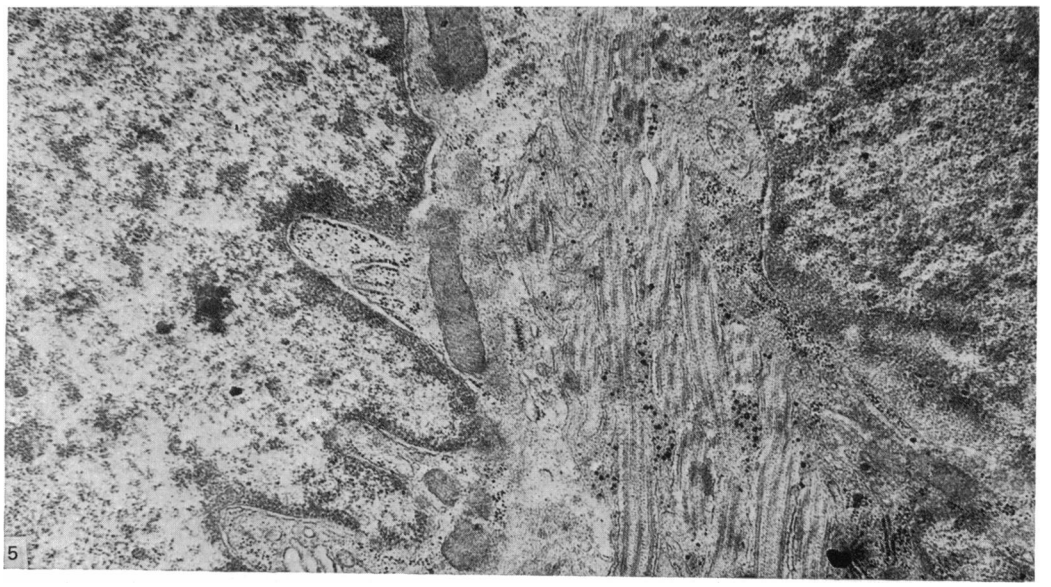


Fig. 4. This micrograph was taken further into the region of fusion than that in Fig. 3. Cilia and microvilli can be seen apparently embedded in the darkly staining, glycogen-rich ground substance. Mouse. 15 days postnatum. × 19200.

Fig. 5. Deeper into the region of fusion very little ground substance and glycogen remain. Mouse. 15 days postnatum. × 12800.

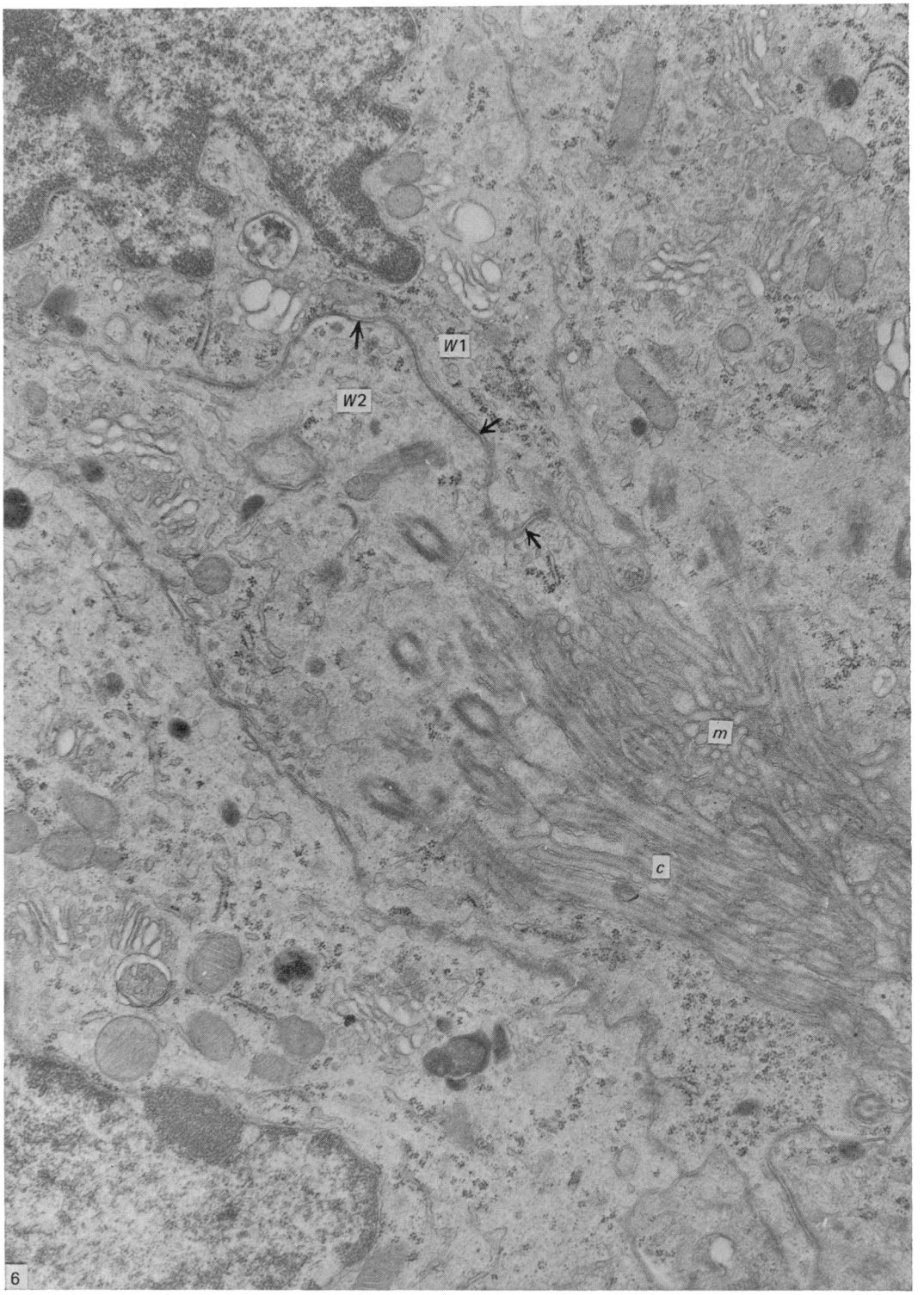


Fig. 6. Lateral ventricular coarctation showing the junction where the closely tangled cilia (*c*) and microvilli (*m*) end and the coarctation is completed by apposed ventricular cells from opposite walls (*W*1, *W*2) losing their surface specialisations and being joined by maculae adherentes (arrows). Mouse. 10 days postnatum. × 19200.

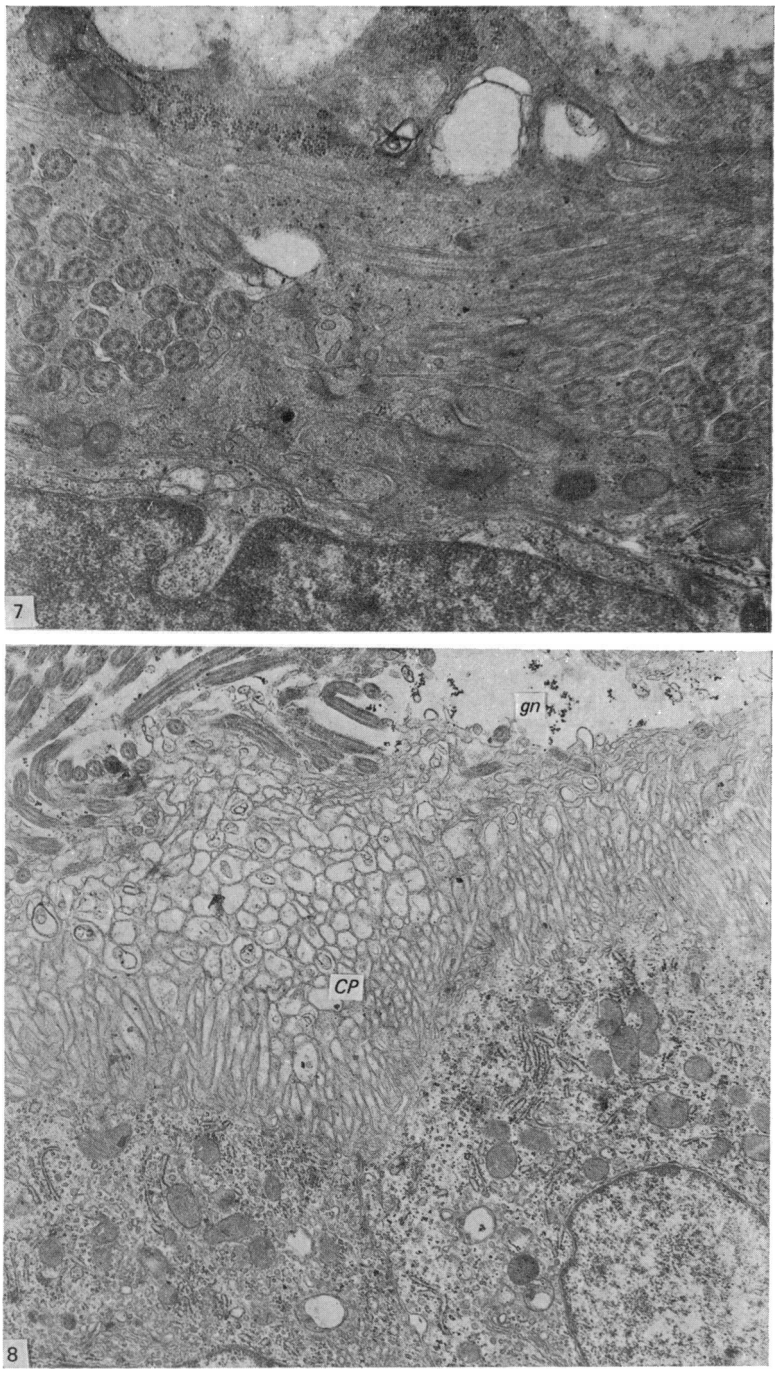
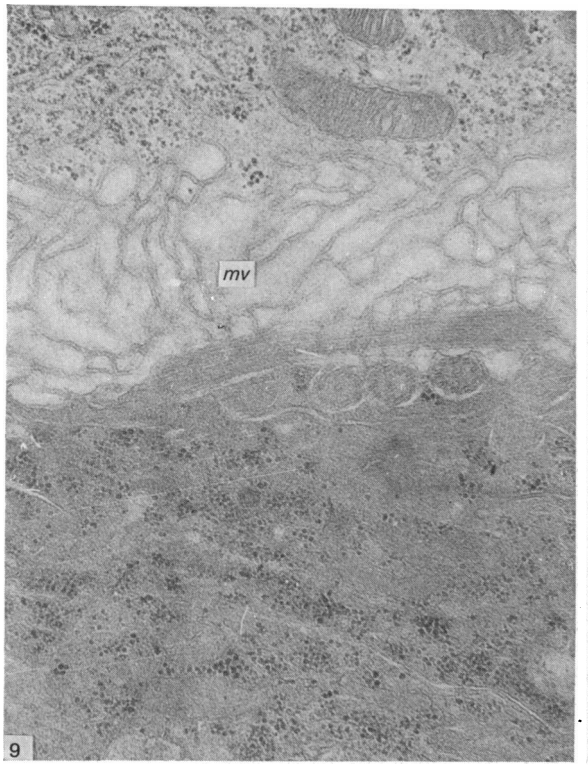


Fig. 7. This micrograph was taken from a region distant from the site of coarctation shown in Fig. 6. The dark ground substance between the tangled cilia contains very few glycogen granules. Mouse. 10 days postnatum. ×12000.

Fig. 8. The lateral edge of an area of fusion between the choroid plexus (*CP*) and the ependyma whose cilia are evident in the upper part of the micrograph. Note the extracellular glycogen (*gn*). Mouse. 15 days postnatum. ×8000.



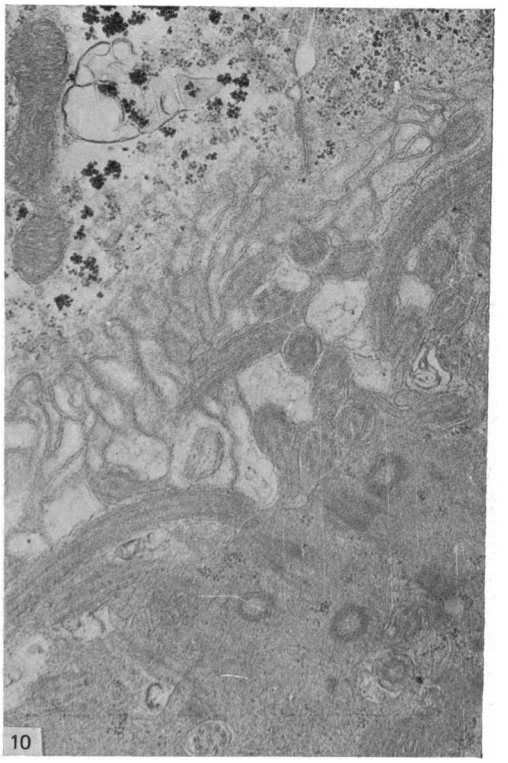


Fig. 9. Relative absence of extracellular glycogen towards the centre of fusion of the ependyma and choroid plexus. The ependymal cilia appear entangled with the choroid plexus microvilli (*mv*). The ependymal cytoplasm is rich in glycogen. Mouse. 15 days postnatum. ×12800.

Fig. 10. In the middle of the area of fusion of the choroid plexus and ependyma no extracellular glycogen is present and very little is present in the cytoplasm of the ependymal cells. In this case 'fusion' seems dependent upon the entanglement of ependymal cilia and choroidal microvilli rather than upon specialised cell to cell contacts such as occur in ventricular coarctation. Mouse. 15 days postnatum. ×12800.

(Fig. 10), which suggests that 'fusion' of the choroid plexus and ependyma is incomplete, unlike coarctation of the ventricular walls where fusion is complete.

DISCUSSION

The appearance of ventricular coarctation in adult members of a number of species has been comprehensively described by Westergaard (1970). He noted that coarctations were absent in newborn mice and newborn rats, but were present in 73 % of newborn guinea-pigs. In his series he found coarctation developed in 3–6 days old mice, which is in keeping with the results of the present study. An examination of 6 μm paraffin serial sections of mouse brain from our own comprehensive series supports his findings (Sturrock, unpublished).

The source of the extracellular glycogen is difficult to determine. In the prenatal and early postnatal brain the richest source of glycogen is undoubtedly the choroid plexus (Goldmann, 1913; Weed, 1917; Tennyson & Pappas, 1968), but coarctation extends much further ventrally than the choroid plexus. The pale amorphous ground substance in Figure 3 could conceivably be cytoplasm of a degenerating choroid

plexus epithelial cell, but it seems unlikely that this is the explanation for the quantity of glycogen found in the darker amorphous ground substance. It is possible that the glycogen is involved in the formation of the glycosaminoglycans of intercellular cement, but further histochemical studies are required to confirm or refute this suggestion. It is, however, interesting that extracellular glycogen is also present in areas of partial fusion between the choroid plexus and ependyma.

The presence of cilia is probably of major importance in the process of ventricular coarctation. A transmission and scanning electron microscopic study of the ependyma of the developing mouse lateral ventricle (Sturrock, in preparation) demonstrates that ependymal cilia are relatively infrequent before birth. It is worth emphasising that ventricular wall apposition is common in mouse embryos, but fusion, as evidenced by the presence of maculae adherentes, does not occur. Westergaard (1970) suggested that the reason why 73 % of guinea-pigs had lateral ventricular coarctations was that their central nervous systems were much more developed than those of mice and rats, owing to their much longer gestation period (64 days in contrast to 19 days for mice and 22 days for rats), and it seems probable that the most important difference is the presence of cilia in large numbers in the newborn guinea-pig.

SUMMARY

Lateral ventricular coarctation and fusion of the choroid and ependyma was studied in prenatal and postnatal mice. Coarctation of the lateral ventricle was preceded by the presence of a large quantity of extracellular glycogen embedded in an electron-dense, amorphous matrix lying between the ventricular walls. As the site of coarctation is approached the quantity of glycogen and ground substance decreases and eventually the cilia and microvilli of the ependymal cells also disappear and adjacent walls are joined by maculae adherentes. The glycogen may be produced by the choroid plexus. The presence of cilia seems necessary for coarctation to occur. Fusion of the choroid plexus with the ependyma appears to be due simply to entanglement of choroidal microvilli and ependymal cilia without any specialised cell to cell connexions being present.

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